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Agradecimientos: Son opcionales y tendrán un máximo de tres renglones para expresar agradecimientos a personas e instituciones que hayan contribuido a la realización del trabajo.
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Determination of Combinatorial Ability and Heterosis in *Capsicum chinense* Jacq., using Line×Tester Analysis Method

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ABSTRACT
Objective: Know the combinatory ability and heterosis in yield parameters and fruit quality, in nine genotypes of Habanero Chilli (*Capsicum chinense* Jacq.).
Design/methodology/approach: In order to know the combinatory ability and heterosis in yield parameters and fruit quality, were crossed nine genotypes of *Capsicum c hinense* Jacq. (Habanero Chilli) in a Line×Tester (7×2) mating design. The parental lines and combination of crosses were evaluated in a randomized complete block design with three replications at the Yucatan Scientific Research Center during the 2018 and 2019.
Results: The results showed the lines 3 and 7, presented a high and positive general combinatory ability (GCA) for most of the parameters studied, the tester 1 presented a high GCA for yield per plant (YP) with 0.45. The cross L1×T2 presented the highest value of specific combining ability (SCA) for YP with 0.50. The highest heterosis (171.01%) was observed in the L1×T1 cross for capsaicin content (CC). Tester 1 was identified as a promising genotype for breeding *Capsicum chinense* Jacq.
Findings/conclusions: Line 7 and Tester 1 was identified as promising genotypes for crop breeding *Capsicum chinense* Jacq.

Keywords: *Capsicum chinense* Jacq., Line×Tester Analysis, F1 hybrids, crop breeding, yield.

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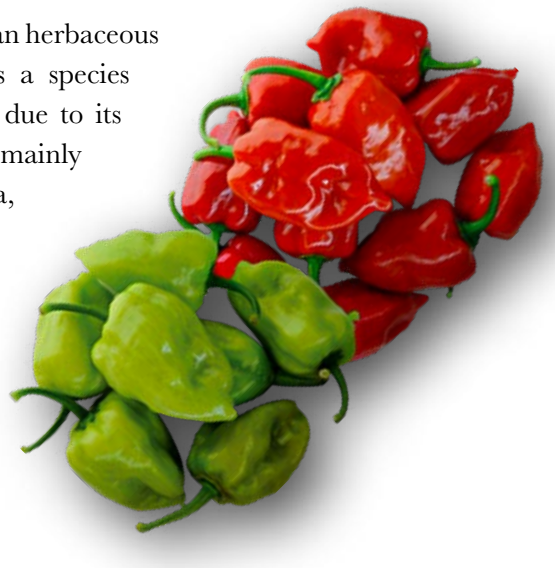
INTRODUCTION

The (*Capsicum chinense* Jacq.) is grown from an herbaceous plant belonging to the genus *Capsicum*, it is a species highly appreciated by international markets, due to its high level of spiciness, flavor and aroma. It is mainly cultivated in Australia, Britain, USA, Sri Lanka, Bangladesh, India, and Mexico, Habanero Chilli the latter country being the one with the greatest genetic diversity of this genus and a growing demand for production (Palma-Orozco *et al.*, 2021), (Cordova *et al.*, 2021).



Plant genetic improvement contributes to improving the sustainability of production systems, through the generation of crops adapted to different environments and consumer demands. However, despite recent scientific discoveries, the need to generate new cultivars persists (Dato *et al.*, 2015)59 accessions belonging to nine different species have been genotyped with a set of ten simple sequence repeats (SSR. The wide genetic diversity of *Capsicum chinense* Jacq. position it as an excellent resource to enter into a genetic improvement program, to obtain better genotypes that increase yield and improve fruit quality.

The main job of plant breeders is to achieve combinations of genes through selection, crossing, mutations and genetic engineering, to obtain the best genotypes, based only the phenotypes (Kempthorne, 1974). By carrying out crosses between various genotypes, we can first obtain F1 hybrids which are the most efficient alternative to satisfy the demands of *Capsicum chinense* Jacq. in the food, pharmaceutical, military, and painting industries, among others (Tag *et al.*, 2014; Martins *et al.*, 2017 and Tyagi *et al.*, 2022). F1 hybrids represent two important advantages, its uniformity and heterosis. However, a great segregation occurs in their progenies, losing the two advantages mentioned and they only maintain these, if they are crossing again with their parents. This is because the F1 hybrids do not reproduce themselves, since their heterozygosity is very high.

The Line×Tester (L×T) analysis is a modified version of the top cross design. The most important advantage of this method is that it allows evaluation between parents with less experimental material compared to other designs. Besides, with the Line×Tester design, the analysis of the level of heterosis and combinatorial ability can be carried out, which are important parameters to define the best parents and introduce them to other genetic improvement programs (Kahriman *et al.*, 2016). Heterosis represents a specific result of the dissimilarity in the constitution of the parents' gametes, which is expressed in greater size, vigor, fecundity manifested by crossed organisms, with respect to inbred organisms (Tuxtla-Andrade *et al.*, 2022).

The present study was carried out with the aim of determining the combinatorial ability and heterosis, as well as knowing the best crosses and their respective parents, in F1 hybrids of Habanero Chilli (*Capsicum chinense* Jacq.), obtained through the Line×Tester genetic improvement design.

MATERIAL AND METHODS

Genetic material

Nine genotypes of *Capsicum chinense* Jacq. were used as parents of which, four are ripe red fruit (T1, L1, L2, L3), two orange (L4, L5), two yellow (L6, L7) and one purple (T2), and were obtained from the CICY (Yucatan Scientific Research Center) gene bank.

Field performance evaluation

The nine parents were crossed in autumn 2018 in a scheme seven lines and two testers (7×2), according to the Line×Tester mating design developed by (Kempthorne, 1974). The process consisted of crossing one parent chosen as tester (P), with the other parents (lines), generating 14 crosses in total (Table 1, Figure 1). For this evaluation, the seeds

Table 1. Design of Line×Tester crosses (7×2) in Habanero Chilli.

Genotypes		
Progenitors	Testers (2)	T1; T2
	Lines (2)	L1; L2; L3; L4; L5; L6; L7
	Crosses (14)	L1×T1; L2×T1: L3×T1; L4×T1; L5×T1; L6×T1; L7×T1; L1×T2; L2×T2; L3×T2; L4×T2; L5×T2; L6×T2; L7×T2

Figure 1. Habanero Chilli genotypes used as testers, in the Line×Tester (7×2) genetic improvement design. A) T1=Tester 1; B) T2=Tester 2.

obtained from the crosses and their respective parents were germinated in commercial substrate Peat moss® (*Spagnum* spp.). The transplant was performed in the summer of 2019, 40 days after sowing in growbags (Pelemix, Guadalajara Jalisco, Mexico) with 1 m a length of, which contains coconut fiber (thick and fine) in a proportion 70:30; the distances between plants were 20 cm and rows were 160 cm, in a greenhouse of the Yucatan Science and Technology Park located in Merida, Yucatan, under hydroponic conditions.

Fruit quality attributes

The fruit quality attributes evaluated in the crosses (F1 hybrids) and their parents were: fruit weight (FW) in grams (g), pericarp thickness (PT), length (FL), width (WF) in centimeters (cm), the parameter yield per plant (YP) in kilograms per plant ($kg \cdot plant^{-1}$) and the number of fruits per plant (NF) were also evaluated, evaluated according to the Capsicum descriptors (IPGR, 1995). The capsaicin content, was expressed in milligrams per gram of dry weight (DW), was calculated with the method reported by (Muñoz-Ramírez *et al*., 2018) which consisted of taking mature fruits picked randomly per row of each genotype and dried in oven with a circulation of air for 48 h, to later be ground until a fine powder was obtained, 100 mg fruit powder were mixed with 40 mL acetonitrile and maintained in water bath at 80 °C for 4 h with periodic agitation. The extracts were centrifuged (Sigma 2-16kl) at 17,968 g for 10 min at 4 °C. The supernatant was collected and filtered through syringe filters with a polytetrafluoroethylene (PTFE) membrane of 0.45 mm (no. 721-1345; Thermo Scientific) in 2 mL vials of amber glass and finally stored at 4 °C until the chromatographic analysis was performed by high-performance liquid chromatography (HPLC) with a brand team mark Agilent series 1200.

Heterosis and combinatorial abilities

Heterosis was estimated based on the average parent, using the following expressions: percentage of heterosis with respect to the average parent;

$$HPM = \left[(F1 - PM) / PM \right] \times 100$$

where: HPM=heterosis mean of the parents, $F1$=Hybrid F1, PM=mean of the parents= $\left[(P1 + P2) / 2 \right]$ proposed by (Martínez-Martínez *et al.*, 2014). Combinatorial abilities were determined as reported by (Kahriman *et al.*, 2016).

Experimental design and statistical analysis

The arrangement of the treatments (parents and crosses) was carried out based on the complete randomized block design, with four repetitions and eight plants as the experimental unit. The data obtained from the evaluated parameters were subjected to an analysis of variance (ANOVA). The means of the cultivars were compared by a multiple comparison of means by the least significant differences (LSD) test, at a significance level of 5% ($p \leq 0.05$). The statistical analysis was calculated using the SAS program version 9.3 for Windows (Rodrigues *et al.*, 2012).

RESULTS Y DISCUSSION

Field performance evaluation

Table 2 shows the fruit yield and quality parameters of Habanero Chilli genotypes. It can be observed that line 7 presented statistically the highest values in fruit weight, pericarp thickness and fruit width (16.82 g, 2.79 mm and 4.32 cm, respectively). The cross between line 3 and tester T1 had the greatest fruit length (FL) and the greatest number of fruits per plant (NF) with 5.37 cm and 278.1 respectively. The L7×T1 cross produced the highest yield per plant (YP) with 3.38 kg·plant^{-1}, whereas line 3 obtained the highest value in capsaicin content in fruit (CC) with 120.38 mg·g^{-1} DW. In general, the crosses with Tester T1 stood out in most of the parameters evaluated.

Previous studies also reported significant differences for these traits evaluated between F1 crosses; for example, (Tarinta *et al.*, 2023) reported values of NF (50.88 to 75.52) from patters and (44.54 to 108.9) regarding the hybrids, in *Capsicum baccatum*. Similarly, (Figueiredo *et al.*, 2015) in *Capsicum annuum* observed the same effect in CC (2.4 to 731.4 μg/g DW) from patters and (1.7 to 123.6 μg/g DW) regarding the hybrids.

General combinatorial ability

The general combinatorial ability (GCA) indicates the behavior of a particular parent before the crosses in which it participated, evidencing additive genetic effects (Rohini *et al.*, 2017). In the present study, the parents revealed good general combinatorial ability in the performance and quality parameters (Table 3), the values of general combining capacity in the parental lines indicate that the best genotypes to improve each parameter are the following: Line 7 for FW (2.18 g), PT (0.34 mm) and WF (0.40 cm). Line 3 for

Table 2. Fruit yield and quality parameters evaluated in genotypes of Habanero Chilli analyzed in the Line×Tester (7×2) design.

Line	FW (g)	PT (mm)	FL (cm)	WF (cm)	NF	YP (kg·plant^{-1})	CC (mg·g^{-1} DW)
L1	12.73^{fg}	1.93^{k}	4.32^{ghi}	3.14^{fg}	184.11^{ef}	2.34^{gh}	15.70^{s}
L2	9.75^{k}	2.39^{de}	4.02^{jk}	2.81^{j}	239.83^{b}	2.28^{hi}	20.34^{r}
L3	6.75^{n}	1.46^{m}	5.28^{ab}	2.42^{k}	219.82^{cd}	1.48^{n}	120.38^{a}
L4	12.70^{fg}	2.52^{b}	4.81^{ef}	2.94^{hi}	182.42^{ef}	2.32^{h}	28.95^{o}
L5	10.46^{j}	2.27^{fg}	5.03^{cd}	3.01g^{h}	239.22^{b}	2.50^{ef}	29.13^{o}
L6	16.68^{a}	2.34^{ef}	4.48^{g}	3.67^{c}	149.06^{i}	2.49e^{fg}	26.51^{p}
L7	16.82^{a}	2.79^{a}	3.62^{l}	4.32^{a}	155.18^{hi}	2.61^{e}	23.81^{q}
Tester							
T1	14.11^{c}	2.20^{gh}	4.90^{def}	3.01^{gh}	169.85^{fg}	2.39^{fgh}	23.68^{q}
T2	13.53^{de}	1.98^{jk}	4.24^{hi}	2.95^{hi}	104.92^{j}	1.42^{n}	44.54^{fg}
Line×Tester							
L1×T1	13.61^{d}	2.41^{cde}	4.47^{g}	3.21^{ef}	205.84^{d}	2.80^{d}	53.38^{d}
L2×T1	12.05^{h}	2.56^{b}	4.77^{f}	3.14^{f}	232.65^{bc}	2.80^{d}	30.29^{n}
L3×T1	11.56^{i}	1.84^{l}	5.37^{a}	2.98^{hi}	278.10^{a}	3.21^{b}	71.85^{c}
L4×T1	13.41^{de}	2.49b^{c}	5.04^{cd}	3.02^{gh}	227.35^{bc}	2.99^{c}	45.12^{f}
L5×T1	12.71^{fg}	2.39^{de}	5.13^{bc}	3.13^{fg}	233.40^{bc}	2.96^{c}	33.61^{l}
L6×T1	12.88^{fg}	2.41^{cde}	4.89^{def}	3.30^{e}	228.49^{bc}	2.94^{cd}	39.52^{j}
L7×T1	15.12^{b}	2.46^{bcd}	3.91^{k}	3.87^{b}	223.56^{c}	3.38^{a}	48.1^{e}
L1×T2	12.52^{g}	2.16^{hi}	4.84^{ef}	3.21^{ef}	232.41^{bc}	2.91^{cd}	44.02^{g}
L2×T2	9.83^{k}	2.19^{gh}	4.19^{ij}	2.97^{hi}	189.81^{e}	1.87^{lm}	32.30^{m}
L3×T2	9.47^{kl}	1.94^{k}	4.98^{cde}	2.87^{ij}	220.72^{c}	2.09^{jk}	85.05^{b}
L4×T2	9.18^{lm}	2.04^{j}	4.41^{gh}	2.96^{hi}	188.25^{e}	1.73^{m}	40.78^{i}
L5×T2	8.91^{m}	2.06^{j}	4.45^{g}	3.11^{fg}	240.36^{b}	2.14^{ij}	38.81^{k}
L6×T2	12.74^{fg}	2.07^{ij}	4.75^{f}	3.51^{d}	164.94^{gh}	2.10^{jk}	41.70^{h}
L7×T2	13.12^{ef}	2.76^{a}	4.13^{ij}	3.28e	152.40^{hi}	2.00^{kl}	33.05^{l}
Mean	12.20	2.25	4.61	3.17	202.73	2.42	42.22
LSD 0.05	0.45	0.09	0.19	0.12	14.34	0.14	0.60

Values followed by the same letter do not differ significantly (LSD, T≤0.05); FW: fruit weight; PT: pericarp thickness; FL: fruit length; WF: fruit width; YP: yield per plant; NF: number of fruits per plant; CC: capsaicin content, dry weight (DW); LSD=least significant difference.

FL (0.51 cm), NF (33.82) and CC (32.88 mg·g^{-1} DW). Finally, Tester T1 for YP (0.45 kg·plant^{-1}). In a preliminary study reported by (Naves *et al*., 2022) in *Capsicum annuum* L. the best parents were: LCA625 (P4) for the number of fruits with 8.76, PKM1 (P5) for the shape of the fruit with 0.12 cm, Arka Lohit (P1) and Pusa Jwala (P6) for capsaicin content with 0.04%.

Table 3. General combinatorial ability of the parameters evaluated in Habanero Chilli used in the Line×Tester (7×2) design.

Line	FW (g)	PT (mm)	FL (cm)	WF (cm)	NF	YP (kg·plant^{-1})	CC (mg·g^{-1} DW)
L1	1.13	0.02	−0.01	0.02	3.54	0.29	3.13
L2	−1.00	0.10	−0.19	−0.13	−4.36	−0.23	−14.27
L3	−1.42	−0.38	0.51	−0.26	33.82	0.09	32.88
L4	−0.64	0.00	0.06	−0.19	−7.79	−0.21	−2.62
L5	−1.13	−0.05	0.12	−0.06	21.29	−0.01	−9.36
L6	0.87	−0.03	0.16	0.22	−18.88	−0.04	−4.95
L7	2.18	0.34	−0.65	0.40	−27.62	0.12	−4.82
SE (GCA_l) line	1.32	0.21	0.33	0.22	20.78	0.17	14.86
Tester							
T1	1.11	0.10	0.13	0.05	17.18	0.45	0.43
T2	−1.11	−0.10	−0.13	−0.05	−17.18	−0.45	−0.43
Line×Tester							
SE (GCAt) Tester	0.63	0.054	0.07	0.026	9.91	0.25	0.24

FW: fruit weight; PT: pericarp thickness; FL: fruit length; WF: fruit width; YP: yield per plant; NF: number of fruits per plant; CC: capsaicin content, dry weight (DW); SE: Standard deviation; GCA: General combinatorial ability.

Specific combinatorial ability

Genetic improvement to obtain hybrids in Habanero Chili is little carried out, largely due to a lack of knowledge about heterosis and parent of origin effects in different hybrid combinations (Pech May *et al*., 2010). The specific combinatorial ability effects (SCA) of the 14 F1 hybrids of *C. chinense* Jacq. are presented in Table 4.

The best crosses for each parameter were the following: L3×T1 for FW (14.08 g), L7×T2 for PT (0.25 mm), L1×T2 for FL (0.32 mm) and NF (30.46), L7×T1 for WF (0.24 cm) and YP (0.24 kg·plant^{-1}), L3×T2 for CC (7.03 mg·g^{-1} DW). The crosses with Tester T2 stood out in specific combinatorial ability for most of the parameters evaluated. [13] in their work with *Capsicum baccatum* report positive effects SCA on the hybrids UENF1629×UENF1732 for YP (0.49 kg·plant^{-1}) UENF1616×UENF1732 for FW (3.08 g) and UENF 1624×UENF 1639 for NF (37.19).

Heterosis

Most of the hybrids were significantly superior to the parents in all the parameters evaluated (Table 5), heterosis was positive in the cross L3×T1 for WF, whereas the cross L7×T1 presented positive values of heterosis for most of the parameters evaluated (Figure 2). On the other hand, the L1×T1 cross presented the highest value of heterosis in CC (171.01%).

Table 4. Specific combinatorial ability of the parameters evaluated in the F1 Habanero Chilli crosses obtained from the Line×Tester (7×2) design.

Crosses (F1)	FW (g)	PT (mm)	FL (cm)	WF (cm)	NF	YP (kg·plant^{-1})	CC (mg·g^{-1} DW)
L1×T1	6.74	0.03	−0.32	−0.05	−30.46	−0.50	4.25
L2×T1	4.76	0.09	0.16	0.03	4.24	0.02	−1.43
L3×T1	14.08	−0.15	0.07	0.00	11.51	0.11	−7.03
L4×T1	5.54	0.13	0.19	−0.02	2.37	0.18	1.74
L5×T1	9.50	0.07	0.21	−0.04	−20.66	−0.04	−3.03
L6×T1	9.62	0.08	−0.06	−0.16	14.59	−0.03	−1.51
L7×T1	10.85	−0.25	−0.24	0.24	18.40	0.24	7.00
L1×T2	−2.82	−0.03	0.32	0.05	30.46	0.50	−4.25
L2×T2	−1.19	−0.09	−0.16	−0.03	−4.24	−0.02	1.43
L3×T2	3.37	0.15	−0.07	0.00	−11.51	−0.11	7.03
L4×T2	3.63	−0.13	−0.19	0.02	−2.37	−0.18	−1.74
L5×T2	7.42	−0.07	−0.21	0.04	20.66	0.04	3.03
L6×T2	5.11	−0.08	0.06	0.16	−14.59	0.03	1.51
L7×T2	2.97	0.25	0.24	−0.24	−18.40	−0.24	−7.00
SE (SCA_{lt}) crosses	0.84	0.024	0.03	0.02	3.30	4.83	0.83

FW: fruit weight; PT: pericarp thickness; FL: fruit length; WF: fruit width; YP: yield per plant; NF: number of fruits per plant; CC: capsaicin content, dry weight (DW); SE: Standard deviation; SCA: specific combinatorial ability.

Figure 2. Cross L7×T1 which presented positive values of heterosis, in four of the seven parameters evaluated. A) Crossing L7×T1; B) Progenitors L7.

As we can see, these outstanding crosses are the product of the cross with tester T1, indicating that this is a good tester to improve these parameters. On the other hand, tester T2 only stood out in the LF parameter, with the highest heterosis (13.1%) obtained from the crossing L1×T2. (Zewdie *et al.*, 2001) in their work indicate that the crosses P2×P3, P2×P6 and P3×P4 presented the highest and most positive values of heterosis for yield

Table 5. Percentage for average heterosis in the parameters evaluated in the F1 crosses of Habanero Chilli obtained from the Line×Tester (7×2) design.

Crosses (F1)	FW (g)	PT (mm)	FL (cm)	WF (cm)	NF	YP (kg·plant^{-1})	CC (mg·g^{-1} DW)
L1×T1	1.48	16.59	−3.08	4.26	16.31	18.38	171.01
L2×T1	0.98	1.15	0.69	0.79	1.36	20.03	37.65
L3×T1	10.83	0.17	5.51	9.65	42.74	65.8	−0.25
L4×T1	0.03	5.52	3.71	1.44	29.08	27.03	71.45
L5×T1	3.45	6.65	3.26	3.93	14.11	21.11	27.39
L6×T1	−16.3	6.21	4.32	−1.27	43.29	20.64	57.50
L7×T1	9.46	17.72	−14.47	29.92	62.72	77.08	41.23
L1×T2	−4.63	10.37	13.1	5.44	60.82	54.51	46.12
L2×T2	−15.56	0.11	1.59	3.21	10.11	0.98	−0.42
L3×T2	−6.56	12.5	4.59	6.91	35.94	44.14	3.15
L4×T2	−30	−9.2	−2.6	0.39	31.03	−7.51	11.00
L5×T2	−25.7	−3.31	−3.93	4.39	39.68	9.17	5.42
L6×T2	−15.66	−4.22	9.03	5.86	29.89	7.63	17.37
L7×T2	−13.57	15.84	5.13	−9.67	17.18	−0.87	−2.49

FW: fruit weight; PT: pericarp thickness; FL: fruit length; WF: fruit width; YP: yield per plant; NF: number of fruits per plant; CC: capsaicin content, dry weight (DW).

(84.5, 46.7 y 44.5 % respectively) the *Capsicum annuum* L. A similar result was also observed in the work of (Martínez-Martínez *et al.*, 2014) who determined a positive heterosis with to the average parent in number and weight of fruits per plant variables associated with yield, this in *Capsicum annuum* L. Regarding capsaicin content, (Zewdie *et al.*, 2001) observed heterosis in the capsaicin content in *Capsicum pubenses* hybrids, which means that F1 hybrids can be used to increase the capsaicin content. (Naves *et al.*, 2022) in their study on heterosis for capsacinoid accumulation in hybrids obtained from the genus *Capsicum*, found considerable heterotic effects specifically for capsaicinoid accumulation in the fruit placenta of hybrids, including those derived from non-spicy parents.

CONCLUSIONS

In this study, the parameters fruit weight, pericarp thickness and fruit width (L7), as well as number of fruits per plant, fruit length and capsaicin content (L3), crossed with the best performance of the evaluator (T1), showed the best general combinatorial capacity. The L7×T1 cross showed the best specific combinatorial ability, as well as the greatest number of positive heterosis values, standing out as a promising F1 hybrid, to be mass produced, just as its parents are excellent material to venture into a *Capsicum chinense* Jacq. genetic improvement program.

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Colegio de Postgraduados

Effect of branch girdling on the alternate bearing, yield, and fruit quality of grapefruit (*Citrus paradisi* Macf.)

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ABSTRACT

Objective: The objective of this research was to evaluate the effect of girdling branches, application of gibberellic acid (GA_3), and foliar urea on the alternating yield and quality of grapefruit (*Citrus paradisi* Macf) fruits.

Design/methodology/approach: The experiment was carried out on trees during abundant harvest ("on" year) and trees with low harvest ("off" year). Foliar applications of GA_3 and foliar urea were performed with a manual sprayer at a rate of 7 L tree^{-1} of solution. Branch girdling (5.0 mm wide) was performed on two-thirds of the secondary branches using a circular-edged knife to avoid damaging the xylem. The experimental design was completely randomized, with 24 factorial arrangements with 16 treatments and 3 repetitions. Each experimental unit was a tree to evaluate fruit yield and quality at the experiment's conclusion.

Results: Branch girdling increased the diameter, the number of fruits, the yield, and the °Brix-Acidity ratio of the juice. Foliar urea applications increased the percentage of juice but delayed ripening. The combination of branch girdling plus foliar urea applications increased fruit weight. The yield increase was attributed to the number of fruits rather than their weight. The treatments did not reduce alternate bearing.

Limitations on study/implications: The commercial cultivation areas for the experiment were limited due to the availability of facilities for testing fruit quality.

Findings/conclusions: To increase the size and number of fruits, with an increase in yield, branch girdling is an effective option. Furthermore, the application of foliar urea increases the percentage of juice and fruit weight, with a slight delay in maduration. However, the treatments did not reduce alternate bearing. Future research is required with other grapefruit varieties at different application times.

Keywords: alternate bearing, GA_3, carbohydrates, grapefruit, foliar urea.

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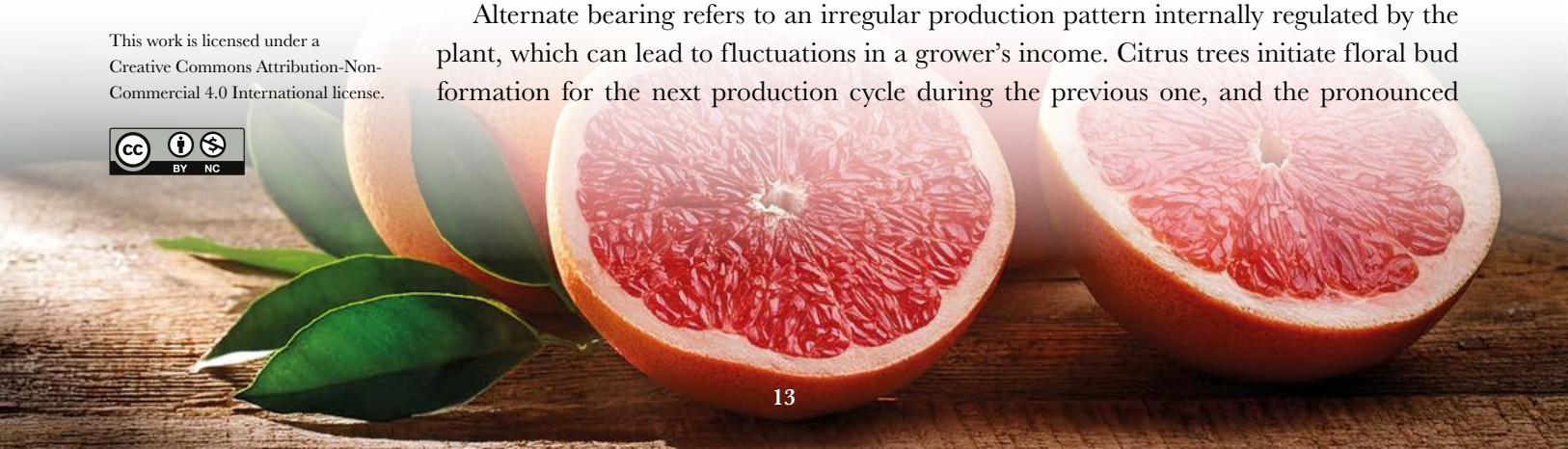
INTRODUCTION

Alternate bearing refers to an irregular production pattern internally regulated by the plant, which can lead to fluctuations in a grower's income. Citrus trees initiate floral bud formation for the next production cycle during the previous one, and the pronounced



alternation between high ("on") and low ("off") harvests is caused by competition between the current crop's production and the floral buds for the next season's harvest. The Rio Red grapefruit variety is highly susceptible to alternate bearing, which may present two consecutive "on" years followed by a low-yield harvest, or occasionally two "off" years followed by one "on" year (Martínez *et al.*, 2012).

Branch girdling or scoring is a technique to increase carbohydrate concentrations in developing fruits while reducing alternate bearing. The changes caused in the endogenous balance of carbohydrates and mineral elements are considered the primary effect of girdling, which aids in fruit set and development (Rivas *et al.*, 2010). A study conducted by Ambriz *et al.* (2018) reported that pruning combined with the application of urea and girdling in September resulted in increased sprouting, flowering, fruit set, as well as higher yield and quality of Persian lime during the winter, compared to the control group. Similarly, Almaguer-Vargas *et al.* (2011) observed an increase in floral differentiation, flowering, and fruit number through the application of pruning, foliar urea, and foliar fertilization in Persian lime. Gaete *et al.* (2007) found an increase in the sugar-acidity ratio by scoring branches in Clementine (*Citrus clementina* Blanco).

The application of foliar urea (0.5% N) in the fall-winter months increased flowering and production in oranges (Albrigo, 1999) and clementines (El-Otmani *et al.*, 1998). In "Washington Navel," it increased fruit set, yield, total fruit number, and the number of commercially sized fruits over three consecutive years, two of which were "on" years and one "off" year (Ali and Lovatt, 1994). During the winter dormancy period, the combined use of gibberellic acid (GA3, at 20-30 ppm) before the flowering of an "on" year and foliar urea (0.5% N) before the flowering of an "off" year can be employed. Both treatments are a good package to partially reduce the effects of alternate bearing (Galván *et al.*, 2006). Agustí *et al.* (1992) performed branch girdling on "Navelate" sweet orange, reporting a 30% increase in the number of harvested fruits and up to 130% in "Clementine" mandarins, increasing yield per hectare. Benhamou *et al.* (2004) observed good control of alternate bearing in "Nour" clementines with the combined application of GA_3 and foliar urea in consecutive "on" and "off" years, respectively. Currently, there is no available information on the effect of branch girdling, GA_3, and foliar urea on grapefruit yield and fruit quality, so research is needed to generate knowledge on this topic.

MATERIALS AND METHODS

The study was conducted in a grapefruit (*Citrus paradisi* Macf) orchard of the Rio Red variety, grafted onto sour orange rootstock (*Citrus aurantium* L.), located at the "Las Anácuas" estate in the municipality of General Terán, Nuevo León, Mexico. The experiment was carried out on trees with abundant harvest ("on" year) and trees with scarce harvest ("off" year). The experimental design used was completely randomized with a 2^4 factorial arrangement with 16 treatments (Table 1) and 3 replications.

The experimental unit was a tree to evaluate fruit yield and quality at the end of the experiment. Healthy trees with full competition were selected. Sample analyses and data collection were carried out at the Faculty of Agronomy in the Campus of Agricultural Sciences at Autonomous University of Nuevo León (UANL).

Table 1. Description of the treatments.

Treatments	Description
1	Tree "on" Without application (control).
2	Tree "on" foliar urea 25 daa, 1 kg 100 L^{-1} of water.
3	Tree "on" GA, 25 daa at 25 ppm.
4	Tree "on" foliar urea 25 daa, 1 kg 100 L^{-1}+GA , 25 daa at 25 ppm.
5	Tree "on" branch girdling, 25 dba.
6	Tree "on" branch girdling, 25 dba+foliar urea, 25 daa, 1 kg 100 L^{-1}.
7	Tree "on" branch girdling, 25 dba+GA , 25 daa at 25 ppm.
8	Tree "on" branch girdling, 25 dba+foliar urea, 25 daa, 1 kg 100 L^{-1}+GA, 25 daa at 25 ppm.
9	Tree "off" Without application (control).
10	Tree "off" foliar urea, 25 daa, 1 kg 100 L^{-1}
11	Tree "off" GA, 25 daa at 25 ppm.
12	Tree "off" foliar urea 25 daa, 1 kg 100 L^{-1}+GA , 25 daa at 25 ppm.
13	Tree "off" branch girdling, 25 dba.
14	Tree "off" branch girdling, 25 dba+foliar urea, 25 daa, 1 kg 100 L^{-1}
15	Tree "off" branch girdling, 25 dba+GA , 25 daa at 25 ppm.
16	Tree "off" branch girdling, 25 dba+foliar urea, 25 daa, 1 kg 100 L^{-1}+GA, 25 daa at 25 ppm.

"on" tree: Tree with abundant harvest. "off" tree: Tree with scarce harvest. dba: Days before anthesis. daa: Days after anthesis. ppm: Parts per million. GA_3: Gibberellic acid.

Foliar applications of GA_3 and foliar urea were carried out with a manual sprayer at a rate of 7 L $tree^{-1}$ of solution. Branch girdling (5.0 mm wide) was performed on two-thirds of the secondary branches using a circular-edged knife to avoid damaging the xylem.

For the analysis of variance, the SPSS (Statistical Package for the Social Sciences) software was used, and for the comparison of means of the variables under study, the experimental designs package, version 1.0 from the Faculty of Agronomy, UANL, was used. Marín N.L. developed by Olivares (1994). The least significant difference (LSD) test ($p \leq 0.05$) was used for the comparison of mean values. The variables measured were diameter (mm), number and weight (g) of fruits, percentage of juice, °Brix-$Acidity^{-1}$ ratio, and yield (t ha^{-1}).

RESULTS AND DISCUSSION

Fruit Diameter (mm)

Girdling increased fruit diameter in "off" trees, while it had no effect on "on" trees (Figure 1). Branch girdling in trees with low harvest increases fruit diameter due to the low competition among fruits, as these are trees with scarce harvest, and due to the redistribution of carbohydrates in the fruits, like what was reported by Martínez *et al.* (2012).

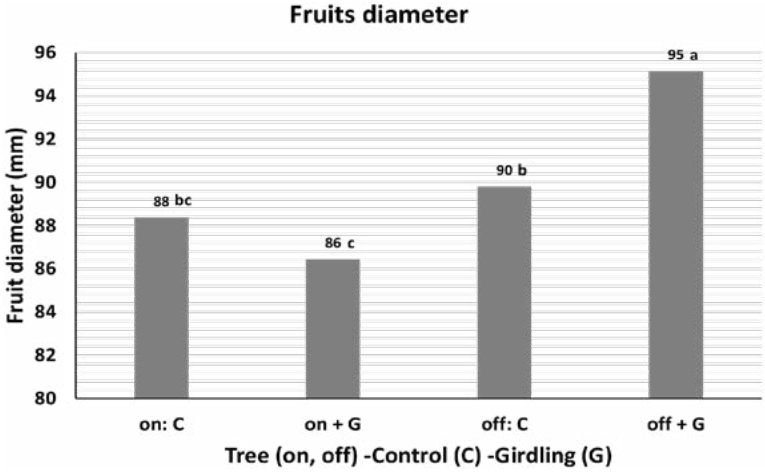


Figure 1. Comparison of means (LSD $p \leq 0.05$) of fruit diameter (mm) in trees with abundant ("on") and scarce ("off") harvest, without (Control: C) and with girdling (G), for the 2014-2015 cycle. Letters that are the same are statistically similar.

Number of fruits

Branch girdling increased the number of fruits (Figure 2), similar to what was reported by Goldschmidt (1999) and Rivas *et al.* (2010). Additionally, there is evidence that girdling has a hormonal effect, increasing GA levels (Mehouachi *et al.*, 2009), altering the GA/ABA ratio and preventing fruit abscission. The girdling treatment was statistically similar to the GA_3 treatment.

Fruit Weight (g)

The treatment with girdling plus urea behaved statistically similarly to the control and differently from the treatments with girdling alone and with only urea application

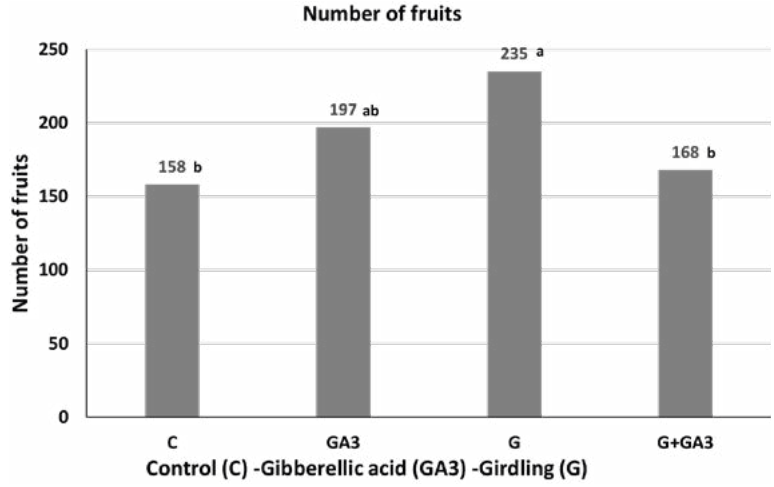


Figure 2. Mean comparison (LSD $p \leq 0.05$) of the number of fruits in trees without application (Control: C), with gibberellic acid (GA_3), and trees with girdling (G), for the 2014-2015 cycle. Letters that are the same are statistically similar.

(Figure 3), with the latter having the lowest fruit weight. The increase in yield is due to the higher number of fruits rather than their individual weight, like the results reported by Martínez *et al.* (2012).

Juice percentage in fruits (%)

Trees that received urea application had a higher juice percentage in fruits but delayed their maturation, possibly due to the nitrogen content in the foliar urea (Figure 4).

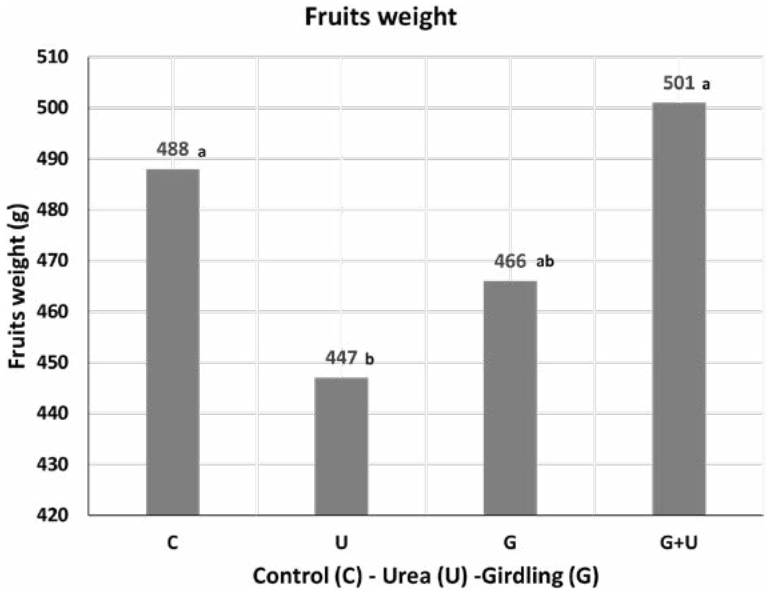


Figure 3. Comparison of means (LSD p≤0.05) of fruit weight (g) in trees without application (Control: C), with urea application (U), and trees with girdling (G), for the 2014-2015 cycle. Letters that are the same are statistically similar.

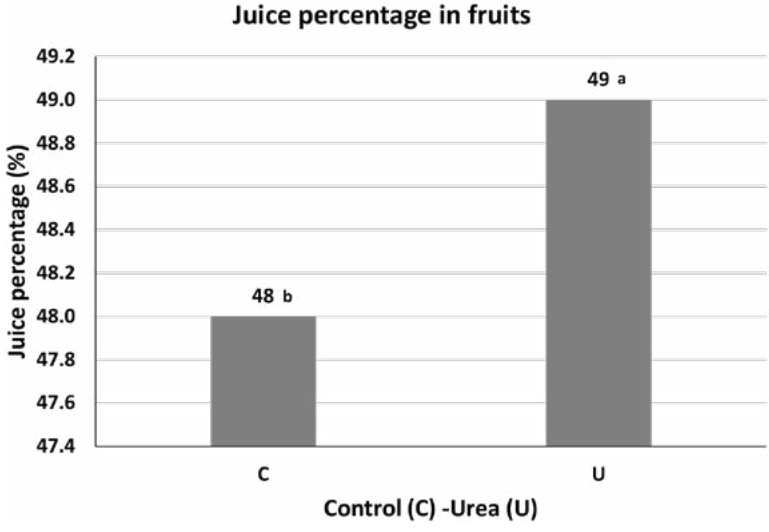


Figure 4. Comparison of means (LSD p≤0.05) of juice percentage in fruits (%), in trees without urea application (Control: C) and trees with urea application (U), for the 2014-2015 cycle. Letters that are the same are statistically similar.

°Brix-Acidity Ratio of the juice

Trees that were only girdled showed a higher °Brix-Acidity ratio (Figure 5), which is consistent with the findings reported by Gaete *et al.* (2007) in "Clementines," where it was mentioned that branch girdling increased this ratio. Additionally, Mehouachi *et al.* (2009) have reported that girdling in citrus increases the carbohydrate concentration in fruits, resulting in earlier maturation.

Yield (t ha^{-1})

The girdling treatment showed the highest yield (tons per hectare), although it was statistically similar to the GA_3 treatment (Figure 6). On the other hand, the control group

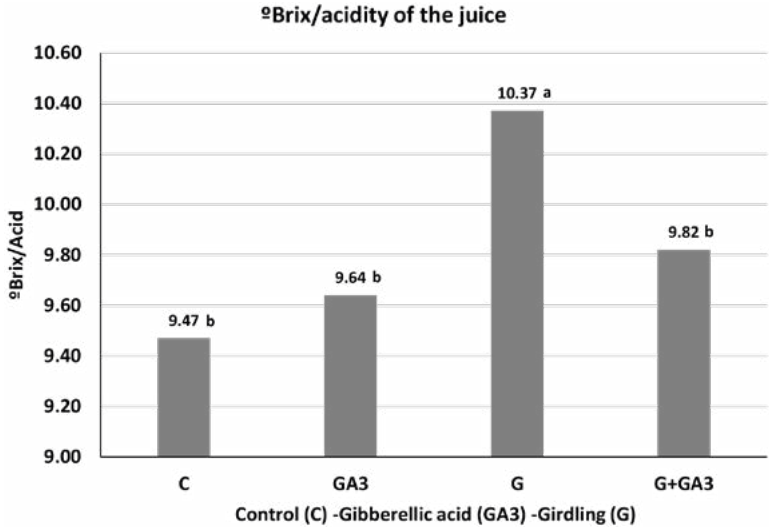


Figure 5. Comparison of means (LSD p≤0.05) of the °Brix-Acidity ratio of the juice, in control trees (T), with gibberellic acid (GA_3), and girdling (A) for the 2014-2015 cycle. Letters that are the same are statistically similar.

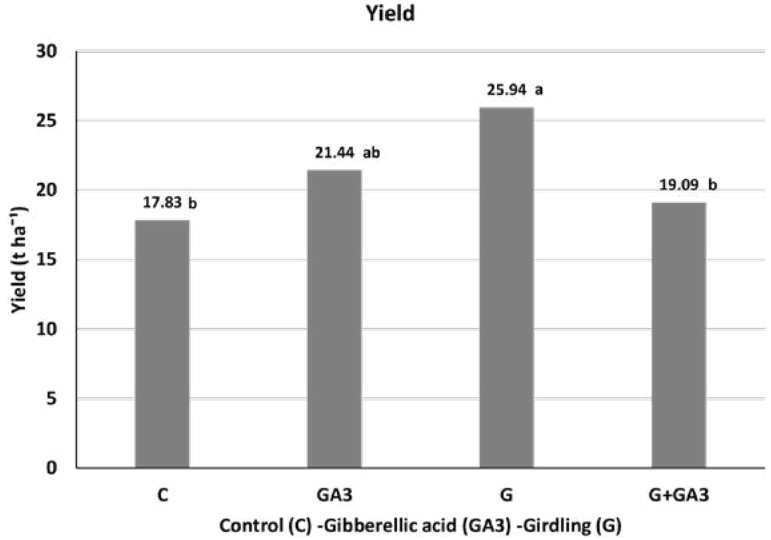


Figure 6. Comparison of means (DMS p≤0.05) of yield (t ha^{-1}) in trees with no application (Control: C), with gibberellic acid (GA_3), and with girdling (G), for the 2014-2015 cycle. Identical letters indicate statistically similar results.

had the lowest yield (tons per hectare). This result agrees with Rivas *et al.* (2010), who report that branch girdling and GA_3 application in citrus positively affect the relationship between gibberellins and abscisic acid, thereby promoting fruit setting. Consequently, an increase in yield was observed due to a higher number of harvested fruits rather than an increase in their individual weight.

CONCLUSION

To increase fruit size and number, and subsequently improve yield, branch girdling is a good option. Additionally, the application of foliar urea increases both the juice percentage and fruit weight, although with a slight delay in ripening. The treatments did not reduce harvest alternation. Future research is needed with other grapefruit varieties and different application timings.

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Impact of planting density and nitrogen on the productivity of warm-climate onion in the Mexican pacific

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ABSTRACT
Objective: To evaluate three population density and nitrogen supply in onion.
Design/methodology/approach: An experiment was developed in the dry tropics of Mexico. Seedlings were produced in nursery and transplanted on 0.9 m planting beds. In addition to crop management with drip irrigation, nitrogen nutrition was planned according to the rational form of supply. Density/nitrogen factors were evaluated in factorial design 3^2. Developmental, productive and qualitative bulb variables were recorded and statistically analyzed.
Results: The interaction of factors was varied, but in the productive, only the density factor was consistent; bulb weight excelled in the low density (14.8 plants m^2) but did not lead to the highest yield, on the other hand, the highest density together with N supply, presented the highest yield per area of 2.14 and 2.17 kg m^2.
Limitations on study/implications: Onions are favored by the Mexican population as they are among the most consumed vegetables due to their bulbs, and they are attributed with various health benefits. Approximately 52 thousand hectares are harvested in the country and Michoacan participates with 8.3% of production. However, yields are lower than their potential, due to the lack of adaptation to current environmental conditions. This leads to consider the exploration of alternative locations and the implementation of strategies to improve yields, where nutrition and spacing are key factors.
Findings/conclusions: The density of 26.9 plants m^2 with N addition produced the highest yield.

Keywords: *Allium cepa* L., bulb, plant spacing, nitrogen fertilization, productivity.

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INTRODUCTION

Onion is one of the main vegetables in the basic food basket in Mexico, making it popular among the country's population of over 100 million people (INEGI, 2020), with a *per capita* consumption of 8.6 kg (SIAP, 2023). Both the fresh shoots or leaves and the bulbs of this vegetable are consumed (Brewster, 2008). Additionally, onions are attributed with various health benefits, such as anticancer properties, platelet aggregation inhibition and anticoagulant effects, as well as antiasthmatic and antibiotic properties (Griffiths *et al.*, 2002).

Globally, Mexico ranks 10th out of 58 onion-producing countries by established area (FAOSTAT, 2022). In 25 of 32 states, 52,024 hectares were harvested, with Michoacán contributing 8.3% of the national production (SIAP, 2023). However, as a temperate climate vegetable, onion yields do not reach their cultivation potential due to environmental issues, which have affected agricultural areas by creating challenges related to adaptation to current conditions. This leads to considering the exploration of alternative locations, where actions in agronomic components, such as planting density and nutrition, especially nitrogen, are implemented to mitigate adversities (Siliquini *et al.*, 2015; Russo, 2008). Regarding population density, the implementation of various planting patterns directly influences bulb size (Brewster, 2008). As planting density per area increases, yield also rises; however, this can reach a limit, as yield tends to decrease when density exceeds the supported level, due to competition for light, nutrients, and available water in the soil (Siliquini *et al.*, 2015). This imbalance is often exploited to produce specific onion sizes. High densities, between 1,000 and 2,000 plants m^2, favor the production of small bulbs for seed-material. Densities between 50 and 100 plants m^2 produce bulbs with diameters between 5 and 7 cm for fresh consumption. In contrast, densities between 25 and 50 plants m^2 produce larger bulbs (Brewster, 2008). Several studies highlight positive effects on onion production through the use of different densities.

By simply increasing the row spacing from 5 to 10 cm, bulb diameter and stem diameter increased by 17% and 15%, respectively (Yemane *et al.*, 2014). Additionally, with a density of 45 plants m^2 on 1.2 m wide planting beds, significant increases in vegetative growth, bulb yield, and bulb quality were observed compared to 0.6 m wide beds at the same density (Abou *et al.*, 2017).

Conversely, yields of 47,540 kg ha^{-1} were associated with a density of 200 plants m^2. However, the preference for smaller bulbs, with a weight of 60 g, is achieved at a lower density of 66.67 plants m^2 (Walle *et al.*, 2018). Thus, managing planting densities is crucial, as long as the level of competition is not reached when plants grow too close together (Siliquini *et al.*, 2015).

Regarding nutrition, meeting the needs of onions efficiently is challenging due to their particular shallow root system (Brewster, 2008). Although various criteria are used to nourish them, little attention is given to soil and foliar analysis, and the different phenological stages are not considered (Bonza-Espinoza *et al.*, 2016). Since onions require nutrients, fertilization is necessary to improve yield, although soil fertility is the foundation for planning the application rates, with the expectation of a response from the crop (Przygocka-Cyna *et al.*, 2020). Thus, nitrogen demand is met by combining soil N with N from fertilizers to ensure optimal growth (Messele, 2016). Since onions are considered an intensive crop, they require high amounts of N (Casella *et al.*, 2022), with 65% of the N concentrated in the bulbs and 35% in the leaves (Geisseler *et al.*, 2022). Since N (NH_4-N or NO_3-N), which is anionic in nature, is easily lost due to its inability to be retained by clay particles, there is a risk of reduced yield. Nitrogen application rates generally range from 50 to 200 kg ha^{-1}, but it should be noted that very low or very high doses can alter or delay the formation and maturation of leaves and bulbs (Tae *et al.*, 2003).

This occurs when nitrogen is applied during the bulb growth phase, between 60 and 75 days after planting, depending on the genotype (Amaya & Méndez, 2013). Therefore, it is necessary to split nitrogen applications between 60 and 80 kg ha^{-1} before planting, and a similar amount when the plants reach about 10 cm in height (Brewster, 2008). Given the need to generate information addressing population density management and nitrogen nutrition in onions, the objective of this study was to evaluate the effect of three population densities and two nitrogen application methods on onions under dry tropical environmental conditions.

MATERIALS AND METHODS

In the Dry Tropics of Michoacán, Mexico, an experiment was conducted at the Valle de Apatzingán Experimental Station of the National Institute of Forestry, Agricultural, and Livestock Research in Antúnez, Michoacán, Mexico (19° 00' 34" N and 102° 13' 44" W), at an elevation of 338 m above sea level. According to García (2004), the predominant climate corresponds to Bs_1, supporting representative species of low deciduous forest, and the soil is classified as pelic vertisol (INEGI, 2016). The climatic variation behaved as specified in Table 1.

In a 3 m^2 seedbed with sandy loam soil, onion seedlings of the Cojumatlán® variety were produced. The preparation involved tilling the soil and applying 90% wettable sulfur at a rate of 10 g m^2. Small furrows were then drawn with a spacing of 15 cm, where onion seeds were sown in a "drill" method, covered with soil, and irrigated by gravity until field capacity was achieved. The seedbed was then covered with black plastic for four days to promote germination. Afterward, it was uncovered, and watering continued until the emergence of the epicotyls. Afterward, the seedlings were watered daily, and ammonium sulfate fertilizer was applied by broadcasting on two occasions. Once the seedlings reached a bulb size similar to that of a cotton swab, after six weeks, they were transplanted. Simultaneously, the experimental area was prepared through tillage, and planting beds were formed with a width of 0.9 m. Additionally, a soil sample was analyzed to determine the status of the main physical and chemical variables, yielding the following results: organic matter 1.43%, pH 7.90, inorganic nitrogen 19.4 mg kg^{-1}, phosphorus 15.5 mg kg^{-1}, potassium 448 mg kg^{-1}, and cation exchange capacity 65.6 mol kg^{-1}.

The management of the experiments was based on cultural practices specific to the crop and phytosanitary management recommended by INIFAP (2015). Additionally, some strategies were based on the guidelines proposed by Khosa and Lee (2018), except for nutrition, which was one of the factors to be tested; these were supplied with nitrogen

Table 1. Climatic variation during the experiment, autumn-winter cycle.

Climate variables	Mean value (September-February)
Maximum Temperature (°C)	34.41
Minimum Temperature (°C)	17.25
Average Temperature (°C)	26.01
Precipitation (mm)	25.61
Evaporation (mm)	4.13

fertilizer sourced from ammonium sulfate (21% N). Irrigation was by drip with daily intervals, consisting of the placement of two irrigation tapes over the planting beds. The emitters, spaced 0.2 m apart, released a flow rate of 0.7 L h^{-1}. The irrigation time varied according to demand, but generally lasted between 2 and 3 h. The crop cycle was approximately 16 weeks.

Using a factorial experimental design, the density factor was evaluated with three treatments, and the nitrogen factor (supply) with two treatments, which together formed six treatments with four replicates. The density factor was set up on beds of 0.9 m with densities of 14.8 (D1), 22.2 (D2), and 29.6 (D3) plants m^2 in two, three, and four rows, respectively. The nitrogen factor consisted of two treatments based on the formula 140-0-0 (N-P-K) kg ha^{-1}, supplied in two parts (N1; before transplanting and 30 days after transplanting) and supplied in one part (N2; 30 days after transplanting), respectively.

At 110 days after transplanting, corresponding to the maturity stage, the development variables were evaluated. Plant height was measured with a tape measure from the base of the soil to the tip of the longest leaf. Stem diameter was measured with a caliper 2.0 cm above soil level.

The number of leaves was recorded by counting the number of leaves produced per plant. At harvest stage, 120 days after transplanting, productive variables such as bulb weight were recorded by weighing the bulbs using a digital scale (Denver Instrument Company Model AA-160); bulb size was measured by the polar and equatorial circumferences with a tape measure; and yield was calculated based on the average bulb weight and the density of plants per area. The qualitative characteristics of the bulbs were obtained from 10 physiologically mature bulbs per treatment. pH was measured by cutting the bulb tissue into pieces and grinding it in a mortar with distilled water; once mixed, the juice was collected in a beaker, and pH values were recorded using a portable pH meter (Hanna® model pHep). Soluble solids were measured using a handheld refractometer (Atago® model HSR-500), where a drop of onion tissue extract was placed on the base of the refractometer and the reading was recorded. The moisture content of the bulbs was obtained by taking 100 g of cut tissue from the bulbs per treatment, which were placed in labeled expanded polystyrene plates and maintained at room temperature for 20 days; the percentage of moisture was calculated based on the difference between initial and final weight.

The obtained data were processed using analysis of variance under a factorial design, and means were compared using Tukey's test (P=0.05). The statistical software used was SAS (2002).

RESULTS AND DISCUSSION

In the development variables, both quantitative and qualitative bulb analyses, the responses were diverse (Table 2). As observed, the development variables did not show changes due to the D*N interaction, and the factor N also did not show changes when considered separately; only the factor D exhibited differences. The treatment D3 increased plant height by 16% and the number of leaves by 12% compared to treatments D1 and D2. However, regarding stem diameter, treatments D1 and D2 were 8% superior to treatment D3 (Table 2).

As for the productive variables, no differences were observed due to the D*N interaction. However, when considering the factors D and N separately, differences were only observed in the polar and equatorial diameters of the bulbs in factor D, with increases of 13% and 10%, respectively, in treatment D2 compared to treatments D1 and D3 (Table 2). For the variables of fruit weight and yield per area, differences were observed due to factors D and N, where treatment D1 and treatment N2 increased fruit weight by 11% and 7%, respectively, compared to the other treatments in each factor. In contrast, treatment D3 and N2 increased yield per area by 32% and 6%, respectively, compared to the rest of the treatments combined (Table 2). Regarding the qualitative bulb variables, no significant changes were detected due to the D*N effect in the variables pH, soluble solids, and dry matter. Similarly, the analysis of the separate effects of factors D and N on the qualitative bulb variables showed no differences (Table 2).

On the other hand, the variance analyses of the combined treatments for the development variables showed statistical differences. The greatest plant height was achieved with the D3-N2 treatment, followed by the D3-N1 treatment, both of which exceeded the others by 16% (Table 3). With a height of 52.3 cm, the D3 (29.6 plants m^2) - N2 (N in one application) treatment aligns with the findings of Seifu *et al.* (2015), who evaluated six nitrogen doses (ranging from 0 to 138 kg ha^{-1}) and four row spacings (ranging from 7.5 to 15 cm). They obtained the greatest plant height (53.69 cm) with a spacing of 15 cm between rows and 138 kg N ha^{-1}, which is consistent with the present findings.

Regarding the stem diameter variable, significant differences were observed, with the highlighted treatments being D1-N2, D2-N2, and D1-N1, which collectively exceeded the treatments at density 3 (29.6 plants m^2) by 10% in both levels of nitrogen supply. However, these treatments integrated at density 3 reflected the highest number of leaves. Similar studies also did not report significant effects on this variable (Seifu *et al.*, 2015; Alemu *et al.*, 2022). In contrast, Amaya and Méndez (2012) reported stem diameters of 1.42 cm, but with low doses of 60 kg ha^{-1} N after 104 days, and they also found no effect on this

Table 2. Analysis of density (D) and nitrogen (N) factors and their interaction on development, productive, and qualitative variables in onion. Antúnez, Michoacán, Mexico.

Variables	D	D1	D2	D3	N	N1	N2	D*N
PH (cm)	**	42.21 b	42.98 b	50.98 a	NS	45.17	45.61	NS
SD (cm)	**	1.36 a	1.30 a	1.22 b	NS	1.27	1.31	NS
NL (No.)	**	7.87 b	8.30 b	9.15 a	NS	8.36	8.51	NS
PD (cm)	**	11.32 b	12.72 a	10.80 b	NS	11.44	11.79	NS
ED (cm)	**	7.95 ab	8.38 a	7.12 b	NS	7.70	7.93	NS
BW (g)	**	83.91 a	76.82 b	73.18 b	**	75.3 b	80.65 a	NS
YA (kg)	***	1.23 c	1.70 b	2.15 a	**	1.64 b	1.75 a	NS
pH	NS	6.55	6.54	6.48	NS	6.49	6.55	NS
SS (°Brix)	NS	5.51	5.30	5.58	NS	5.53	5.40	NS
DM (%)	NS	11.33	11.08	11.26	NS	11.22	11.23	NS

PH (plant height); SD (stem diameter); NL (number of leaves); PD (polar diameter), ED (equatorial diameter); BW (bulb weight); YA (yield per area); pH (Hydrogen potential); SS (soluble solids); DM (dry matter); NS=Not Significant; *P≤0.05; **P≤0.01; ***P≤0.0001.

Table 3. Analysis of plant height, stem diameter, and leaf count variables in onion, affected by plant density and nitrogen. Antúnez, Michoacán, Mexico.

Density (D) / Nitrogen (N)	Height (cm)	Diameter (cm)	Leaves (No.)
D1-N1	43.00 bc	1.35 ab	7.77 b
D1-N2	41.42 c	1.37 a	7.97 ab
D2-N1	42.85 bc	1.25 bc	8.25 ab
D2-N2	43.12 bc	1.35 ab	8.35 ab
D3-N1	49.67 ab	1.22 c	9.07 ab
D3-N2	52.3 a	1.22 c	9.22 a
C.V.	7.28	4.04	6.90
Significance	**	**	**

C.V.=Coefficient of variation; **P≤0.01; ***P≤0.0001.

variable from the supply of other nutrients. The above indicates that stem diameter is not a prominent trait in response to nutrient supplies, as would be desirable in other species; in fact, onions with thinner stems are preferred.

The analysis of variance for the variable "leaves per plant" showed significant differences. The density of 26.9 plants m^2 (D3) and the single N application (N2) resulted in an average of 9.22 leaves (Table 3). It is important to note that the combination of plant density and nitrogen supply influences this variable. However, nitrogen application, either alone or in combination, also promotes leaf emission, as reported by Singh *et al.* (2017). They achieved 11.96 leaves per plant using the fertilizer formula 120-60-80 (N-P-K) kg ha^{-1} combined with *Azospirillum*. This result is similar to the findings of the present study, although only in trend.

Similarly, the variance analyses detected significant differences in the combined treatments of productive variables (Table 4). For the polar and equatorial diameter variables, the D2-N2 treatment exceeded the average of the D3 treatment in both nitrogen levels by 18% and 16%, respectively. Since bulb size depends on density and nitrogen nutrition, the densities of 14.8 and 22.2 plants per m^2, along with nitrogen, presented the

Table 4. Analysis of bulb size, weight, and yield variables in onion, by the effect of density and nitrogen. Antúnez, Michoacán, Mexico.

Density (D) / Nitrogen (N)	Polar diameter (cm)	Equatorial diameter (cm)	Weight (g)	Yield (kg m^2)
D1-N1	11.02 b	7.97 ab	80.90 ab	1.19 c
D1-N2	11.62 ab	7.92 ab	86.92 a	1.28 c
D2-N1	12.35 ab	8.32 ab	72.32 b	1.60 b
D2-N2	13.10 a	8.45 a	81.32 ab	1.80 b
D3-N1	10.95 b	6.82 b	72.67 b	2.14 a
D3-N2	10.65 b	7.42 ab	73.70 b	2.17 a
C.V.	7.10	8.64	5.83	5.82
Significance	**	*	**	***

C.V.=Coefficient of variation; *P≤0.05; **P≤0.01; ***P≤0.0001.

largest bulb sizes, ranging from 11.02 to 13.10 cm in polar diameter and 7.92 to 8.45 cm in equatorial diameter, respectively, compared to treatments with higher density (Table 4). Therefore, having more plants per area may have reduced bulb size. Some studies reported similar behavior. Singh *et al.* (2017) highlighted the influence of N on bulb size, showing that both N alone or in combination increased bulb length and diameter by 5.13 cm and 5.85 cm, respectively. Similarly, Russo (2008) evaluated two onion varieties with three different densities and two N doses. He found that increasing the density to 102,000 plants ha^{-1} produced more bulbs, but of smaller size in short-day onions. This response was similar to the results obtained in this study, where higher density led to smaller bulbs. In fact, Siliquini *et al.* (2015) concluded that bulb size decreases as plant density increases due to competition for resources.

On the other hand, the bulb weight variable showed significant differences, with treatment D1 standing out, achieving 86.2 g, surpassing the other treatments by 12%. However, this was not reflected in the yield variable, as the most outstanding treatments corresponded to density D3 at both nitrogen levels, which together exceeded the other treatments by 32% (Table 4). It is important to highlight that for the bulb weight and yield variables, the outstanding treatments were not necessarily those with the largest bulb size. The highest bulb weight corresponded to the treatment with the lowest density of 14.8 plants m^2, with a value exceeding 80 g per bulb. However, the highest yield was achieved with the density of 29.6 plants m^2, with a value exceeding 2 kg m^2 (Table 4). In contrast, the lower density affected bulb weight, while the higher density impacted yield. Additionally, treatments with the addition of nitrogen improved yield. Various reports suggest improvements in bulb production through adjustments in density and nitrogen. Seifu *et al.* (2015) evaluated six nitrogen doses and four row spacings. The highest yield was achieved with 15 cm spacing combined with 138 kg ha^{-1} of nitrogen; similarly, Simon *et al.* (2014) reported a yield of 2.72 t ha^{-1} with 69 kg ha^{-1} of nitrogen and 46 kg ha^{-1} of P_2O_5, which is comparable to the yield reported in this study. In their case, the lower nitrogen supply was compensated by the addition of phosphorus. On the other hand, Kahsay *et al.* (2014) tested three different spacings and four onion varieties. They found that the average bulb weight increased from 49.86 to 81.31 g when the spacing between rows was increased from 5 to 10 cm, achieving a yield of 36.14 t ha^{-1}, which is similar to the results obtained in this study. This aligns with the findings of Gebretsadik and Dechassa (2018), who evaluated nitrogen supply from 0 to 150 kg ha^{-1} and plant spacing from 4 to 10 cm. The highest yield of 26.72 t ha^{-1} was achieved with a nitrogen dose of 100 kg ha^{-1} and a spacing of 6×20 cm (833,300 plants ha^{-1}), which aligns with previous reports. Similarly, Walle *et al.* (2018) tested six densities ranging from 25 to 200 plants m^2 and two onion varieties, finding that the highest density (200 plants m^2) produced yields of 47,540 kg ha^{-1}. However, they noted a preference for bulbs weighing around 60 g, which were best produced at a density of 66.67 plants m^2.

Regarding the integrated treatments of the qualitative variables, the variance analyses did not show differences in pH, soluble solids, and dry matter, although their average values corresponded to 6.52, 5.46, and 11.22%, respectively (Table 5). This behavior is consistent with that reported by Walle *et al.* (2018), who tested six densities and two onion

Table 5. Analysis of qualitative variables pH, soluble solids, and dry matter in onion bulbs, by the effect of density and nitrogen. Antúnez, Michoacán, Mexico.

Density (D) / Nitrogen (N)	pH	Soluble solids (°Brix)	Dry matter (%)
D1-N1	6.67	5.57	11.20
D1-N2	6.43	5.45	11.47
D2-N1	6.40	5.27	11.07
D2-N2	6.68	5.32	11.10
D3-N1	6.42	5.75	11.40
D3-N2	6.55	5.42	11.12
C.V.	3.98	5.84	6.20
Significance	NS	NS	NS

C.V.=Coefficient of variation; NS=Not Significant.

varieties. They found no differences in the variable soluble solids, either by separate factors or by the cultivar/density interaction. This aligns with what has been reported for this variable; however, the supply of elements influences biomass production (Przygocka-Cyna *et al.*, 2020). This was also found by Singh *et al.* (2017), who tested a chemical formula and biofertilizers and found that the combination of the dose 120-60-80 (N-P-K) kg ha^{-1} with *Azospirillum* generated 4,510 kg ha^{-1} of dry matter from bulbs, which corresponds to 10% dry matter. This is similar to what was found in the present study. However, the choice of varieties also influences different purposes; for example, for tissue dehydration, the materials should contain between 17% and 20% dry matter, compared to 10% to 12% for the production of bulbs for fresh consumption (Brewster, 2008), which corresponds to the dry matter content found.

In onion cultivation management, various strategies are necessary to improve yields. However, the choice of onion cultivar, nitrogen supply, and planting distances are crucial as they influence outcomes (Awad *et al.*, 2022; Mahmood and Khan, 2007; Gamiely *et al.*, 1991). Thus, the results obtained provided insights for enhancing the production system or, if necessary, discarding practices that may be detrimental.

CONCLUSIONS

The interaction of factors and the independent factors were significant in the productive variables, primarily due to the effect of planting density. The higher density of 29.6 plants m^2 favored plant height, reaching 49.67 cm and 52.3 cm, as well as the number of leaves with 9.07 and 9.22 leaves, but did not influence stem diameter, which corresponded to the lower density of 14.8 plants m^2. Even with nitrogen supply, the highest density of 26.9 plants m^2 reduced bulb size. In contrast, the lowest density produced the highest bulb weights of 80.9 g and 86.92 g, while the highest yield per area of 2.14 and 2.17 kg m^2 corresponded to the treatment with the highest density of 26.9 plants m^2 along with nitrogen supply. The qualitative characteristics of the bulbs were not statistically influenced by the treatments tested.

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Colegio de Postgraduados

Alternative Solution to Estimate Cucumber Crop Evapotranspiration in Shade Nets for the Valle de Culiacán, Mexico

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ABSTRACT

Objective: This work aimed to estimate the evapotranspiration of cucumber crop grown in a shade house in Valle de Culiacán, México.

Design/methodology/approach: The FAO56 method was used with a variation of the FAO Penman-Monteith equation, and a non-conventional evaporimeter to measure evaporation inside the shade house. Using the estimated reference evapotranspiration and the measured evaporation, pan coefficients were calculated, and a mathematical model was proposed to calculate this coefficient based on the meteorological parameters: air temperature, relative humidity, and net solar radiation.

Results: The estimated crop evapotranspiration was 417.6 mm. The linear regression between the reference evapotranspiration estimated with FAO56 and calculated with evaporation and adjusted evaporimeter pan coefficient yielded excellent statistical estimators (R^2>0.9).

Findings/conclusions: The analysis of the evaporimeter pan coefficient's dependence on meteorological parameters indicates a strong dependence on air temperature, net solar radiation, and vapor pressure deficit, but not on relative humidity.

Keywords: *Cucumis sativus*; evaporimeter pan coefficient; FAO56; protected agriculture; reference evapotranspiration.

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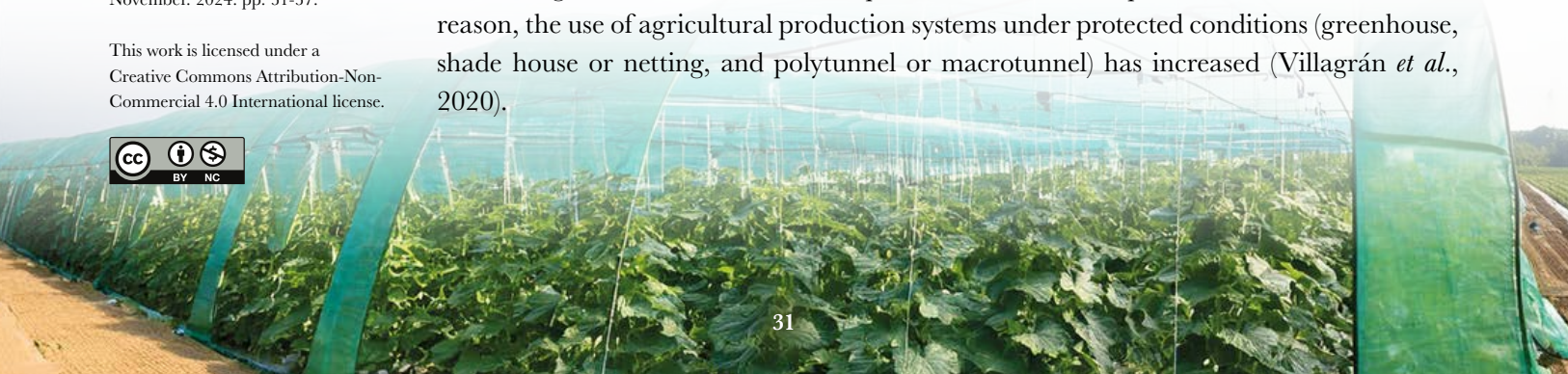
INTRODUCTION

The global increase in food demand pressures production systems to increase crop yields despite water resource limitations. Therefore, there is growing interest in more sustainable practices and optimized operations for agricultural systems that allow efficient use of water resources (Ghiat *et al.*, 2021). However, crop water consumption is affected by meteorological conditions that are impossible to control in open-field conditions. For this reason, the use of agricultural production systems under protected conditions (greenhouse, shade house or netting, and polytunnel or macrotunnel) has increased (Villagrán *et al.*, 2020).



For the period 2019-2022, in the central agricultural region of Sinaloa, which includes the municipalities of Culiacán and Navolato, the area covered with shade nets is 4,593 ha, representing 61.6% of the state total. Regarding cucumber crops (*Cucumis sativus* L.) grown under shade nets, Sinaloa has 1,388 ha established (42.3% of the national total), of which 917 ha (66.1%) are located in the central region of the state (SIAP, 2024). On the other hand, the average net water applied to cucumber crops is 77.8 cm (agricultural cycles 2021-2022 and 2022-2023) in the central region of the state, corresponding to irrigation district 010 Culiacán-Humaya (CONAGUA, 2024).

In this regard, it should be noted that there is a lack of studies analyzing the applicability of evapotranspiration models in closed agricultural systems, and that provide methods and guidelines to improve the estimation of crop water requirements for producers or technicians in charge of irrigation management in shade-net production systems. Therefore, the objective of this study was to estimate the evapotranspiration of cucumber crops in shade nets using the FAO56 methodology, with a variation of the FAO Penman-Monteith equation and the calibration of a non-conventional evaporimeter pan that is easily accessible for producers and field technicians.

MATERIALS AND METHODS

The research was conducted in a shade-net production system located at the facilities of the Faculty of Agronomy of the Autonomous University of Sinaloa, geographically situated in northwestern Mexico, with central coordinates of 24° 37' 24.23" N and 107° 26' 38.43" W. This area is characterized by a warm semi-arid climate (Bsh, Köppen classification) with an average monthly temperature of 24.6 °C, recording maximum temperatures of 43 °C and a minimum of 0.6 °C. The average annual precipitation is 705 mm, with a rainy season from July to September. The total growing cycle of the crop was 118 days, with 28 days in the seedling stage and 90 days after transplant (DAT). The study took place from February 29 to May 28, 2024 (DAT). The area used for the study was 280 m^2, and the shade net allows 75-80% of net solar radiation to pass through. The soil is clayey, containing 69% clay, 16% silt, and 15% sand. The soil bulk density is 1.23 g cm^{-3}, with an organic matter content of 0.8%. The soil's hydrodynamic characterization shows a field capacity moisture content of 46.7 cm^3 cm^{-3} and a permanent wilting point of 35.1 cm^3 cm^{-3}.

In this study, Persian cucumber variety was established. Four beds were constructed, with a separation of 1.5 m between them, each 20 m long and 0.6 m wide. The spacing between plants was 0.3 m, and irrigation was done by drip using emitters with a flow rate of 1 L h^{-1}, spaced 0.3 m apart. The bed was covered with silver-colored plastic mulch.

To measure the meteorological variables required by the FAO56 methodology (Allen *et al.*, 1998), a station with sensors was used to measure air temperature, relative humidity, net solar radiation, soil heat flux, precipitation, and barometric pressure. Given the low or negligible wind speed conditions inside the shade net, the reference evapotranspiration (ET_g) was calculated using Equation (1) established by FAO Penman-Monteith, adjusted for the internal conditions of a greenhouse as used by Liu *et al.* (2020).

$$ET_0 = \frac{0.408\Delta(R_n - G) + \gamma \frac{628}{T+273}(e_a - e_s)}{\Delta + 1.24\gamma} \quad \text{(Equation 1)}$$

Where ET_0 is the reference evapotranspiration (mm d^{-1}), R_n is the net solar radiation (MJ m^{-2} d^{-1}), G is the soil heat flux (MJ m^{-2} d^{-1}), T is the mean air temperature (°C), $(e_a - e_s)$ is the vapor pressure deficit (kPa), Δ is the slope of the vapor pressure curve (kPa °C^{-1}), and γ is the psychrometric constant (kPa °C^{-1}).

To measure evaporation inside the shade net, four non-conventional evaporimeters (mini-evaporimeters) were installed. These consisted of a 4-inch nominal diameter PVC cap, with an effective inner diameter of 100.8 mm and a height of 35 mm, in white color. The evaporimeters were placed on the aisles of the shade net between the cultivation beds, on a wooden structure, at a height of 13 cm above the soil level. The initial water level was 30 mm, leaving 5 mm of freeboard in the container, which was reached by adding 239 ml of water. The water in the mini-evaporimeter was changed every third day, adding the initial 239 ml. Evaporation was determined by weight, considering a measured water density of 0.979 g cm^{-3}, which represents 7.8 g mm^{-1} of evaporated water. Daily weight measurements were taken for each container, and the average of the four mini-evaporimeters was calculated to determine the weight difference, applying Equation (2).

$$E = \frac{P_i - P_{i-1}}{7.8} \quad \text{(Equation 2)}$$

Where E is the daily evaporation (mm), P_i is the weight of the container on the current day (g), and P_{i-1} is the weight of the container with water from the previous day (g). Considering Equation (3), where K_p is the evaporimeter pan coefficient.

$$ET_0 = E \cdot K_p \quad \text{(Equation 3)}$$

Allen *et al.* (1998) suggests that the values of K_p depend on the type of evaporimeter pan used, and that if evaporation values are measured with an alternative pan, the values of K_p should be determined by relating the evaporation to the reference evapotranspiration (ET_0) estimated with FAO56. This is achieved using Equation (4).

$$K_p = \frac{ET_0}{E} \quad \text{(Equation 4)}$$

To calculate the water requirement for cucumbers, a single Kc model was used, applying the Kc values proposed by Allen *et al.* (1998) (Table 1).

Table 1. Crop Coefficients (Kc) used.

	Ini	Med	Fin
Kc	0.60	1.15	0.75

To obtain the mathematical model representing the behavior of K_p as a function of meteorological parameters, multiple linear regression was applied. Meanwhile, to evaluate the individual dependence of K_p on meteorological parameters, simple linear regression was used. For statistical analysis, Pearson's correlation coefficient (R), the coefficient of determination (R^2), Willmott's concordance index (d), and the root mean square error (RMSE) were employed.

RESULTS AND DISCUSSION

ET_0 ranged from 1.9 to 6 mm d^{-1}, with a total estimated at 405.3 mm. In contrast, the ETc was 417.6 mm accumulated, with a maximum and minimum of 6.4 mm d^{-1} and 1.5 mm d^{-1}, respectively, resulting in a ratio (ETc/ET_0) of 1.03. Meanwhile, the total evaporation measured with the mini-evaporimeters was 615.4 mm, yielding a ratio (ET_0/E) of 0.66. The maximum recorded evaporation was 7.6 mm d^{-1} and the minimum was 5.9 mm d^{-1}, respectively (Figure 1).

The estimated ETc in this study represents 53.7% of the net water application considered for this crop in the Valle de Culiacán irrigation area (CONAGUA, 2024). Ike *et al.* (2019) obtained an ETc for cucumber in hydroponics of 289.2 mm, considering a 110-day growing cycle in Nigeria, with an ETc/ET_0 ratio of 0.91, given that the prevailing meteorological conditions, cultivation conditions, and crop development periods in both areas are completely different. On the other hand, Yaghi *et al.* (2013) estimated the ETc

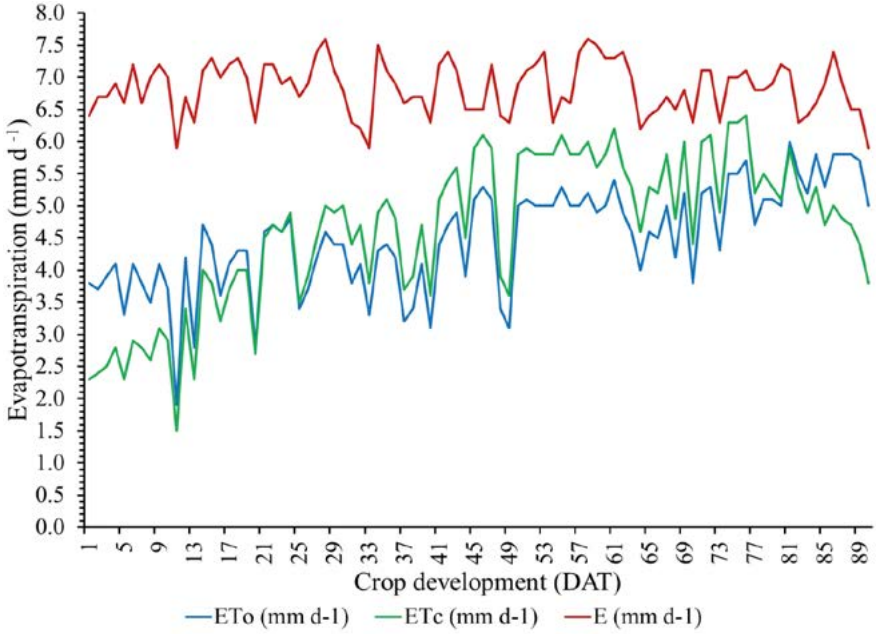


Figure 1. Behavior of evaporation and evapotranspiration during crop development.

for cucumber under open field conditions using furrow irrigation and drip irrigation with and without plastic mulching, reporting values of 673.2 mm, 243.9 mm, and 288.2 mm, respectively. The growing cycle considered was 92 days under prevailing meteorological conditions in Syria. These researchers reported an ETc/ET_0 ratio of 0.37 for drip irrigation with plastic mulching, 0.44 for drip irrigation without plastic mulching, and 1.02 for furrow irrigation; while the ET_0/E ratio was 0.74, higher than that reported in this study.

Based on the ETg estimated with the FAO56 methodology and the evaporation measured with the non-conventional evaporimeter, the K_p values were estimated according to Equation 4 in this study. Subsequently, considering that K_p results from the interaction with different meteorological variables, a multiple linear regression was performed, using K_p as the dependent variable and the interaction of air temperature, relative humidity, and net solar radiation. This yielded an excellent correlation with a coefficient of determination (R^2) of 0.97, an error of 0.018, and a Willmott concordance index of 0.99 (Figure 2).

Other studies have explored the possibility of using non-conventional evaporimeters with different pan diameters. For example, Sujitha *et al.* (2020) used pan with diameters of 60 and 20 cm to measure evaporation inside a greenhouse, obtaining acceptable correlation coefficients when comparing the ET_0 obtained with the Class A evaporimeter pan. In a statistical analysis of the daily ET_0 estimation, excellent results were achieved in the RMSE, R^2, R, and d indicators (Figure 3).

In an individual dependence analysis of K_p with respect to T, HR, Rn, and DPV, a good correlation was found between K_p and Rn (R^2=0.68) and T (R^2=0.68), moderate with respect to DPV (R^2=0.53), and low with respect to HR (R^2<0.1). This contrasts with what Antensay (2020) reported when analyzing the dependence of the Class A evaporimeter pan coefficient on meteorological parameters in open fields (T, HR, Rn, and DPV), where the correlation was very low with R^2<0.1.

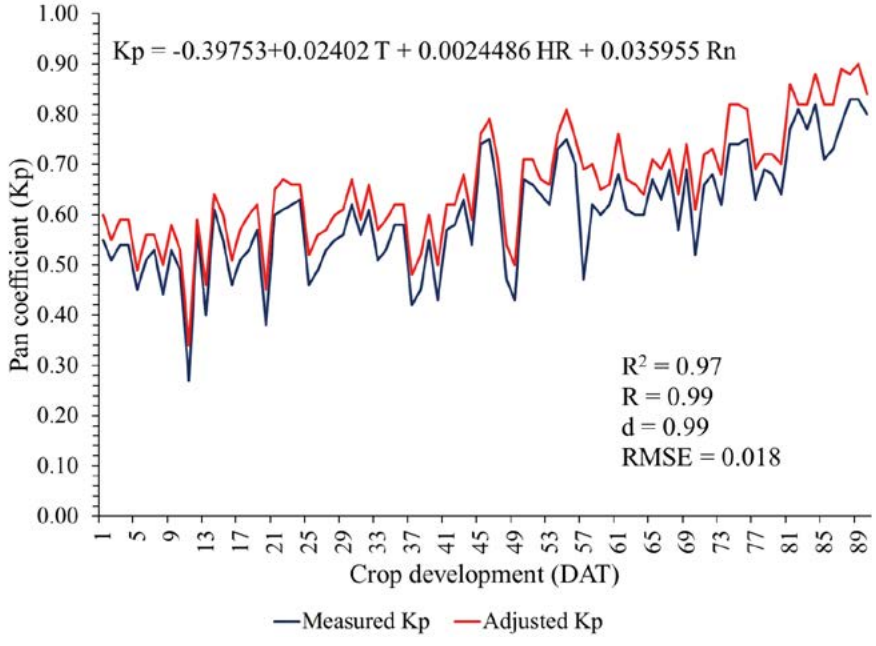


Figure 2. Evolution of the evaporimeter pan coefficient (K_p) during Crop Development.

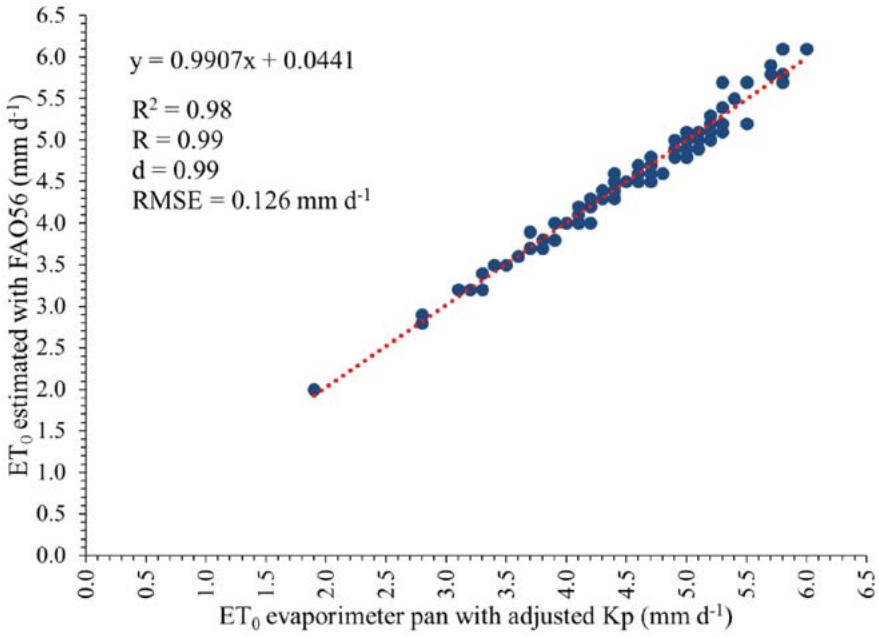


Figure 3. Statistical analysis of ET_0 estimated with FAO56 and evaporimeter pan.

CONCLUSIONS

The evapotranspiration of cucumber crops under shade netting in the Valle de Culiacán, Mexico, was estimated using the FAO56 methodology with a variation of the FAO Penman-Monteith equation for reference evapotranspiration in protected agriculture conditions. The total water consumed by the crop was 417.6 mm. Additionally, the use of a low-cost, accessible, and easy-to-operate unconventional evaporimeter pan is proposed for local agricultural producers, requiring minimal meteorological data. To estimate the reference evapotranspiration, a mathematical model is suggested to calculate the evaporimeter pan coefficient simply and with good, statistically validated precision. Further validation of this methodology under different protected agriculture conditions and for various crops is recommended.

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Colegio de Postgraduados

Effectiveness of Chemical Fungicides for the *in vitro* Control of *Fusarium* spp. Causing Basal Rot in Onion in Sinaloa, Mexico

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ABSTRACT
Objective: To identify the in vitro effectiveness of five chemical fungicides and the percentage of mycelial growth inhibition of *Fusarium falciforme*, *Fusarium brachygibbosum*, and *Fusarium oxysporum* in onion production.
Design/methodology/approach: Isolates were subjected to an *in vitro* sensitivity test with five fungicides at concentrations of 1, 10, 100, and 1000 ppm in PDA culture medium. Disks of 0.5 cm in diameter from the isolates were transferred to the center of Petri dishes containing the cul-ture medium impregnated with the fungicides. A completely randomized design was established with five treatments and a control, with three rep-lications per concentration. Mycelial growth was measured, and the per-centage of mycelial growth inhibition was calculated.
Results: Differences in the percentage of mycelial inhibition were ob-served among fungicides and species, with tubeconazole being the most effective. Furthermore, it was found that higher fungicide doses resulted in lower mycelial growth inhibition. The most effective fungicides for *F. falciforme* and *F. brachygibbosum* were boscalid and verango at low con-centrations, and tubeconazole and thiabendazole at high concentrations. For *F. oxysporum*, boscalid and verango were most effective at low con-centrations.
Findings/conclusions: Overall, *F. oxysporum* showed greater sensitivity to all fungicides.

Keywords: Effect; Chemical products; *Fusarium* spp.; Onion.

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INTRODUCTION

Agriculture worldwide is affected by various phytopathogenic fungi, causing diseases in vegetable, cereal, and fruit crops, as well as significant post-harvest losses (Alburqueque and Gusqui, 2018). The genus *Fusarium* is of great importance in agriculture due to its ability to cause a wide range of diseases in different crops. This pathogen has a remarkable capacity for adaptation, reproduction, and dispersal, and is found in soils around the world (Garcia, 2004; Kauffman, 2012).

Additionally, it can inhabit seeds, plant tissues, fruits, and weed plants (Davila, 2000). Currently, Mexico contributes 9.3% of the global onion production. However, this production is limited by basal rot disease caused by *Fusarium* spp., which leads to

significant losses in agricultural yields. This disease interferes with nutrient absorption and translocation, causing premature plant death (Delgado *et al.*, 2016) (Figure 1).

The most commonly used method to mitigate damage caused by *Fusarium* is the use of chemical fungicides, primarily those based on benzimidazoles and azoles (Rios *et al.*, 2021). Other commercial chemical fungicides used include difenoconazole, copper oxychloride, mancozeb, zineb, and azoxystrobin (Minag, 2007). However, the use of chemical products poses several issues that must be considered: the emergence of resistant strains, increased production costs, and risks to the environment and public health.

Fusarium has the ability to survive in the soil, and eradicating it once contaminated is difficult. For this reason, producers often resort to increasing the application of agrochemicals for phytosanitary control of crops in an attempt to combat the pathogen's persistence (Al-Hatmi *et al.*, 2019; Marx *et al.*, 2020). In Mexico, the most commonly used fungicides are mancozeb and chlorothalonil, as well as benzimidazoles, triazoles, strobilurins, and anilopyrimidines (Romero and Sutton, 1997; Hernández, 2019). Therefore, it is of utmost importance to conduct research that addresses the effect of chemical fungicides on the control of basal rot disease, using appropriate dosages.

It is crucial to highlight that there is a lack of information on fungicides that show a positive effect in controlling this pathogen (Dugan *et al.*, 2007), not to mention the potential resistance that pathogens may develop against these chemical products. The objective of this study was to assess the *in vitro* effectiveness of five chemical fungicides, based on the percentage of mycelial growth inhibition of the pathogen, for controlling basal rot disease in onion crops caused by *F. falciforme*, *F. brachygibbosum*, and *F. oxysporum* isolated in Sinaloa, Mexico.

MATERIALS Y METHODS

The monosporic isolates used were *F. falciforme* (Ff05), *F. brachygibbosum* (Fb20), and *F. oxysporum* (Fo24), provided by the fungal collection of the Faculty of Agronomy at the

Figure 1. Basal rot in onion crops. A, necrosis at the basal part of the onion bulb; B, damage to the leaves, from the tips downward, and rot in the bulbs; C, damage at the basal part of the developed bulb.

Universidad Autónoma de Sinaloa. These isolates were cultured on PDA medium and were previously identified both morphologically and molecularly. Additionally, they are registered in GenBank with accession numbers MH041264, MH041261, and MH041263, respectively. These isolates have been reported as the causal agents of basal rot in onion crops in Sinaloa, Mexico (Tirado *et al.*, 2021).

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The isolates were subjected to an *in vitro* susceptibility test with different fungicides at concentrations of 1, 10, 100, and 1000 ppm in potato dextrose agar (PDA) medium (Gálvez *et al.*, 2016), following the poisoned food technique (Dingran and Sinclair, 1985). The culture medium was prepared in Erlenmeyer flasks sterilized at 120 °C for 15 minutes in an autoclave, allowed to cool to 40 °C before adding the fungicides at the corresponding concentrations to 9 cm diameter Petri dishes.

The pathogen isolates were incubated for 7 days at 28 °C in darkness to inoculate the different treatments, by placing a 0.5 cm diameter disk. The plates were incubated at 28 °C for the first 7 days, during which the control treatment occupied 100% of the Petri dish growth (Muiño *et al.*, 2010). The diameter of the colony was measured in two perpendicular directions using a digital Vernier. The growth of the pathogen colonies was compared with the growth of the control treatment, following the methodology described by the radial growth method (FAO, 1982).

A completely randomized design was established, consisting of five treatments and a control without fungicide. Each treatment was applied at four doses and with three replications. The experiment was repeated twice. The effectiveness of the treatments was evaluated using the variable of mycelial growth inhibition percentage (MGIP), according to the formula proposed by Pandey *et al.* (1982).

Table 1. Description of Fungicides Used.

Fungicide	Chemical group
Thiabendazole	Benzimidazole
Boscalid	Pyridinecarboxamides
Tebuconazole	Triazole
Chlorothalonil	Chloronitrile
Verango	Chlorinated benzimide

$$MGIP = \frac{Diameter\ of\ the\ control\ colony - Diameter\ of\ the\ colony\ at\ the\ concentration}{Control\ colony\ growth\ diameter} \times 100$$

An analysis of variance and Tukey's multiple comparison test were conducted on the mycelial growth inhibition percentages (MGIP) obtained from the fungicide treatments. This analysis was performed separately for each species. Subsequently, a separate analysis of variance and Tukey's multiple comparison test were carried out to evaluate the effectiveness of the fungicides, considering both the concentration of each fungicide and the *Fusarium* species.

RESULTS AND DISCUSSION

The MGIP results by species revealed that the fungicide that inhibited the most mycelial growth in the three species under study was Tubeconazole, with values of 95.54% for *F. falciforme*, 94.7% for *F. oxysporum*, and 94.13% for *F. brachygibbosum*, showing a significant difference compared to the other fungicides for each species (Figure 2). These results were similar to those published by Jahanshir and Dzhalilov (2010), who reported that the triazole chemical group, such as the fungicide tubeconazole, is the most effective in controlling Fusarium species in both cereal grains and various crops, as it inhibits the formation of the cell wall in these pathogens.

Overall, the fungicide that inhibited mycelial growth the least in the three *Fusarium* species was boscalid, with values of 70.35%, 86%, and 67.56% for *F. falciforme*, *F. oxysporum*, and *F. brachygibbosum*, respectively. It is worth noting that *F. brachygibbosum* showed the lowest inhibition percentage with the fungicide chlorothalonil, with a value of 58.75% (Figure 2). This can be attributed to the fact that boscalid is not recommended for *Fusarium* species (The chemical company BASF, 2014). These results are consistent with those reported by Parada *et al.* (2013), who evaluated the *in vitro* growth of *Fusarium* isolated from corn and beans, obtaining a lower MGIP with boscalid. However, the present study revealed that boscalid at low concentrations presented a high MGIP.

On average, across concentrations, the three *Fusarium* species showed a high percentage of mycelial growth inhibition (MGIP). However, as the concentrations of fungicides increased, MGIP decreased in all *Fusarium* species (Figure 3). This finding demonstrates that the higher the fungicide concentration, the lower its effectiveness. These results are of great social and economic importance. According to Chin *et al.* (2001), it is crucial to reduce fungicide applications, whether systemic or contact-based, as they increase production costs and pose environmental risks. Additionally, there is the risk that the pathogen may develop resistance to the fungicide (FRAC, 2010) when doses are increased or application frequencies are intensified (Martínez *et al.*, 2012).

The *in vitro* effectiveness of the fungicides depended on the fungicide concentration and the *Fusarium* species studied. In the case of *F. falciforme*, the most effective fungicide was boscalid at a concentration of 10 ppm, followed by tubeconazole at concentrations of 100 and 1000 ppm, and finally, tiabendazole at a concentration of 100 ppm, showing inhibition percentages of 100%, 100%, 99.16%, and 99.16%, respectively. These

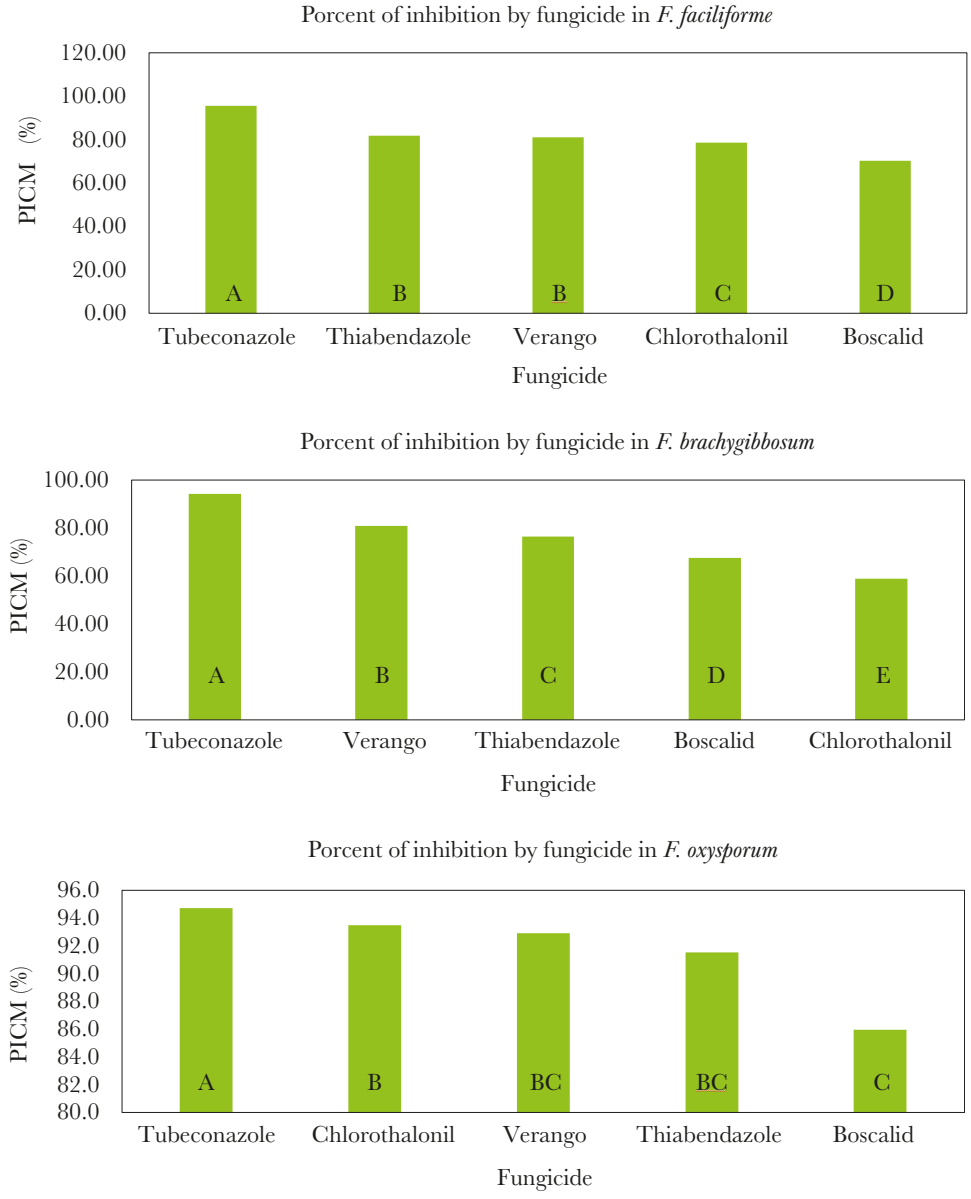

Figure 2. Percentage of Mycelial Growth Inhibition (MGIP) by fungicide in *F. falciforme*, *F. brachygibbosum*, and *F. oxysporum*. MGIP is expressed as a percentage, and the comparison of means was performed using Tukey's test with $p \leq 0.05$.

treatments showed a significant difference compared to the other treatments. In the case of *F. brachygibbosum*, the most effective fungicides were tiabendazole at 100 ppm, boscalid and verango at 1 ppm, tubeconazole at 1000 and 100 ppm, and boscalid at 10 ppm, showing inhibition percentages of 100%, 100%, 100%, 99.62%, 99.25%, and 99.23%, respectively. These treatments showed a significant difference compared to the other treatments. Lastly, for *F. oxysporum*, the fungicides boscalid and verango at 1 ppm presented the highest inhibition percentages and showed a significant difference compared to the other treatments (Table 2).

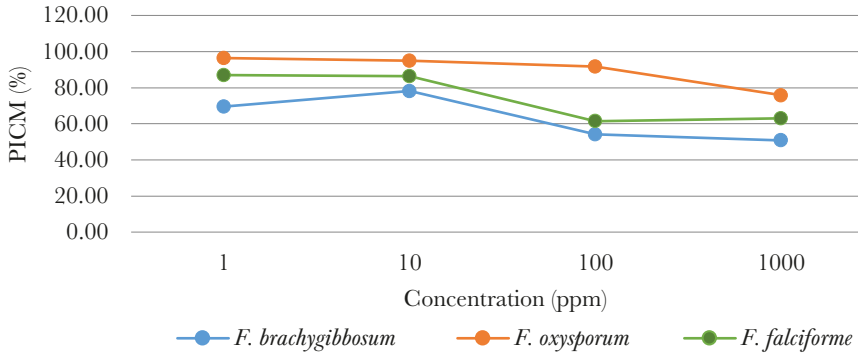

Figure 3. Percentage of mycelial growth inhibition (MGIP) by concentration in parts per million of *F. falciforme*, *F. brachygibbosum*, and *F. oxysporum*.

These results are consistent with those reported by Jahanshir and Dzhalilov (2010), who state that tubeconazole is effective in controlling *Fusarium* species by influencing the ergosterol biosynthesis process, which affects the fluidity and permeability of the pathogen's cells, essential for cell survival (Lepesheva and Waterman, 2004). Dubos *et al.*

Table 2. Analysis of variance of fungicide treatments on *Fusarium* spp. causing basal rot in onion crops.

Treatments	***Fusarium* spp causing basal rot in onion crops**					
Concentration / Fungicide	***F. falciforme***		***F. brachygibbosum***		***F. oxysporum***	
	Average[a]	**Group[b]**	**Average[a]**	**Group[b]**	**Average[a]**	**Group[b]**
Boscalid - 10 ppm	100.00	A	99.23	A	97.78	AB
Tubeconazole - 100 ppm	100.00	A	99.25	A	95.00	ABC
Tubeconazole - 1000 ppm	99.16	A	99.62	A	93.89	ABC
Thiabendazole - 100 ppm	99.16	A	100	A	97.78	AB
Boscalid - 1 ppm	98.72	AB	100	A	100.00	A
Verango -1 ppm	98.29	AB	100	A	100.00	A
Verango -10 ppm	95.42	ABC	99.23	A	99.44	AB
Tubeconazle -10	95.42	ABC	89.66	CD	94.44	ABC
Thiabendazole - 1000 ppm	94.09	BC	91.22	C	93.33	ABC
Thiabendazole - 10 ppm	91.67	CD	95.02	B	92.22	BC
Tubeconazole - 1 ppm	87.61	DE	87.98	D	95.56	ABC
Chlorothalonil - 100 ppm	84.81	EF	68.17	E	94.44	ABC
Chlorothalonil - 1000 ppm	81.43	F	70.61	E	94.44	ABC
Chlorothalonil - 1 ppm	75.21	G	39.15	I	92.78	ABC
Chlorothalonil - 10 ppm	72.50	G	57.09	H	92.22	BC
Verango - 100 ppm	65.40	H	63.67	F	92.78	ABC
Verango - 1000 ppm	64.98	H	60.31	G	79.44	E
Boscalid - 1000 ppm	44.73	I	30.92	J	57.22	F
Thiabendazole - 1 ppm	42.74	I	19.77	K	82.78	DE
Boscalid - 100 ppm	37.97	J	40.08	I	88.89	CD

[a] Mycelial growth inhibition percentage.
[b] Percentages followed by different letters indicate significant difference according to Tukey's test, $p \leq 0.05$.

(2011) report that *Fusarium* is more sensitive to fungicides that alter cell wall biosynthesis. On the other hand, benzimidazoles, such as thiabendazole, have a systemic, protective, and curative effect, causing abnormalities in cells (Deepak and Lal, 2009). Additionally, they inhibit mitosis, affecting the growth and development of the pathogen (FRAC, 2017). In the present study, tubeconazole and thiabendazole showed high MGIP only at high concentrations, which is agronomically significant, as resistance to these chemical groups has been reported in different species such as *C. gloeosporioides*, *C. acutatum*, and *Colletotrichum fragarie* (De los Santos and Romero, 2002).

Arie (2019) reports numerous cases of resistance in various forma specialis of *F. oxysporum* when applying benzimidazoles. The repeated use of chemicals with the same mode of action could lead to the selection of resistant strains, with reports of low sensitivity to tebuconazole in Germany (Klix *et al.*, 2007) and China (Yin *et al.*, 2009). The fungicides that showed the least effectiveness were boscalid and verango at concentrations of 100 and 1000 ppm, showing the same behavior across the three species with MGIP values below 57% (Table 2). In the case of boscalid, the results are consistent with those reported by Masiello *et al.* (2019), who evaluated different fungicides *in vitro* against various *Fusarium* species causing corn ear rot, reporting that after ten days, all *Fusarium* species were able to grow at both low and high concentrations of boscalid. These results are attributed to boscalid's systemic and translaminar activity, which inhibits spore germination, disrupts the cytochrome complex, deprives the cells of their energy source, and prevents the synthesis of essential components (The Chemical Company BASF, 2014). In the case of Verango, it is reported as a nematicide that disrupts the flow of electrons in the pathogen's mitochondria, affecting ATP production, which is the main energy source for all biochemical processes. This product was considered due to its use and effectiveness in controlling *Fusarium* diseases in various crops in the field of Sinaloa (Bayer, 2022). Studies on the efficacy of new products, and even those registered for other crops, are very useful (Masiello *et al.*, 2019), as according to Blandino and Reyneri (2009) and Edwards *et al.* (2001), they can control *Fusarium*-associated diseases in different crops. *F. oxysporum* showed greater sensitivity to all treatments, demonstrating a higher percentage of mycelial growth inhibition, such as with the fungicide thiabendazole, which at a concentration of 1 ppm, showed lower effectiveness for *F. falciforme* and *F. brachygibbosum* with values of 42.74% and 19.77%, respectively; however, for *F. oxysporum*, it presented a MGIP value of 82.78% (Table 2). Similar results were observed for all treatments with the fungicide chlorothalonil, where *F. oxysporum* showed greater sensitivity compared to the other species. These results are consistent with those reported by Alburqueque and Gusqui (2018), where *F. oxysporum* had the highest MGIP compared to other pathogenic species. The fungicide chlorothalonil is a contact fungicide with multisite activity that inhibits the respiration of fungal cells. The fungicide molecules bind to the sulfhydryl group of amino acids, deactivating enzymes that affect the Krebs cycle, leading to a lack of ATP production and the death of the pathogen (Bacmaga *et al.*, 2018). Masiello *et al.* (2019) evaluated different *Fusarium* species causing maize ear rot with eleven fungicides, reporting that *F. verticillioides*, *F. graminearum*, and *F. proliferatum* showed considerable variability in sensitivity to the concentrations and to all the fungicides. Petkar *et al.* (2017) discuss the susceptibility of *F. oxysporum* to the fungicide

due to the position 200 of the gene; however, other authors dismiss this (González and Iglesias, 2022). Misiello *et al.* (2019) suggest considering the geoclimatic adaptability of *Fusarium* species for their antifungal effects; however, the high genetic variability within different *Fusarium* species makes it difficult to select the best fungicides for their control (Fravel *et al.*, 2005; Kopacki and Wagner, 2006; Chen and Zhou, 2009; Deepak and Lal, 2009; Srivastava *et al.*, 2011). This study provides new information on the sensitivity of *Fusarium* species causing basal rot in onion crops in Sinaloa, Mexico, to different chemical fungicides. It suggests that field evaluations of these products should be conducted due to various factors to consider; however, if a product is not effective *in vitro*, it is unlikely to be useful in the field (González, 2005).

CONCLUSIONS

There are differences among the groups of fungicides and *Fusarium* species in inhibiting mycelial growth *in vitro* and, consequently, in controlling basal rot disease in onion crops in Sinaloa, Mexico. This study found that higher concentrations of chemical fungicides result in lower effectiveness in terms of mycelial growth inhibition percentage (MGIP) in vitro. The most effective fungicides for controlling *F. falciforme* are boscalid, tubeconazole, and tiabendazole. For *F. brachygibbosum*, the most effective fungicides are tiabendazole, boscalid, verango, and tubeconazole, while for controlling *F. oxysporum*, the most effective are boscalid and verango. In this study, *F. oxysporum* was the most sensitive to the mycelial growth inhibition percentage compared to *F. falciforme* and *F. brachygibbosum*.

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Light Quality Produced by LED Combinations on the Growth of Cucumber Seedlings (*Cucumis sativus* L.)

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ABSTRACT

Objective: To determine the influence of light quality on the growth of cucumber plants (*Cucumis sativus* L.), through the intensity of expression of different characteristics.

Design/methodology/approach: A completely randomized block design was used with four treatments and 10 repetitions. The treatments consisted of combinations of white (B), red (R) and blue (A) LEDs placed in growth chambers, with percentages of: 100B-0R-0A, 70B-30R-0A, 80B-0R- 20A and 60B-27R-13A, growth chambers with LED-based lighting systems lamps were used. Seeds of the 'Top 1056' cultivar, Persian type, were sown. The response variables evaluated in the cucumber plants were plant height, stem diameter, leaf greenness, leaf area, fresh and dry biomass of leaves, stem and root of the plants.

Results: The light spectrum emitted by the LEDs influenced the morphology of the cucumber seedlings. With the 80B-0R-20A treatment, where there was greater blue light emission, greater leaf greenness and stem diameter were achieved. In contrast, the 70B-30R-0A treatment, with more red light, increased plant height and leaf area. Fresh and dry biomass of leaves and stem were also modified by light quality. Plants grown in the 70B-30R-0A treatment produced the greatest amounts of fresh and dry biomass, both stem and leaves.

Limitations on study/implications: The use of artificial lighting systems, with different spectral compositions for production in controlled environments presents a viable opportunity to enhance crop growth. Therefore, it is important to investigate how the light spectrum of different LED combinations affects the growth of cucumber seedlings.

Findings/conclusions: The light spectrum emitted by LED combinations influenced the morphology of cucumber seedlings, since with 80B-0R-20A treatment, resulted in greater leaf greenness and stem diameter, while the 70B-30R-0A treatment increased the height and leaf area of the plants.

Keywords: white ligh, red light, blue light.

INTRODUCTION

Light-emitting diodes (LEDs) represent a promising technology for the greenhouse industry and are currently being tested for horticultural applications (Mitchell *et al.*, 2012). A LED is a unique type of semiconductor diode. The wavelength of the emitted light (light color) depends on the properties of the semiconductor material.

LEDs can have peak emission wavelengths ranging from UV-C (~250 nm) to infrared (~1000 nm) and are the first light source capable of controlling the spectral composition. This allows wavelengths to be matched with plant photoreceptors to enhance optimization, production, and influence the morphology and composition of plants (Bourget, 2008). Plant species have the ability to respond in various ways to light quality (color or wavelength), light intensity (photon flux density or irradiance), and the combination of both (Nguy-Robertson *et al.*, 2015).

The plant response to the received light spectrum is determined by the action of different photoreceptors. According to Xie *et al.* (2019), these can be grouped based on the region of the electromagnetic spectrum they detect: phytochromes detect red (600 to 700 nm) and far-red (700 to 750 nm) light in a dynamic photoequilibrium relationship, while cryptochromes and phototropins respond to blue light from 350 to 500 nm (Fantini *et al.*, 2019).

Light quality affects plant growth, development, and morphology (Fukuda *et al.*, 2008). The photosynthetic organs of plants (leaves and green stems) absorb photons more efficiently in the blue and red regions of the incident visible radiation spectrum, while absorption in the green and infrared regions is minimal, as most of these photons are reflected as diffuse radiation (Lazo and Ascencio, 2010). On the other hand, tomato and pepper seedlings grown under blue light, either alone or in combination with red light, exhibit reduced stem height (Javanmardi and Shandiz, 2013). Additionally, blue light supplementation promotes the growth of spinach, radish, and lettuce under red light (Yorio *et al.*, 2001).

MATERIALS AND METHODS

The research was conducted at the Plant Physiology and Anatomy Laboratory of the Faculty of Agronomy, Autonomous University of Sinaloa, in Culiacán, Sinaloa, Mexico. Growth chambers with LED-based lighting systems were used. Cucumber seeds cv. 'Top 1056', a Persian type, were sown in polystyrene trays with 128 cavities.

A completely randomized block design was used with four treatments and ten replications. The treatments consisted of: 100% white light, 0% red, and 0% blue (100B-0R-0A) emitted by white LEDs (B); 70% white light, 30% red, and 0% blue (70B-30R-0A) from a combination of white and red LEDs (R); 80% white light, 0% red, and 20% blue (80B-0R-20A) achieved by combining white and blue LEDs; and 60% white light, 27% red, and 13% blue (60B-27R-13A), generated by using a mix of white, red, and blue LEDs. These treatments were applied in the respective growth chambers, where the percentages of light for each treatment were determined based on the number and types of LEDs installed.

The spectral distribution achieved with the LED combinations used is shown in Figure 1. Additionally, the absolute quantities of photosynthetic photon flux (PPF), red light (RL),

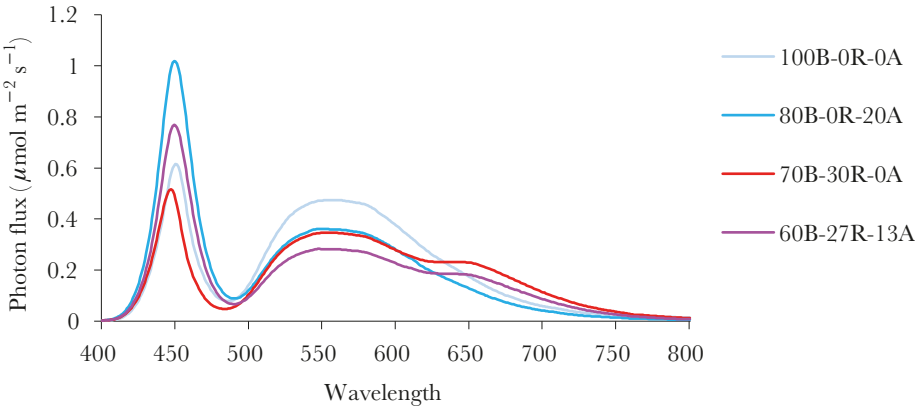

Figure 1. Spectral distribution (400-800 nm) of light emitted by combinations of white (B), red (R), and blue (A) LEDs.

far-red light (FRL), and blue light (BL), as well as the relative amounts of RL and BL with respect to PPF, and the proportions between RL, BL, and FRL, are detailed in Table 1.

The light quality (Table 1), expressed in terms of the corresponding PPF, for the wavelength intervals of 400 to 700 nm (photosynthetically active radiation), 400 to 500 nm (blue light), 600 to 700 nm (red light), and 700 to 800 nm (far-red light) was determined using a spectroradiometer (FieldSpec Pro®VNIR, Analytical Spectral Devices, USA). The combination of white and red LEDs (70B-30R-0A) emitted more red light (20.87 μmol m^{-2} s^{-1}), which was 52.78%, 26.1%, and 12.69% higher than the respective combinations of white and blue LEDs (80B-0R-20A), white, red, and blue LEDs (60B-27R-13A), and white LEDs alone (100B-0R-0A). With 70B-30R-0A, conditions had the highest amount of far-red light (4.48 μmol m^{-2} s^{-1}), followed by the quantities of μmol m^{-2} s^{-1} generated by 60B-27R-13A, 100B-0R-0A, and 80B-0R-20A.

Table 1. Spectral characteristics of light emitted by LED combinations.

Parameters	Treatment			
	100B-0R-0A	**70B-30R-0A**	**80B-0R-20[a]**	**60B-27R-13[a]**
FFF (400-700 nm)[x]	81.31	70.51	79.15	68.76
LA (400-500 nm)[x]	19.67	15.73	33.08	24.85
LR (600-700 nm)[x]	18.52	20.87	13.66	16.55
RL (700-800 nm)[x]	2.78	4.48	1.68	3.26
LA: FFF [(400-500/400-700 nm)*100][y]	24.20	22.31	41.80	36.13
LR: FFF [(600-700/400-700 nm)*100][y]	22.77	29.60	17.26	24.06
LA: LR (400-500/600-700 nm)[z]	1.06	0.75	2.42	1.50
LA: LRL (400-500/700-800 nm)[z]	7.07	3.51	19.70	7.62
LR: LA (600-700/400-500 nm)[z]	0.94	1.33	0.41	0.67
LR: LRL (600-700/700-800 nm)[z]	6.66	4.56	8.13	5.07

PPF=Photosynthetic Photon Flux. BL=Blue Light, RL=Red Light, FRL=Far-Red Light. B=% White LEDs, R=% Red LEDs, A=% Blue LEDs. Absolute quantities × (μmol m^{-2} s^{-1}), relativey (%), and proportionalz (dimensionless).

Regarding blue light emission, the combination of LEDs 80B-0R-20A produced 33.08 μmol m^{-2} s^{-1}, which was 110.3%, 68.17%, and 33.12% higher than the blue light emitted by the combinations of LEDs 70B-30R-0A, 100B-0R-0A, and 60B-27R-13A, respectively. The photosynthetic photon flux varied from 68.76 μmol m^{-2} s^{-1} to 81.31 μmol m^{-2} s^{-1}.

The response variables evaluated in the cucumber plants were: plant height, measured with a tape measure; stem diameter, obtained with a digital caliper (6MP, Truper Tools, Mexico); leaf greenness, estimated with a chlorophyll meter (SPAD 502, Konica Minolta, Japan); leaf area, calculated using the formula:

$$LA_{leaf} = (Lenght * Width) \wedge 0.851$$

proposed by Blanco and Follegati (2003); and fresh and dry biomass of leaves, stem, and roots, determined using a precision balance (CP622, Sartorius, Germany), after drying in an oven (292, Felisa, Mexico) at 70 °C until a constant dry weight was achieved. The data obtained were subjected to analysis of variance and mean comparison using Tukey's test at 95% confidence level, using the Minitab 19 statistical package.

RESULTS AND DISCUSSION

The quality of light emitted by the LED combinations had significant effects ($P \leq 0.05$) on the height, greenness, and leaf area of cucumber plants (Table 2). The highest amount of red light (20.87 μmol m^{-2} s^{-1}), the highest red light to photosynthetic photon flux ratio (29.60%), and the highest red light to blue light ratio (1.33), emitted by the LED combination with 70B-30R-0A, caused plant height to increase by 20.6%, 12.1%, and 3.2% compared to the height achieved by those grown under the LED combinations of 80B-0R-20A, 100B-0R-0A, and 60B-27R-13A, respectively. These results are consistent with those of Ding *et al.* (2010), who observed that *Paeonia suffruticosa* seedlings were taller when grown under red light, as well as with those of Juwei *et al.* (2016), who reported that *Morus alba* plants exhibited greater stem length under red light.

The results show that higher absolute quantities (Table 2), relative, and proportional (Table 1) of red light promoted increased stem length, while the highest amount of blue light in the light environment had the opposite effect. This is because blue light directs

Table 2. Influence of light quality emitted by white, blue, and red LEDs on stem length and diameter, leaf greenness, and leaf area in Persian cucumber seedlings 'Top 1056'.

Tratamiento	Plant height (cm)	Stem diameter (mm)	Greenery (Spad units)	Leaf area (cm^2/plant)
100B-0R-0A	7.72±1.29 ab	2.81±0.19 a	30.77±2.12 ab	23.81±4.14 b
70B-30R-0A	8.65±0.59 a	2.87±0.28 a	27.85±1.98 c	27.98±3.65 a
80B-0R-20A	7.17±1.44b	2.62±0.21 a	32.38±2.45 a	16.25±2.02 c
60B-27R-13A	8.38±1.38 ab	2.66±0.39 a	28.82±2.78 bc	15.31±3.01 c

*B=% White LEDs, R=% Red LEDs, A=% Blue LEDs. =Means ± standard deviation; values with the same letter within each column are statistically similar (Tukey, $p \leq 0.05$).

plant behavior towards photosynthetic efficiency rather than stem elongation, resulting in more compact and efficient plants. This reduction in stem growth is consistent with the findings of Dougher and Bugbee (2001), who reported that under high light intensities, blue light strongly inhibits stem elongation. Similarly, Javanmardi and Shandiz (2013) found that tomato and pepper seedlings had shorter heights when grown under blue light, either alone or in combination with red light. In terms of stem diameter, the results were inverse to those for plant height, although no statistical differences were observed (Table 2). However, leaf greenness was more intense in plants that received more blue light (33.08 μmol m^{-2} s^{-1}, as indicated in Table 1) from the 80B-0R-20A LED combination, with SPAD values exceeding those of plants that received less blue light (15.73 μmol m^{-2} s^{-1}) from the 70B-30R-0A combination by 16.2%. Light quality also caused significant modifications in leaf dimensions. Thus, the leaf area of plants grown with 70B-30R-0A, which had the highest amount of red light (20.87 μmol m^{-2} s^{-1}), a high red light: PFF ratio (29.60%), and a red light: blue light ratio (1.33), was 72.1% and 82.7% larger than that of plants grown under the influence of 80B-0R-20A and 60B-27R-13A, respectively. The latter combinations had the highest absolute, relative, and proportional amounts of blue light and the lowest amounts of red light, which is often associated with increases in the transmission of photosynthetically active radiation and blue light (Costa *et al.*, 2010; Hogewoning *et al.*, 2010; Souza *et al.*, 2011). With a relatively low PFF (100 μmol m^{-2} s^{-1}), blue light can alter leaf morphology and photosynthesis (Hogewoning *et al.*, 2010; Terfa *et al.*, 2013).

Table 3 shows that the production of fresh biomass exhibited significant differences ($P \leq 0.05$) due to light quality. Plants grown under 80B-0R-20A conditions accumulated 36.8% more fresh weight in leaves compared to those grown under 60B-27R-13A. The same table also shows that fresh biomass accumulation in stems varied significantly; plants grown in the 70B-30R-0A environment produced 38.18% more fresh weight in stems compared to those grown under 80B-0R-20A. No statistical differences were observed in the fresh biomass of roots.

However, in relation to dry weight (Table 4) of the organs in question, it was found that plants grown in an environment with a higher amount of red light (20.87 μmol m^{-2} s^{-1}), emitted by the LED combination of 70B-30R-0A, produced 63.5% more dry weight of leaves compared to the leaves from plants grown under 80B-0R-20A.

Table 3. Influence of light quality emitted by combinations of white, blue, and red LEDs on the fresh biomass of Persian cucumber seedlings 'Top 1056'.

Treatment	Fresh weight (g)		
	Leaves	Stem	Root
100B-0R-0A	0.258±0.059 ab	0.435±0.052 a	0.508±0.217 a
70B-30R-0A	0.233±0.029 ab	0.470±0.050 a	0.313±0.069 a
80B-0R-20A	0.368±0.302 a	0.330±0.065 b	0.333±0.170 a
60B-27R-13A	0.162±0.044 b	0.305±0.066 b	0.364±0.177 a

B=% of white LEDs, R=% of red LEDs, A=% of blue LEDs. Means ± standard deviation with the same letter within each column are statistically similar (Tukey, $p \leq 0.05$).

Table 4. Influence of Light Quality Emitted by Combinations of White, Blue, and Red LEDs on the Dry Biomass of Persian Cucumber 'Top 1056' Seedlings.

Treatment	Dry Weight (g)		
	Leaves	Stem	Root
100B-0R-0A	0.0239±0.0053 a	0.0127±0.0026a	0.0215±0.0120 a
70B-30R-0A	0.0224±0.0041 ab	0.0115±0.0038 a	0.0134±0.0031 ab
80B-0R-20A	0.0137±0.0058 c	0.0084±0.0048 a	0.0158±0.0076 ab
60B-27R-13A	0.0170±0.0060bc	0.0243±0.0095 a	0.0110±0.0035 b

B=% of white LEDs, R=% of red LEDs, A=% of blue LEDs. Means ± standard deviation with the same letter within each column are statistically similar (Tukey, $p \leq 0.05$).

Although no statistical differences were found in the dry weight of the stem, the 70B-30R-0A treatment resulted in a 36.9% increase compared to that produced with 80B-0R-20A. Meanwhile, for root dry weight, a high ratio of blue light to red light (2.42 μmol m^{-2} s^{-1}, indicated in Table 1) emitted by 80B-0R-20A influenced the plants to produce 17.91% and 43.63% more root biomass compared to that produced by plants grown under the respective conditions of 70B-30R-0A or 60B-27R-13A, which emitted lower amounts of blue light (15.73 or 24.85 μmol m^{-2} s^{-1}, respectively).

The results can be linked to the fact that red light increases the photosynthetic rate of plants, leading to increases in dry weight (Nishimura *et al.*, 2009). This is why authors like Ayala-Tafoya *et al.* (2015) found that the dry weight of leaves and stems in pepper plants increased when cultivated under red netting, due to the interaction of higher fluxes of total radiation, photosynthetically active radiation, and red light. Similarly, Casierra-Posada *et al.* (2012) have noted that under blue light conditions, there is a decrease in the total dry weight of plants such as strawberry, beetroot, and broccoli.

CONCLUSIONS

The light spectrum emitted by combinations of white (B), red (R), and blue (A) LEDs influenced the morphology of cucumber seedlings. With 80B-0R-20A, where there was higher blue light emission, greater leaf greenness and stem diameter were achieved. Conversely, with 70B-30R-0A, which provided more red light, plant height and leaf area were increased. This influence of light quality was also observed in the characteristics of fresh and dry biomass of leaves and stems, as plants grown under 70B-30R-0A illumination showed the highest average biomass. However, this was not the case for the root system.

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Colegio de Postgraduados

Cabbage plant (*Brassica oleracea* var. *capitata* L.) quantification cultivated under different soil covers using aerial photographs

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ABSTRACT

Objective: Evaluate the efficiency of cabbage plants (*Brassica olaraceae* var. *capitata* L.) quantification cultivated under different types of mulching, using aerial images captured by RPAS (Remotely Piloted Aircraft System).

Design/methodology/approach: The cabbage plantation used for the study was established under a completely randomized block design with different types of mulch as treatments: black plastic, white plastic, straw, and bare soil. Manual plant counts and automated estimates were performed using two agricultural artificial intelligence platforms (Platforms A and B). The relationship was evaluated using linear regression correlation (R^2), and the following indicators were subsequently used: estimation accuracy (Ps), estimation error percentage (Es), mean absolute error (MAE), and root mean square error (RMSE).

Results: Platform A showed a correlation coefficient range of R^2=0.41 to 0.91. Platform B obtained R^2 values ranging from 0.77 to 0.88. Platform A exhibited the highest estimation accuracy (Ps) with 98.3% and an estimation error (Es) of −1.7% for straw mulch, with a mean absolute error (MAE) of 2.0% and a root mean square error (RMSE) of 1 for bare soil. Both platforms showed underestimations in the number of detected plants, ranging from −6.7% to −1.7%.

Limitations on study/implications: The use of RPAS was limited by atmospheric conditions such as wind and rain.

Findings/conclusions: The effectiveness of counting cabbage plants using RPAS was validated.

Keywords: Precision agriculture, Remotely Piloted Aircraft System (RPAS), drone, Unmanned Aerial Vehicle (UAV).

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INTRODUCTION

Cabbage (*Brassica oleracea* L. var. *capitata*) is a cruciferous plant that is consumed worldwide, it is one of the main vegetables in the human diet and is prescribed by nutrition specialists as a source of nutrients and fiber, with potentially positive effects (Galanty *et al*., 2024). Also, cabbage crop can be achieved either by direct seeding (placing the seed

on the soil) or by transplanting (placing previously produced seedlings). Regardless of the strategy the producer uses to grow cabbage, various factors could interfere with adequate plant density, the most common being the presence of pests and diseases (Ngosong *et al.*, 2021; Isaq *et al.*, 2023). It is also known that in warm climate regions, the establishment of appropriate plant density is affected by high temperatures, with the consequent need for plant replacement with live ones (replanting) (Adilov *et al.*, 2021; Osmani *et al.*, 2023). Therefore, locating and counting live plants in the plot is necessary to plan the replanting strategy. One of the current alternatives available for the producers is using aerial photographs and artificial intelligence algorithms for Precision Agriculture (PA) (Maurya *et al.*, 2024).

PA focuses on the efficient use of resources applied to agricultural crops at various stages throughout the management of the agricultural production cycle (Chin *et al.*, 2023; Sangeetha *et al.*, 2024 and Mehedi *et al.*, 2024). Among the technologies used to achieve PA, the use of remote sensors through aerial photographs stands out for detecting, counting, and monitoring cultivated plants (Sangeetha *et al.*, 2024 and Mehedi *et al.*, 2024).

The information on the distribution and location of plants within a plot, as well as the timely determination of the quantity of existing elements, allows the decision-making for crop management (Thakur and Srinivasan, 2024). Therefore, the objective of the present research was to evaluate the effectiveness of the quantification of cabbage plants (*Brassica oleracea* L. var. *capitata*) grown under different soil covers using aerial photographs taken by RPAS (Remotely Piloted Aircraft System).

MATERIALS AND METHODS

Description of the area of study

This study was conducted at the experimental station of the Instituto de Ciencias Agricolas de la Universidad Autonoma de Baja California (ICA-UABC), located at the coordinates 32.407319° north latitude and −115.198853° west longitude. The soil in the experimental plot is of the salic Vertisol type, subtype sodic saline Regosol, belonging to the physiographic subprovince of the San Sebastián Vizcaino Desert (VRs-zwca+RGsoszw/2) (INEGI, 2007 and 2021b). The climate of the region is described as very dry and hot with summer rains, with temperatures ranging from 13 °C to 33.5 °C (BW(h')hw(x')) (INEGI, 2020 and 2021a).

Experimental design and description of treatments

A plot with a cabbage crop was established with a completely randomized block design with three replicates. The cabbage crop was established with four treatments, which were: black plastic mulch, white plastic mulch, straw mulch, and bare soil. Each treatment consisted of two crop beds. The crop beds were oriented north-south, with a length of 6.0 m, separated by 1.8 m, with a height of 0.2 m. Each bed had a pressurized irrigation system with a double drippers watering line. Commercial drip tape with an average water discharge of 1.0 L ha^{-1} per dripper was used. Each dripper was spaced at 0.2 m.

Crop establishment

The crop material was cabbage (*Brassica oleracea* L. var. *capitata*) of the Supreme Vantage® variety [Sakata Seed America, Inc. USA]. The seeds were germinated in commercial polystyrene trays with 338 cavities. 45 days after germination, the transplant was performed on September 27, 2023. The crop design had a triangular staggered distribution, with 0.4 m spacing between plants and 0.5 m between rows (Escobosa *et al.*, 2024).

Agronomic management

The land preparation tasks consisted of one pass with a harrow and the formation of the planting beds. Subsequently, trenches were made where the pipes for the irrigation system were installed. The detailing of the planting beds, as well as the installation of covers and the irrigation system, was done manually. During the soil preparation tasks, Paraquat (dimethyl-4,4-bipyridylium dichloride-1; DRAGOCSON® Dragón, Mexico) was applied to control Bermuda grass (*Cynodon dactylon*). Broadleaf weed control was performed manually and mechanically. Irrigation management consisted of weekly applications. The pests that appeared were thrips (*Thrips tabaci*) and Bagrada bug (*Bagrada hilaris*). These were controlled through weekly applications of systemic insecticide (thiamethoxam, chlorantraniliprole; Durivo®, Syngenta Group, Mexico).

Fertilization was applied weekly through the irrigation system. The fertilization dose per hectare consisted of 330, 100, 150, 40, and 15 kg ha^{-1} of nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), and magnesium (Mg), respectively (Escobosa *et al.*, 2024). The fertilizer sources were urea [$CO_2(NH_2)_2$], phosphoric acid (H_3PO_4), potassium sulfate (K_2SO_4), calcium nitrate [$Ca(NO_3)_2$], and magnesium sulfate ($MgSO_4$).

Acquisition of aerial images and processing

The images were obtained on October 24, 2023, 27 days after the transplant (DAT). A DJI® Phantom 4 RTK multispectral RPAS was used (DJI, 2019b). The flight path was designed in the native application for the iOS system, DJI Ground Station Pro (DJI GS Pro) (DJI, 2019a). The flight parameters used were perpendicular flight of the course, stationary image capture, forward speed of 1 m s^{-1}, flight altitude of 38.5 m, 80% front and side overlap, and a gimbal angle of −84.1°. The images were georeferenced using five ground control points positioned with a GNSS RTK differential GPS, South Galaxy G7 (Prado *et al.*, 2020; SOUTH, 2024).

The obtained aerial images were used to generate the orthomosaic composed of multispectral bands. These images underwent radiometric correction using PIX4DFields software (Pix4D, 2024), used under an academic license with (key: 61b1b106). The obtained bands in the composite orthomosaic were: blue (B: 450±16 nm), green (G: 560±16 nm), red (R: 650±16 nm), red edge (RE: 730±16 nm), and near-infrared (NIR: 840±26 nm) (DJI, 2019c). The orthomosaic was analyzed using the open-source Geographic Information System (GIS) software QGIS v.3.22.10 (Qgis, 2023).

The first count was performed manually, visually identifying the cabbage plants present in the image (García *et al.*, 2020). For the count, the agricultural-type orthomosaic

composition was used, requiring the combination of the red (R), near-infrared (NIR), and blue (B) bands. This image was overlapped with 40% transparency over the Modified Soil Adjusted Vegetation Index (MSAVI2) (Equation 1). This index minimized the effect of bare soil, which allowed the visual differentiation of vegetation (Suman *et al.*, 2024).

Finally, a point shapefile was created and used in edit mode to mark each visible plant with a vertix.

$$MSAVI2 = \left(\frac{1}{2}\right) \times \left(2(NIR+1) - \sqrt{(2 \times NIR+1)^2 - 8 \times \left(NIR - Rojo^2\right)}\right) \qquad \text{(Equation 1)}$$

Automated quantification and identification of plants were performed using two online platforms focusing on artificial intelligence for precision agriculture. The platforms used were Agremo (Platform A) (Agremo, 2024b) and Solvi (Platform B) (Solvi, 2024c). In Platform A, the estimation procedure involved: uploading the orthomosaic, specifying the type of crop to be analyzed, providing the planting density used, and finally running the quantification tool (Agremo, 2024a). For Platform B, the procedure for quantification involved: uploading the multispectral orthomosaic. Subsequently, a training sample representing the treatments used was selected. The sample consisted of a rectangular section containing 22% of the plants from the experiment. Each present plant was marked within the training area (Figure 1). Finally, the quantification instruction was executed (Kitano *et al.*, 2019; Solvi, 2024a).

Once the quantification tools were executed on both platforms, the results were exported. Platform A provided the result in Portable Network Graphics (PNG) format. This file was georeferenced and vectorized within the initial project where the manual quantification had been done (Qgis, 2024a). Platform B allowed the export of the identified objects in shape format (shp). This format is compatible with major GIS software. Subsequently, a polygon was created in shape format (shp), which delimited each crop bed. This vector file was the input to use the point counting tool within a polygon (Qgis, 2024b). This way, the number of detected plants was obtained for each platform and each planting bed.

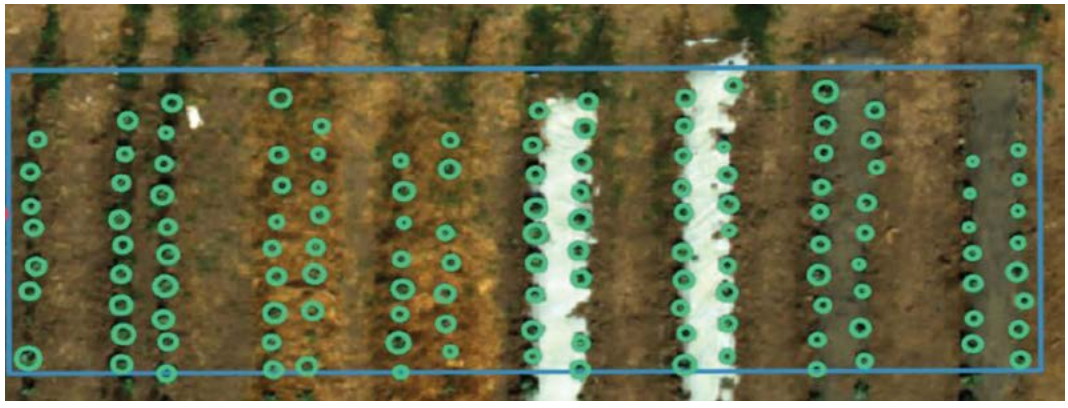

Figure 1. Training surface of Platform B.

Data Statistical Analysis

The statistical analysis was performed by comparing estimates from both platforms. For this purpose, the correlation method (R^2) was used through linear regression in Minitab v18 software (Minitab, 2021). Subsequently, the estimates were evaluated using the following indicators: estimation accuracy (Ps) (Equation 2); percentage error in estimation (Es) (Equation 3); mean absolute error (MAE) (Equation 4); and root mean square error (RMSE) (Equation 5) (Kitano *et al.*, 2019; García *et al.*, 2020 and Li *et al.*, 2023). The equations used are presented below.

$$Ps = \left(Estimated\ plants\right) / \left(Plants\ counted\right) \quad \text{(Equation 2)}$$

$$Es = \left(Estimated\ plants - Plants\ counted\right) / \left(Plants\ counted\right) \quad \text{(Equation 3)}$$

$$MAE = 1 / N \sum_{i=1}^{N} \left| Es_i \right| \quad \text{(Equation 4)}$$

$$RMSE = \sqrt{\frac{\sum_{i=1}^{N} \left(\left(Estimated\ plants - Plants\ counted\right)^2 \right)}{N}} \quad \text{(Equation 5)}$$

RESULTS AND DISCUSSION

Manual quantification identified 567 cabbage plants. Figure 2 shows the spatial distribution. The plants were transplanted on a surface of 200 m^2, which corresponded to a planting density of 2.8 plants m^{-2}. Table 1 indicated the number of plants counted manually and the estimation made by both platforms, as well as their presence in each type of mulching.

The estimates made by both platforms correlated positively with the manual counts (Figure 3). The correlation coefficients were in ranges above $R^2=0.77$. Except for the estimate made by platform A in black plastic mulching ($R^2=0.41$) (Figure 3A). Additionally, platform A presented the highest correlation coefficient with an $R^2=0.91$ (Figure 3G) in bare soil. Therefore, platform A had the widest range of correlation coefficient variability. Like platform A, platform B had its minimum $R^2=0.77$ (Figure 3B) in black plastic mulching; and the maximum correlation value ($R^2=0.88$) (Figure 3H) in bare soil. According to Aziz *et al.*, (2023), correlation is affected by the presence of false positives, mainly corresponding to the presence of shadows, weeds and rocks.

The reliability indicators obtained during the experiment (Table 2) show that platform A achieved the highest estimation accuracy (Ps) in straw mulching with 98.3%. Platform B achieved its highest accuracy in treatments with black plastic mulching and straw mulching, both with (Ps=96.8%). Both estimation platforms achieved their lowest accuracy in white plastic mulching (Platform A Ps=97%; Platform B Ps=87%). According to Li *et al.*, (2024), the high reflectance provided by white plastic covers reduces accuracy in plant identification, as their reflectance values are lower than those of white surfaces.

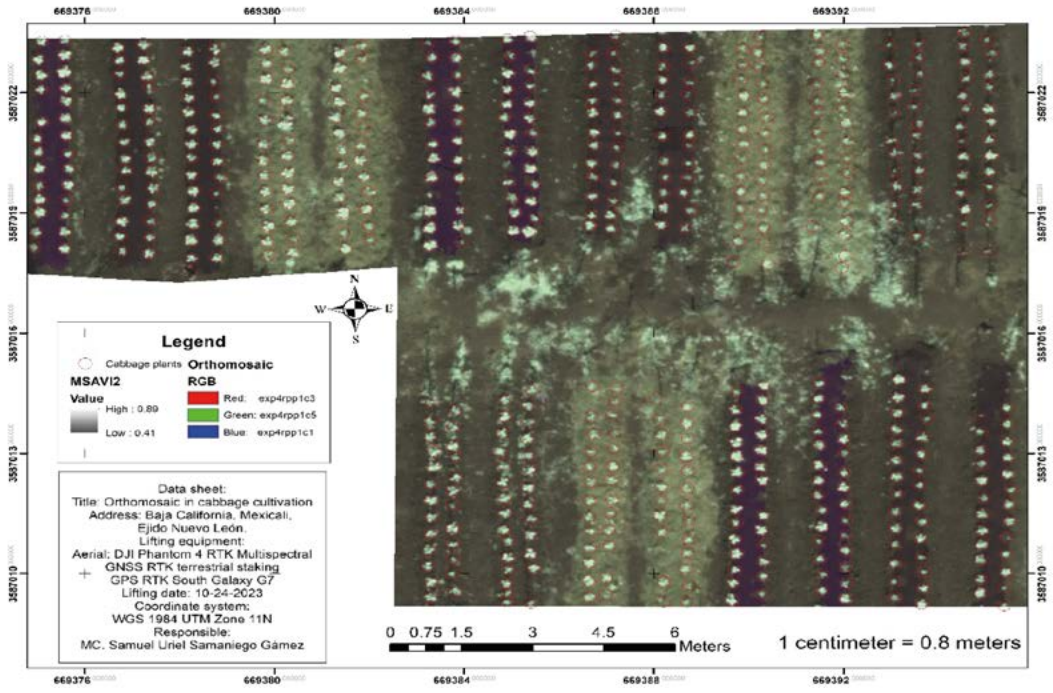

Figure 2. Spatial distribution of cabbage plants identified manually different types of mulching.

Table 1. Plants counted and detected in the mulch treatments.

Mulch treatment	Manual counting	Plants counted by:	
		Platform A	Platform B
Black plastic	163	159	158
White plastic	136	132	154
Straw	162	159	157
Bare soil	106	104	99
Total	567	554	568

Table 2. Reliability indicators obtained in the experiment.

Platform	Mulch treatment	Ps (%)	Es (%)	MAE (%)	RMSE
A	Black plastic	97.6	−2.4	2.4	1.291
	White plastic	97.0	−3.0	3.0	1.095
	Straw	98.3	−1.7	3.1	1.080
	Bare soil	98.0	−2.0	2.0	1.000
B	Black plastic	96.8	−3.2	3.2	1.472
	White plastic	87.0	13.0	13.0	4.099
	Straw	96.8	−3.2	4.5	1.354
	Bare soil	93.3	−6.7	6.7	1.936

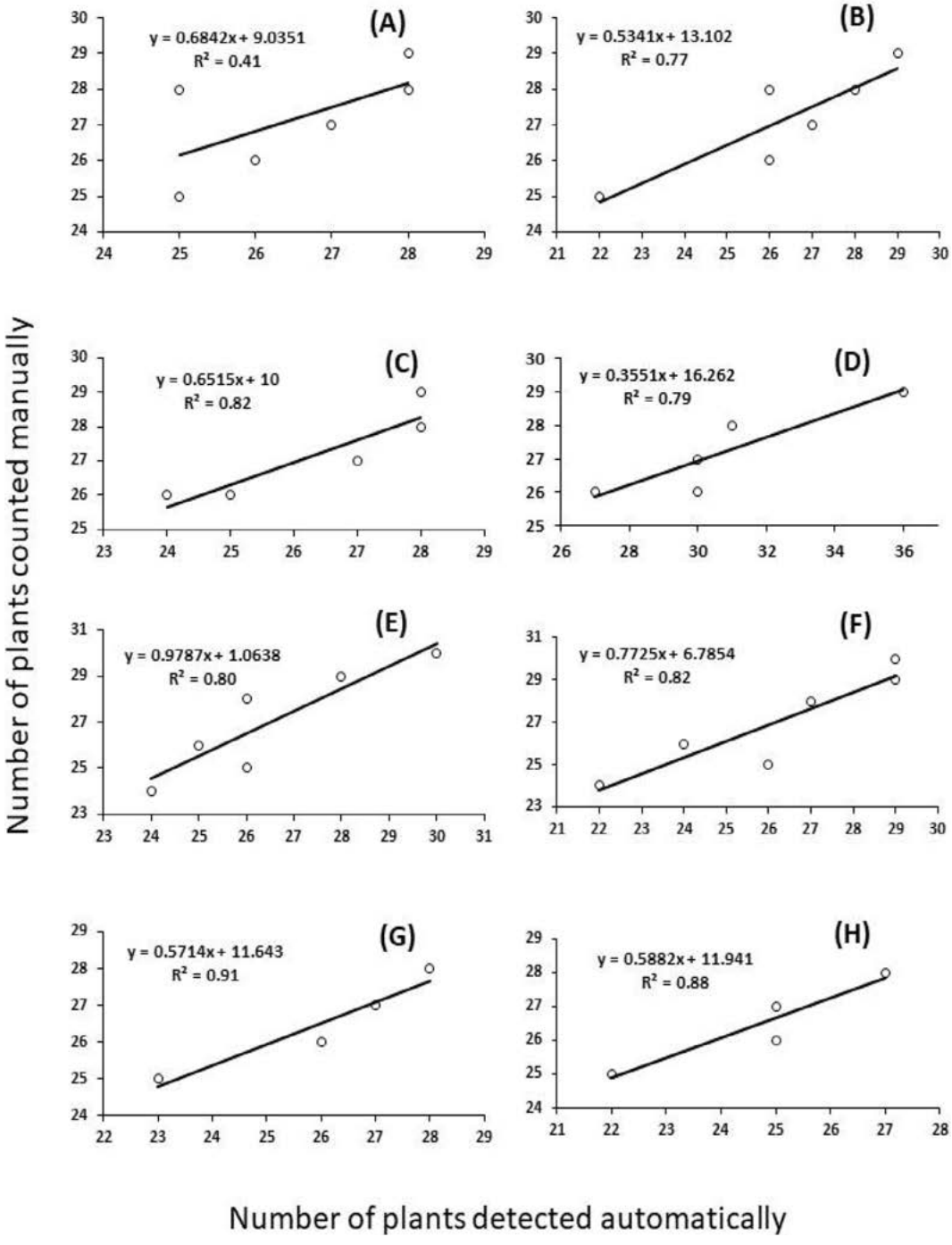

Figure 3. Linear regression for both estimation platforms: platform A in black mulching (A); platform B in black mulching (B); platform A in white mulching (C); platform B in white mulching (D); platform A in straw mulching (E); platform B in straw mulching (F); platform A in bare soil (G); platform B in bare soil (H).

The accuracy values in plant number estimation match those reported by other authors (Neupane *et al.*, 2019; García *et al.*, 2020 and Prado *et al.*, 2020).

Both platforms showed underestimations in the number of cabbage plants detected. The range of underestimation (Es) was from −1.7% to −6.7%. Platform B was the only one that

showed overestimation (Es=13.0%) in white plastic mulching. This overestimation affected the total value of the mean absolute error indicator for platform B (MAE=3.2 to 13%). The RMSE ranged from 1 to 4.09. According to Du *et al.* (2024), the detection of elements in plastic covers is affected by sample sizes, the presence of shadows, and surrounding vegetation; increasing the number of repetitions, with different sample sizes, will result in greater estimation accuracy.

CONCLUSIONS

The counting and location of plants has been carried out in different studies. These will focus on the development of tools for quantification, and on the reliability of different cameras and flight parameters (Paz, and Medrano, 2016; Chu *et al.*, 2019; Jiang *et al.*, 2019; Koh *et al.*, 2019; Jang *et al.*, 2020; Shirzadifar *et al.*, 2020; Valente *et al.*, 2020 and Villareal *et al.*, 2020). In the current research, aerial photographs taken by RPAS proved to be a reliable resource for quantifying transplanted cabbage plants under different soil covers.

The two AI platforms used for plant detection and quantification showed varying degrees of reliability, with platform A exhibiting the lowest degree of error in estimations.

The cover soil material or the absence of mulching, influenced in the reliability of the plant quantification, where the white plastic cover showed lower degrees of reliability for the estimation of plants.

The results obtained from RPAS images and processed by AI platforms should be verified by humans; this is because the estimations made are still not entirely accurate.

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"Conceptualization, S.G.S.U. y N.R.; methodology, S.G.; software, S.G.S.U.; validation, Y.J., N.R., P.A.; formal analysis, V.G.R.E.; research, V.G.R.E.; resources, N.R.; data curation, P.A.; writing—original draft preparation, S.G.S.U.; writing—review and editing, N.R.; visualization, V.G.R.E.; supervision, N.R.; project management, S.G.B.Y.

All authors have read and approved the final version of the manuscript."

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Commercial amino acids for yield and its components of common bean Azufrado Reyna in Northern Sinaloa

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ABSTRACT

Objective: To investigate the effect of foliar commercial amino acids on yield and its components of common bean Azufrado Reyna under field conditions.

Design/methodology/approach: Randomized complete block design with four replicates and four treatments (three commercial amino acids and a control). Grain yield, aboveground biomass, number of pods and normal seeds per unit surface, number of grains per pod, number of normal seeds per pod, weight of 100 seeds, and individual weight of seed were evaluated.

Results: All biostimulants influenced the increase in grain yield, aboveground biomass, number of normal pods and seeds per unit surface as well as the number of seeds per pod with respect to the control.

Limitations on study/implications: The study only assessed the evaluation of agronomic variables; therefore, it is necessary the measurement of morpho-physiological traits that provide evidence about the benefits of foliar aminoacids on yield and the formation of each component under field conditions.

Findings/conclusions: Foliar biostimulant application had a positive effect on grain yield and some of its components as compared to the control. The number of normal seeds per normal pod was the variable that showed the highest correlation with grain yield.

Keywords: amino acids, yield, aboveground biomass.

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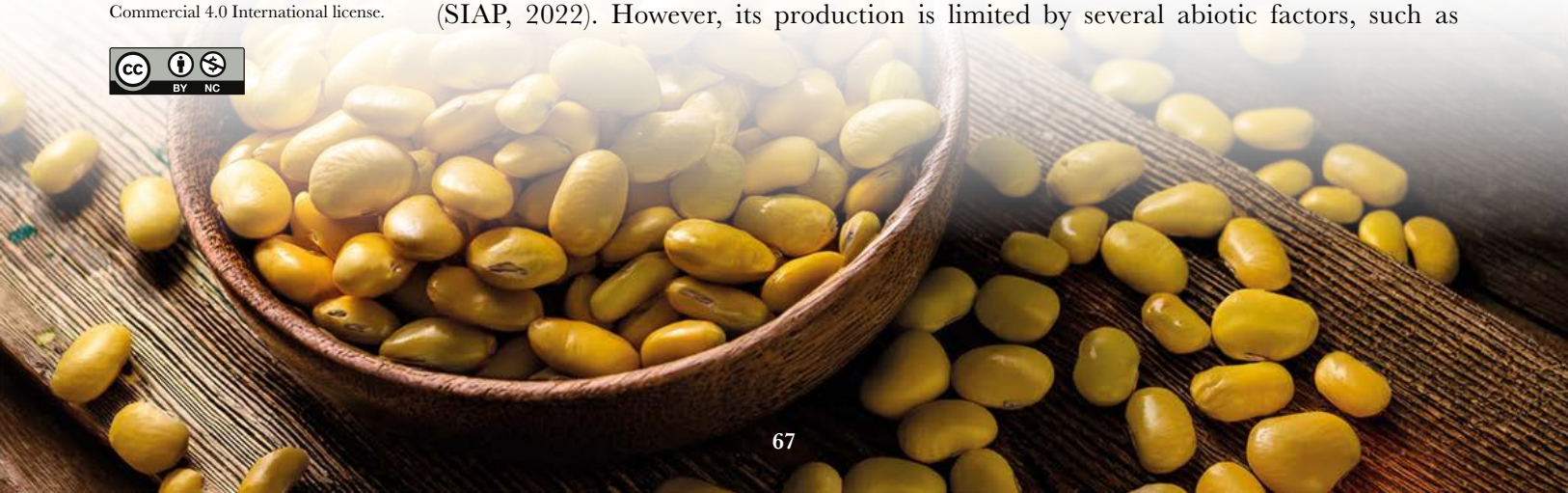
INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is a significant crop for human consumption because it is a good source of minerals, proteins and fiber (Siwakoti *et al.*, 2023). The state of Sinaloa ranked fourth in planting and harvesting surface (85,924.98 ha), with a production of 165,474.96 tons and an average yield of 1.93 t ha^{-1} by 2020 (SIAP, 2022). However, its production is limited by several abiotic factors, such as



drought, salinity, cold, freezing, and high temperatures that affect crop growth and development (El-Nasharty *et al.*, 2021). Thus, agronomic management practices based on biostimulant application represent an ecological tool to prevent or ameliorate stress (Kocira *et al.*, 2020). Amino acids (animal or plant source) have benefits on several physiological processes such as photosynthetic capacity, availability and translocation of nutrients, and quality parameters (Malécange *et al.*, 2023). Besides, physiological processes in plants are regulated by short-chain free amino acids (Rai, 2002). They are basic components of protein synthesis, promote active functions in plant development, participate in their response to environmental stress, and act as precursors of primary and secondary metabolites (Trovato *et al.*, 2021). Furthermore, amino acid application at the beginning of flowering enhances the yield of common bean and improves the nutrient use efficiency (Moreira and Moraes, 2017). Several studies show that amino acid application in crops has positive effects on growth parameters (Ismail and Fayed, 2020; Zaky *et al.*, 2021; El-Hay *et al.*, 2022) and yield (Zewail, 2014; Teixeira *et al.*, 2018; Baqir *et al.*, 2019). Based on this background, the objective of this study was to evaluate the effect of commercial amino acids on the yield and components of common bean Azufrado Reyna under field conditions.

MATERIALS AND METHODS

Location of the experiment

A field experiment (season 2023-2024) was conducted at the experimental site within Facultad de Agricultura del Valle del Fuerte, Universidad Autonoma de Sinaloa (25° 45' 20.88" N, 108° 50' 22.16" W and 14 m above sea level). Soil tillage techniques were those employed by growers of the region. A composite surface 30 cm soil sample was collected before fertilization for fertility evaluation. The soil was classified as clay loam (50% clay, 30% silt and 20% sand), low organic matter content (0.48%), bulk density of 1.15 g cm^{-3}, pH (6.3), E.C (1.38 dS m^{-1}), NO_3^{-1} (365 ppm), PO_4^{-1} (12 ppm), soluble K (28 ppm), exchangeable K (387 ppm), exchangeable Ca (1900 ppm), exchangeable Mg (540 ppm), Na (184 ppm), Fe (8.60 ppm), Mn (12.32 ppm), Cu (1.60 ppm) and Zn (1.23 ppm). The planting was done on moistened soil (November 3rd, 2023), and approximately 12 seeds m^{-1} of variety Azufrado Reyna were planted. These plants have determinate growth habit, can reach up to 40 cm in height, and are well adapted in northern and center Sinaloa and south Sonora. Their seed testa color is yellow, round-oval shape, and resistant to oxidation. The crop begins its flowering at approximately 52 days after planting and has good yield levels in irrigated areas, especially in northern Sinaloa (Iramfra, 2021). The fertilization rate was 500 kg ha^{-1} of Nitrofoska®Triple16. Irrigations were applied at the beginning of flowering (38 dap) and pod filling (67 dap). Besides, insecticide application (Imidacloprid®) was performed to control whitefly (*Bemisia tabaci*) and aphids at a rate of 2.5 L ha^{-1}, and weeds were manually controlled throughout the season.

The experiment consisted of four treatments (three commercial amino acids and the control): T1 without amino acid; T2 Aminocel 500® (1 kg ha^{-1}), animal derived

product that contains 50% free amino acids, 10% N, 8% P_2O_5, 10% K, 0.1% Fe, 0.003% Zn, 0.02 Mn, 0.01% Mg, 0.005% Mo and 0.01% B; T3 Amino 80® (1 kg ha^{-1}), plant derived product that contains 80% free amino acids, 12% N and <4% ammonium; T4) Fito-maat® (300 g ha^{-1}) plant derived product that contains 8% glycine betaine+1% proline, 9% N and 50% C.

All biostimulants were in powder form and were foliar applied at 35, 49 and 74 days after planting using a 20 L sprayer pump. The treatments were arranged in a randomized complete block design with four replicates. The experimental unit consisted of seven rows (0.8 m between rows and 5 m long) with a density of 12 plants m^{-1}. The useful plot consisted of two rows, and plants were taken from an area of 2.4 m^2.

Response variables

Seed yield (SY, kg ha^{-1}) was determined by taking the seed weight ratio to the harvested area (2.4 m^{-2}). Aboveground biomass at physiological maturity (DB, kg ha^{-1}) was determined by weighing the plants from each useful plot. Normal pods (NP m^2) were estimated by counting the total number of pods in each useful plot. A normal pod was considered to have at least one seed with the size and color of Azufrado Reyna Variety.

Seeds per pod (SP) was determined by counting the average number of seeds in 40 randomly selected pods from the sample used to determine seed yield.

Normal seeds (NS m^{-2}) were estimated by taking the ratio of total pods and the harvested area (2.4 m^{-2}). The number of normal seeds per normal pod (NSNP) was determined by taking the ratio of seeds per pod and total number of pods. Weight of 100 seeds (W100S, g) 100 seeds were randomly selected and weighted on a digital scale (Ohaus®). Individual seed weight (ISW, mg) was estimated by taking the ratio between the weight of 100 seeds and 100.

Statistical analysis

All data was subject to normality test (Shapiro and Wilk, 1965) and to an appropriate analysis of variance. Pearson correlation models were fitted and tested on significance levels ($p<0.0.05$) (Infostat®, 2020).

RESULTS AND DISCUSSION

Seed yield and its components were statistically ($p\leq0.05$) affected by the applied biostimulants, except for the weight of 100 seeds and individual seed weight that were similar. The application of Fito-maat® showed higher seed yield (19%). However, it was similar to treatments with Aminocel500® (15%) and Amino80® (3%), and with respect to the control (Table 1). The biostimulant Fito-maat® contains glycine-betaine, an amino acid that has been found to promote higher seed yield even in drought conditions, since it influences the increase of yield components, membrane stability, photosynthetic performance, and the antioxidant system (Ahmed *et al.*, 2019).

Aboveground biomass and number of normal pods were similar in all treatments but higher than the control. Accordingly, dry biomass was 21, 21 and 16% higher in

Table 1. Yield and its components of common bean Azufrado Reyna.

Treatment	SY kg ha^{-1}	DB kg ha^{-1}	NP m^2	SP	NS m^2	NSNP	W100S g	ISW mg
Fito-maat®	4342.3 a	6447.5 a	395 a	4.11 a	927 a	2188	46.7	47
Aminocel500®	4161.7 ab	6449.7 a	334 a	4.17 a	958 a	2264	44.7	45
Amino80®	3645.7 ab	6075.7 a	334 a	4.04 ab	834 ab	2001	48.0	48
Control	3535.0 b	5076.3 b	228 b	3.76 b	701 b	1954	45.3	45
Tukey ($p \leq 0.05$)	762	538	81	0.33	171	469	4.80	0.04

SY=Seed yield; DB=aboveground biomass; NP=normal pods; SP=Seeds per pod; NS=normal seeds; NSNP=number of normal seeds per normal pod; W100S=weight of 100 seeds; ISW=Individual seed weight.

treatments with Fito-maat®, Aminocel500® and Amino80® with respect to the control; while the number of normal pods was approximately 42, 32 and 32% higher for the same treatments respectively. Previous studies showed a similar trend when applying foliar amino acids as compared to the control. In that aspect, EL-Hay *et al.* (2022) observed higher seed yield, aboveground biomass and pods as increasing the amino acid concentration in common bean cv. Poulista. Ismail and Fayed (2020) also observed higher seed yield, dry biomass and normal pods (m^2) in the same crop. The number of seeds per pod and normal seeds was higher on treatments with Aminocel500® (10%) and Fito-maat® (9%), followed by Amino80®. In a similar manner, Espinoza-Galaviz *et al.* (2023) found an increase in the number of seeds per pod (11%) when applying amino acids to common bean Azufrado Higuera. The number of normal seeds per pod, as well as the seed weight, were not statistically different for all treatments. These results coincide with those found by Gonçalves *et al.* (2012) who evaluated different treatments based on amino acids and nutrients on common bean cultivars. Finally, Bianchi *et al.* (2020) stated that an increase in the number of seeds per pod in soybean plants (*Glycine max*) enhanced seed yield regardless of the number of pods and plant density.

Relationship between seed yield and its components

The correlation analysis showed a positive relationship but not statistically different between yield and aboveground biomass (Figure 1A), yield and number of seeds per pod (Figure 1B), as well as yield and number of normal seeds (Figure 1C). There was only a positive and significant relationship between yield and normal seeds/normal pod (NSNP) [$SY = 2.44\ (NSNP) - 1198.8$, $r = 0.92$, $p = 0.02$] (Figure 1 D).

Previous studies have shown a positive and significant relationship between yield and the number of seeds per pod in peas (*Pisum sativum*) cultivars (Naeem *et al.*, 2020; Kaur *et al.*, 2023). Other works by Bianchi *et al.* (2020) mention that the number of seeds per pod is a relatively stable component of yield, while the number of pods is strongly influenced by environmental and management factors.

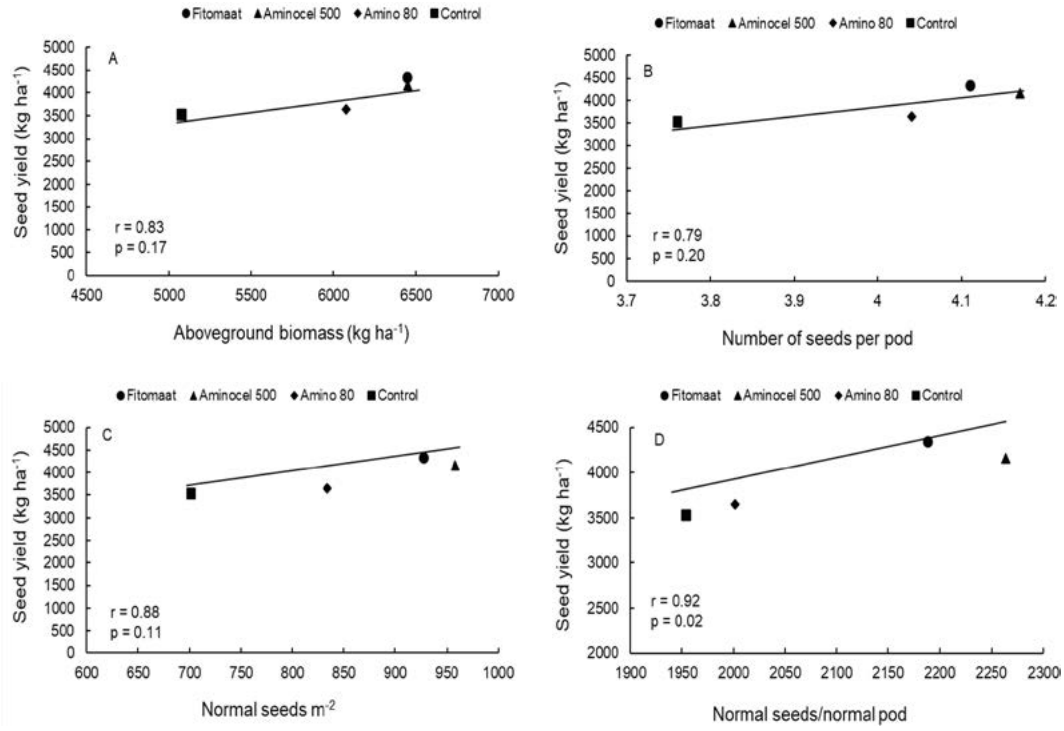


Figure 1. Correlation analysis A) SY-DM); B) SY-SP; C) SY-NS; D) SY-NSNP.

CONCLUSIONS

Foliar amino acid application showed a positive effect on yield, biomass, number of normal pods, and number of seeds per pod on the common bean variety Azufrado Reyna.

The number of seeds per normal pod was the component with the highest relationship with yield.

It is suggested the application of amino acids in specific stages (pre-bloom, flowering and beginning of pod filling) to enhance the overall growth and yield potential of common bean.

The biostimulant Fito-maat® performed better in crop characteristics in the rates recommended by the manufacturer.

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Relationship between Vegetation Indices and Pinoxaden Toxicity in Two Populations of *Avena fatua* L.

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ABSTRACT

Objective: To study the relationship between vegetation indices and the toxicity of the herbicide pinoxaden on two populations of *Avena fatua* L.

Design/methodology/approach: A completely randomized design with a 2×4 factorial arrangement was used in the laboratory, with factor A being the two populations of *Avena fatua* and factor B being the four concentrations of the pinoxaden herbicide (0, 30, 60 and 120 g a.i. ha^{-1}). The percentage of control, plant height and the GA and GGA vegetation indices were evaluated. The data were analyzed with an ANOVA and a comparison of means was performed with the Tukey test (α=0.05). The relationship between the control percentage and vegetation indices was determined by Pearson correlation analysis.

Results: There was a higher percentage of control, plant height, GA index and lower GGA index in the *Avena fatua* population from alfalfa compared to the wheat population, indicating that pinoxaden has greater phytotoxicity for the alfalfa population. A negative correlation was observed between the control percentage and the GA index for the two populations regardless of the evaluation time, a similar negative correlation was found for the GGA index in both populations. This indicates that the GA and GGA indices decrease as the control percentage increases.

Findings/conclusions: The GA and GGA indices were inversely correlated with the control percentage of the herbicide pinoxaden. The GA and GGA indices obtained through digital camera images are feasible to estimate the toxicity levels of the herbicide pinoxaden.

Keywords: Pinoxaden, vegetation index, wild oats, digital image.

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INTRODUCTION

Wild oats (*Avena fatua* L.) are considered one of the major phytosanitary problems in wheat cultivation worldwide (Tidemann, 2021). This weed reduces wheat yield due to its high competition and, in extreme cases, causes total crop loss (Jäck *et al.*, 2017). Management of *Avena fatua* in this crop is primarily conducted using ACCase-inhibiting herbicides (Scursoni *et al.*, 2011; Sasanfar *et al.*, 2017).

Therefore, it is crucial to study the susceptibility of *Avena fatua* to ACCase inhibitors. These studies are conducted both in the field and in the laboratory, typically using the visual scale of the European Weed Research Society (Qasem, 2007; Scursoni *et al.*, 2011). However, this method is unreliable, as visual observation depends on the observer's perception, which makes the results subjective. For this reason, it is necessary to have more reliable, highly sensitive, and consistent methodologies to differentiate between plants treated with herbicides.

Recent studies involve the use of multispectral imaging to measure herbicide toxicity (Yao *et al.*, 2012; Huang *et al.*, 2016), but these measurements are performed using specialized equipment (spectroradiometers) that are generally not accessible to producers. On the other hand, the use of conventional digital cameras can be a useful tool in detecting herbicide toxicity. Digital cameras allow for high-resolution images, and through these images, vegetation indices can be determined based on the saturation, intensity, and tonality of the image (Casadesus and Villegas, 2014).

Vegetation indices allow for the quantification of plant characteristics such as leaf area, leaf senescence, grass cover, and turf quality (Li *et al.*, 2005; Lati *et al.*, 2011). The use of digital cameras could represent an additional alternative for herbicide toxicity studies by determining vegetation indices, such as Green Area (GA) and More Green Area (GGA) using Breedpix® 0.1 software. This would estimate weed control based on the increase or decrease in vegetation (Velasco-López *et al.*, 2020). Therefore, this study aimed to investigate the relationship between vegetation indices and the toxicity of the herbicide pinoxaden on two populations of *Avena fatua* L.

MATERIALS AND METHODS

Biological Material

Two populations of *Avena fatua* were collected: one from alfalfa crops and the other from wheat crops, in the Mexicali Valley, Baja California. Seeds from both populations were germinated on filter paper moistened with 10 mL of distilled water in Petri dishes. They were kept at 4 °C for 48 hours. Subsequently, they were incubated at room temperature (23±2 °C) in darkness for 24 hours. Finally, they were placed in a growth chamber at a temperature of 21.1 °C, with a photoperiod of 16 hours light and 8 hours darkness.

Seven *Avena fatua* plants with 1 cm of hypocotyl were transplanted into 14 cm diameter×10.57 cm depth (1.2 L volume) plastic pots. A substrate composed of a mixture of organic matter and loamy soil in a 5:3 v/v ratio was used. The plants were fertilized from the first leaf stage to the 3-4 leaf stage with commercial Poly Feed (19-19-19+M.E) fertilizer at a dose of 10 g L^{-1} of water.

Experimental Design

The experiment involved evaluating three concentrations (30, 60, and 120 g a.i. ha^{-1}) of the herbicide pinoxaden and a control (water only) on the two populations of *Avena fatua*. The experiment was conducted under controlled laboratory conditions (temperature 23±2 °C and a 16:8 h light photoperiod), using a completely randomized

design with a 2×4 factorial arrangement. Factor A corresponds to the two populations of *Avena fatua*, while Factor B represents the three herbicide concentrations and the control. Three replications were included, each represented by a pot containing seven *Avena fatua* plants.

The 3-4 true leaf stage was considered the criterion for applying the herbicide treatments. The herbicide solution was prepared with distilled water, and 3 mL L^{-1} of the adjuvant Inex A (Fatty Alcohol Ethoxylate and Polydimethylsiloxane) was added. The herbicide solutions were applied with a hand sprayer fitted with a flat fan nozzle (Teejet 8002), adapted and connected to an air compressor, calibrated at a pressure of 35 PSI and a water flow rate of 400 L ha^{-1}.

Evaluations of the treatments on both *Avena fatua* populations were conducted at 6, 12, and 18 days after application (DAA). Herbicide toxicity was assessed by estimating the percentage of visual control for each experimental unit using the scale proposed by the European Weed Research Society (EWRS) (Ciba-Geigy ag, 1992). Plant height was measured using a graduated ruler in centimeters (Burril *et al.*, 1977). Digital images were captured with a 12.1-megapixel digital camera (SONY Cyber-shot DSCW200) and processed with Breedpix® 0.1 software to estimate the GA and GGA vegetation indices.

Statistical Analysis

The data from the toxicity experiment, plant height, and vegetation indices were analyzed using ANOVA with a completely randomized design and a 2×4 factorial arrangement using PROC GLM. Least squares means were compared with Tukey's test at a significance level of 0.05 (SAS, 2001). The relationship between toxicity and vegetation indices was analyzed using Pearson correlation with PROC CORR (SAS, 2001).

RESULTS AND DISCUSSION

The analysis of variance indicated that the effects of the factors population of *Avena fatua*, pinoxaden concentration, and their interactions were significant for percentage of control and plant height in both populations of *Avena fatua* at 6 DAA ($F=102.46$; $df=3$; $p=0.0001$; $F=0.001$; $df=3$; $p=0.0001$, respectively) and 12 DAA ($F=35.34$; $df=3$; $p=0.0001$; $F=8.54$; $df=3$; $p=0.0013$, respectively). However, for the evaluation at 18 DAA, the interaction of these factors was not significant ($F=3.03$; $df=3$; $p=0.0597$; $F=2.88$; $df=3$; $p=0.0683$, respectively). For the variables GA and GGA, the analysis of variance indicated that the effect of the interaction between *Avena fatua* population and herbicide concentration was not significant at 6 DAA ($F=0.52$; $df=3$; $p=0.6811$; $F=0.81$; $df=3$; $p=0.0510$, respectively), 12 DAA ($F=2.26$; $df=3$; $p=0.1204$; $F=2.44$; $df=3$; $p=0.1022$, respectively), and 18 DAA ($F=2.44$; $df=3$; $p=0.1034$; $F=1.51$; $df=3$; $p=0.2483$, respectively). However, the main effects of the factors were significant for the GA and GGA indices in both *Avena fatua* populations.

In general, the concentrations of 30, 60, and 120 g a.i. ha^{-1} of pinoxaden applied to the alfalfa population exhibited a higher percentage of control compared to when applied to the wheat population (Table 1) at 6 DAA ($F=732.30$; $df=1$; $p=0.0001$), 12 DAA ($F=63.18$; $df=1$; $p=0.0001$), and 18 DAA ($F=6.20$; $df=1$; $p=0.0242$).

The wheat population showed a higher percentage of control as the concentration of pinoxaden increased (Table 1) at 6 DAA ($F=599.24$; $df=3$; $p=0.0001$), 12 DAA ($F=352.39$; $df=3$; $p=0.0001$), and 18 DAA ($F=79.94$; $df=3$; $p=0.0001$). In contrast, in the alfalfa population, the three herbicide concentrations resulted in similar percentages of control at 12 and 18 DAA (Table 1).

The results of this study suggest that there is a difference in susceptibility between the two *Avena fatua* populations, indicating that pinoxaden has greater phytotoxicity for the alfalfa population compared to the wheat population (Table 1). The alfalfa population achieved more than 80% control at 6 DAA regardless of the concentration evaluated. At 12 and 18 DAA, it reached more than 97% and 100% control, respectively, at all three tested concentrations, suggesting that pinoxaden has a rapid control action on this *Avena fatua* population. These results are consistent with those reported by Scursoni *et al.* (2021) when evaluating pinoxaden on *Avena fatua*.

On the other hand, the wheat population exhibited less than 60% control with all three evaluated concentrations at 6 DAA. Although the percentage of control increased at 12 and 18 DAA, only the 120 g a.i. ha^{-1} concentration of pinoxaden achieved 100% control at 18 DAA (Table 3). This indicates that this population shows greater tolerance to this herbicide, likely due to pinoxaden (an ACCase inhibitor) being one of the most commonly used herbicides in the Mexicali Valley for controlling this weed in wheat cultivation (Tafoya-Razo *et al.*, 2017).

As a result, the *Avena fatua* population from wheat may have experienced greater selection pressure for this herbicide, thereby decreasing its susceptibility to pinoxaden (Cruz-Hipolito *et al.*, 2011). In contrast, in alfalfa cultivation, weed control is achieved

Table 1. Effect of three concentrations of pinoxaden on the percentage of control in two populations of *Avena fatua* L. during three evaluation periods after treatment application.

Evaluation Period	Treament (g a.i. ha^{-1})	Control (%)	
		Alfalfa	Wheat
6 DAA	Control	0.000fCx	0.000fADx
	30	83.093bBx	18.333eCy
	60	88.807aAx	43.333dBy
	120	90.000aAx	55.000cAy
12 DAA	Control	0.000dBx	0.000dDx
	30	97.380aAx	36.667cCy
	60	97.857aAx	85.000bBy
	120	99.333aAx	97.000aAx
18 DAA	Control	0.00Bx	0.000Cx
	30	100.00Ax	61.67By
	60	100.00Ax	86.67Ax
	120	100.00Ax	100.00Ax

Letters A-D indicate the comparison between concentrations for a population and an evaluation time as columns. Letters a-d represent the interaction of populations and concentrations for an evaluation time. Letters x-y show the comparison between populations for a concentration and evaluation time in a linear manner.

using various herbicides such as carfentrazone, diuron, flumioxazin, hexazinone, imazethapyr, metribuzin, pendimethalin, paraquat, and saflufenacil (Adjesiwor and Prather, 2022). This prevents selection pressure for a specific herbicide group, contributing to the high susceptibility of the *Avena fatua* population from alfalfa cultivation to pinoxaden.

All three concentrations of pinoxaden resulted in shorter plant height compared to the control (no application) in both populations of *Avena fatua* (Table 2) at 6 (F=51.01; df=3; p=0.0001), 12 (F=72.23; df=3; p=0.0001), and 18 days after application (DDA) (F=81.72; df=3; p=0.0001). The alfalfa population exhibited shorter plant height compared to the wheat population at 6 DDA (F=114.01; df=1; p=0.0001), 12 (F=63.51; df=1; p=0.0001), and 18 DDA (F=24.70; df=1; p=0.0001), regardless of the pinoxaden concentration (Table 2).

At 6 days after application (DDA), the concentrations of 30, 60, and 120 g a.i. ha^{-1} of pinoxaden reduced the height of the alfalfa population by 30.71%, 32.91%, and 40.14%, respectively, compared to the control treatment. For the wheat population, the reduction in height was 45.59%, 54.08%, and 55.21% for the concentrations of 30, 60, and 120 g a.i. ha^{-1} of the herbicide, respectively (Table 2). Similarly, the reduction in plant height in both populations increased at 12 days after application (DDA), with average percentages of 63.80% and 63.39% for the alfalfa and wheat populations, respectively. A similar pattern was observed at 18 DDA, with average percentages of 77% and 61.39% for the alfalfa and wheat populations, respectively. This reduction in plant height in both *Avena fatua* populations may be related to the death of meristematic tissue caused by pinoxaden (Kukorelli *et al.*, 2013).

Table 2. Effect of three concentrations of pinoxaden on plant height in two populations of *Avena fatua* L. during three evaluation periods after treatment application.

Evaluation Period	Treament (g a.i. ha^{-1})	Plant height (cm)	
		Alfalfa	Wheat
6 DAA	Control	17.797bAy	37.193aAx
	30	12.330cBy	20.233bBx
	60	11.940cBy	17.080bBx
	120	10.653cBy	16.657bBx
12 DAA	Control	30.673bAy	55.687aAx
	30	11.760cBy	29.033bBx
	60	10.700cBy	16.783cCx
	120	10.837cBy	15.353cCx
18 DAA	Control	48.01Ax	55.63Ax
	30	11.34By	31.63Bx
	60	10.65By	17.73Cx
	120	9.77By	15.07Cx

Letters A-D indicate the comparison between concentrations for a population and an evaluation period in column format. Letters a-d show the interaction of populations and concentrations for a specific evaluation period. Letters x-y denote the comparison between populations for a concentration and evaluation period in a linear fashion.

The inhibition of lipid synthesis, which constitutes the membranes of these growing cells, leads to cell death (Kaundun, 2014), which reduces the growth of new leaves in the treated plants (Kukorelli *et al.*, 2013), and consequently decreases plant height in both studied populations.

Although there is a significant reduction in plant height due to pinoxaden in both populations, the effect was less pronounced in the wheat population across all three evaluation dates and herbicide concentrations. The plant height of the wheat population was significantly greater than that of the alfalfa population, with the concentration of 30 g a.i. ha^{-1} showing that the height of the wheat population exceeded that of the alfalfa population by 39.06%, 59.49%, and 64.15% at 6, 12, and 18 days after application, respectively. This indicates that this concentration was not sufficient to cause the death of the meristematic tissue. Additionally, it is possible that the wheat population plants, due to their higher tolerance, might metabolize the herbicide molecule and recover from the toxic effects of pinoxaden, leading to greater growth. Another aspect that could explain this result is that the concentration of 30 g a.i. ha^{-1} is half of the recommended concentration for controlling *Avena fatua* in wheat cultivation (60 g a.i. ha^{-1}), which may also have contributed to the lesser reduction in plant height. This aligns with the findings of Scursoni *et al.* (2021), who reported that reducing the field dose of the herbicide fenoxaprop (an ACCase inhibitor) by 50% results in a lesser effect on biomass production in *Avena fatua*, which could translate to greater plant height, as observed in this study.

The concentration of 120 g a.i. ha^{-1} of pinoxaden exhibited lower GA compared to the control, 30, and 60 g a.i. ha^{-1} in both populations of *Avena fatua* (Table 3) at 6 DAA ($F=4.94$; $df=3$; $p=0.0003$). Meanwhile, all three herbicide concentrations showed lower

Table 3. Effect of three concentrations of pinoxaden on the green area (GA index) in two populations of *Avena fatua* L. during three evaluation periods after treatment application.

Evaluation Period	Treament (g a.i. ha^{-1})	GA Index	
		Alfalfa	Wheat
6 DAA	Control	0.1600Ax	0.1867Ax
	30	0.1267Ay	0.1833Ax
	60	0.1233Ay	0.1733ABx
	120	0.1033By	0.1433Bx
12 DAA	Control	0.1567Ax	0.2200Ax
	30	0.0533By	0.2000Ax
	60	0.0300By	0.1100Bx
	120	0.0267By	0.0667Bx
18 DAA	Control	0.1167Ay	0.1967Ax
	30	0.0433By	0.1233Bx
	60	0.0233By	0.0933BCx
	120	0.0133By	0.0333Cx

Letters A-D show the comparison between concentrations for a single population and an evaluation time in column format. Letters x-y show the comparison between populations for a concentration and an evaluation time in a linear manner.

GA compared to the control at 12 (F=17.28; df=3; p=0.0001) and 18 DAA (F=13.20; df=3; p=0.0001) in both populations. Lower GA was observed in the alfalfa population compared to the wheat population (Table 3) for the concentrations of 30, 60, and 120 g a.i. ha^{-1} of pinoxaden at 6 (F=21.67; df=1; p=0.0127), 12 (F=29.38; df=1; p=0.0001), and 18 DAA (F=11.40; df=1; p=0.0039).

For the alfalfa population, the three herbicide concentrations showed lower GGA compared to the control at 12 (F=12.30; df=3; p=0.0002) and 18 DAA (F=9.85; df=3; p=0.0006). At 6 DAA (F=8.29; df=3; p=0.0016), the control and 30 g a.i. ha^{-1} of pinoxaden had higher GGA compared to the 60 and 120 g a.i. ha^{-1} herbicide concentrations (Table 4).

For the wheat population, the 60 and 120 g a.i. ha^{-1} concentrations showed lower GGA values compared to the control and the 30 g a.i. ha^{-1} pinoxaden concentration at 12 (F=12.30; df=3; p=0.0002) and 18 DAA (F=9.85; df=3; p=0.0006). Lower GGA was observed in the alfalfa population compared to the wheat population for all three pinoxaden concentrations (Table 4) at 6 (F=80.00; df=1; p=0.0001), 12 (F=27.04; df=1; p=0.0001), and 18 DAA (F=15.67; df=1; p=0.0011).

Vegetative indices (GA and GGA) have been used to estimate the green biomass of plants (Casadesús *et al.*, 2007), which is related to the growth of healthy plants. Therefore, these indices can differentiate between plants growing under optimal conditions and plants under stress (Fiorani and Schurr, 2013). In this study, the GA and GGA indices differentiated between *Avena fatua* plants affected by pinoxaden concentrations and untreated (control) plants in both *Avena fatua* populations. Both populations showed a gradual decrease in the vegetative index values as the pinoxaden concentration increased (Tables 3 and 4). These results were consistent across the different evaluation times.

Table 4. Effect of three concentrations of pinoxaden on the greenest area (GGA index) in two populations of *Avena fatua* L. during three evaluation periods after treatment application.

Evaluation Period	Treament (g a.i. ha^{-1})	GGA Index	
		Alfalfa	Wheat
6 DAA	Control	0.1033Ay	0.1500Ax
	30	0.0767Ay	0.1433Ax
	60	0.0667By	0.1400Ax
	120	0.0533By	0.1067Bx
12 DAA	Control	0.0967Ay	0.1667Ax
	30	0.0233By	0.1533Ax
	60	0.0133By	0.0733Bx
	120	0.0100By	0.0367Bx
18 DDA	Control	0.0633Ay	0.1303Ax
	30	0.0200ABy	0.0833ABx
	60	0.0100By	0.0633Bx
	120	0.0057By	0.0097Cx

Letters A-D indicate the comparison between concentrations for a population and an evaluation time as columns. Letters x-y indicate the comparison between populations for a concentration and an evaluation time in a linear manner.

Although both populations of *Avena fatua* exhibited lower levels of GA and GGA, the alfalfa population showed lower values for these vegetative indices compared to the wheat population, regardless of concentration and evaluation time (Tables 3 and 4). This fact could be related to the greater susceptibility of this population to the different concentrations of pinoxaden evaluated in this study. Correlation analyses showed a negative correlation between the percentage of control and the GA index for both *Avena fatua* populations, regardless of the evaluation time. Similarly, both populations showed a negative correlation for GGA (Table 5), indicating that the GA and GGA indices decrease as the percentage of control increases.

Table 5. Pearson correlation between visual control percentage and digital tools in two populations of *Avena fatua* L. from the Valley of Mexicali.

Population	Index	DAA		
		6	12	18
		% Control		
Alfalfa	GA	−0.72141 0.0081	−0.92768 <.0001	−0.86976 0.0003
	GGA	−0.80110 0.0017	−0.95329 <.0001	−0.86555 0.0003
Wheat	GA	−0.57351 0.0512	−0.81531 0.0012	−0.64865 0.0223
	GGA	−0.61926 0.0318	−0.79578 0.0020	−0.59833 0.0399

The alfalfa population showed a stronger correlation compared to the wheat population for the GA index, with correlation coefficients of −0.72141, −0.92768, and −0.86976 for 6, 12, and 18 DAA, respectively. Similarly, for the alfalfa population, the GGA index was strongly correlated with the control percentage, with correlation coefficients of −0.80110, −0.95329, and −0.86555 for 6, 12, and 18 DAA, respectively.

These results differ from those found by Huang *et al.* (2016), who reported a low correlation between vegetative indices and dicamba toxicity in soybean crops, concluding that vegetative indices are not suitable for estimating the sensitivity to the herbicide dicamba. In contrast, in this study, the high correlation between control percentage and the GA and GGA indices suggests that they are suitable for estimating the sensitivity of *Avena fatua* to the herbicide pinoxaden under laboratory conditions. Additionally, they suggest that these vegetative indices represent a promising tool for assessing herbicide susceptibility in laboratory experiments, providing complementary information or even replacing the visual evaluation of herbicide toxicity. This type of visual assessment is subjective and depends on the operator conducting it, which can result in imprecise outcomes. Therefore, it is crucial to have tools that can determine herbicide toxicity in an objective, repeatable, and reliable manner, such as the GA and GGA indices obtained through digital images using conventional digital cameras.

CONCLUSIONS

According to the results of this research, it is concluded that the values of the GA and GGA vegetation indices were inversely correlated with the herbicide pinoxaden's control percentage (toxicity). Additionally, it is concluded that the GA and GGA indices obtained through digital camera images are feasible tools for estimating the toxicity levels of the herbicide pinoxaden.

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Colegio de Postgraduados

Endophytic Mycorrhiza-mediated Pathogen resistance in plants

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ABSTRACT

Objective: Mycorrhizal fungi are a group of microorganisms that live insithusants, thus, maintaining perceptible associations with their host plants in certain parts of their life cycle. They can be characterized by their capacity to synthesize secondary metabolites and to promote growth and induce plant-disease resistance, therefore, gaining greater biotechnological importance in pest and diseases management for crops of agricultural relevance. The study of these microorganisms has been a widely researched area subject for more than half a century.

Design/methodology/approach: Their biology and molecular relationships in plant-microorganism interactions, on the other hand, have only recently begun to gain relevance for understanding the colonization process in recent decades. There has been observed a complexity in the generation of formulations that can guarantee the permanence of fungi outside the host plant.

Findings/conclusions: This review article will address topics related to their biology, ecological role, possible negative effects on commercially important animals, and successful cases in Mexico regarding biotechnological products based on these microorganisms.

Keywords: Plant-endophyte interaction, mycorrhizae, biological control.

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INTRODUCTION

At the end of the 20th century, the Mycology Committee of the American Phytopathological Society (APS) held one of the first discussions on the presence of endophytic fungi in woody plants and grasses. This event marked the beginning of a series of publications aimed at highlighting the phenomenon of endophytism in these species to the scientific community (Backman & Sikora, 2008). In 1996, a book on the ecology and evolution of endophytes was published, mostly focused on clavicipitaceous fungi, which were frequently associated with the reduction in grass consumption due to insects and some mammals (Redlin & Carris, 1996).

Endophytes are microorganisms that reside wholly or partially within the tissues of living plants without causing negative symptoms in their hosts; their internal presence can be demonstrated. Unlike mycorrhizae, endophytic fungi lack external structures such as hyphae or mycelia and can utilize various nutrient sources (Ownley *et al.*, 2010).

The objective of this work was to conduct a literature review on the role of endomycorrhizae in promoting resistance to different phytopathogens when they are in a plant-microorganism association.

MATERIALS AND METHODS

The development of this work was based on a search and review of articles dealing with the associations between mycorrhizal fungi published with the most up-to-date information. The information from studies conducted in Mexico is presented from these articles, providing insights into important concepts of mycorrhizal symbiosis and the mechanisms involved in the interaction between these endomycorrhizae and the host species. The search included experimental and review studies from databases such as SCOPUS, Web of Science, Forest Science, PubMed, and Scielo. The searches were conducted using Spanish keywords: "micorrizas" and "México".

RESULTS AND DISCUSSION

As a result of this review, the information is structured into the following sections: 1) Biology of endomycorrhizae; 2) Endomycorrhizae and their ecological role; 3) Endomycorrhizae and their classification; 4) Mechanisms of action; 5) Adverse effects of some mycorrhizae; 6) Dual effect of endomycorrhizae; 7) Endomycorrhizae in Mexico and success study-cases.

Biology of Endomycorrhizae

Fungi are among the most important and abundant microorganisms on Earth. Their origin dates back more than 400 million years, when interactions between these microorganisms and aquatic plants began the colonization of soils, resulting in our current knowledge. They are present in almost all groups of terrestrial plants (Simon, 1996). The interactions between fungi and plants have changed the Earth's ecology (Berbee *et al.*, 2017). Fungi are characterized by their great diversity, including microscopic fungi, unicellular and macroscopic multicellular fungi (Schoch *et al.*, 2014). Fungi are generally saprophytic, maintaining ecosystem functions and forming a key part of food chains. These qualities make them important for biotechnological applications such as fertilizers, natural pigments, cosmetics, and in the food industry (Muzzarelli *et al.*, 2012).

Currently, there are several types of mycorrhizal fungi, based on the characteristics of their infection and the organisms with which they establish mutualistic relationships. However, for practical purposes, they are distinguished as ectomycorrhiza, endomycorrhiza, and a third intermediate group between these two former groups (Franco-Navarro, 2002).

The term endophyte refers to fungal internal association with plant tissues without causing harmful damage (Petrini, 1991). Endophytic fungi penetrate the cells of the root cortex. They are a group of fungi often grouped according to their physiological condition,

method of infection, secondary metabolism, stages of development and evolution, colonization patterns, and the taxonomic relationship of the symbiosis. This symbiosis is continuous and balanced between the fungus and the host, ranging from mutualism, parasitism, and commensalism (Aly *et al.*, 2011). Their interactions involve a balance of antagonisms independent of the plant organs they infect and encompass almost all taxonomic classifications of plants, especially those of agricultural interest, such as wheat, salvia, thyme, among others (Schulz & Boyle, 2005).

Endomycorrhizae and Their Ecological Role

Endomycorrhizae are involved in the mechanisms of acquired resistance to different types of stress. Stress is defined as external factors that negatively influence the plant and can be biotic, such as pathogens and heavy metals, or abiotic, such as water, salt, thermal, light excess, anoxia, and oxidative stress (Rodriguez & Redman, 2008). Additionally, mycorrhizae improve nutrient uptake, produce phytohormones, and participate in the synthesis of compounds with biological activity, such as antibiotics and secondary metabolites like antioxidants, anticancer, antidiabetic, and immunosuppressive compounds (Aly *et al.*, 2011; Chamkhi *et al.*, 2018; Tanabe *et al.*, 2004). Therefore, it is said that endomycorrhizae have profound effects on the environments where they are found, in addition to evolving alongside the plant species they colonize.

Mycorrhizae have the ability to absorb carbohydrates from the plant roots, and in this absorption process, they exchange minerals with the plant's vascular system. In the absorption of phosphorus, these microorganisms play a decisive role. It has been shown that inoculation with fungal isolates provides protection to plants in soils with lead and zinc toxicity due to phosphorus solubilization (Díaz *et al.*, 2016).

Endomycorrhizae and Their Classification

The current classification of endomycorrhizae is divided into Clavicipitaceous, which are mutualistic organisms that, together with their host, promote defense against herbivorous insect attacks and also colonize grasses (Clay & Schardl, 2002), and non-Clavicipitaceous, which are characterized by colonizing non-vascular plants such as conifers, ferns, and angiosperms (Rodriguez & Redman, 2008).

Class I Clavicipitaceous are related to a few phylogenetically related species with host selectivity (Rodriguez & Redman, 2008). These promote the development of plant biomass, confer resistance to water stress, and produce insecticidal compounds for herbivorous animals. They are divided into three types:

- Type I: The life cycle involves symptomatic and pathogenic species. Their propagation is through ascospores, they are heterothallic and require the transfer of spermatia for successful reproduction.
- Type II: The reproduction of fungi in this classification in already colonized plants involves the formation of fungal fruiting bodies and plant inflorescences as a mechanism of mixed interaction. They are the so-called pleiotropic symbionts transmitted vertically by seeds and horizontally by sporulation.

- Type III: They remain within the plant tissue throughout their life, including the flowering of the host. They have an asymptomatic life cycle and do not produce sexual spores, so there is no genetic recombination for these fungi (Clay & Schardl, 2002).

Non-Clavicipitaceous endomycorrhizae include:

- Class II: (Ascomycota and Basidiomycota) which enhance the morphogenesis of their host, root biomass, synthesis of hormones, growth-regulating enzymes, and provide protection to their host (Campanile *et al.*, 2007).
- Class III: Which grow in fruiting bodies above ground with horizontal transmission in plant organs such as flowers, fruits, and stems (Tejesvi *et al.*, 2007).
- Type IV: Distinguished as fungi with dark melanized septa, mainly ascomycetes that produce conidia forming intercellular and intracellular hyphae in plant roots.

Mechanisms of Action

Salicylic Acid-Mediated Signaling

Secondary plant metabolites serve to prevent or mitigate different sources of stress in plants (Glazebrook, 2005). In this section, we analyze those produced by known attackers, such as secondary metabolites produced by biotrophic agents, which are known for salicylic acid signaling. Necrotrophs, on the other hand, induce programmed cell death (Thomma *et al.*, 1998).

Salicylic acid acts as a "signaling molecule" that triggers the initiation of the acquired resistance mechanism in healthy tissues, in addition to the genetic expression of coding messengers related to pathogenesis (Pieterse *et al.*, 2009). Various experiments in mutant and transgenic plants in these genes are unable to develop the acquired resistance mechanism, as the activation of PR genes is not observed when an infection by a pathogenic organism is present (Durrant & Dong, 2004). This allows us to conclude the fundamental role of salicylic acid as an intermediary in the signaling pathway, with the NPR1 protein being an important transducer of the salicylic acid signaling pathway (Dong, 2004).

Jasmonic Acid and Ethylene-Mediated Signaling

The mechanism of action of salicylic acid represents the interaction of plants with pathogenic organisms (Pieterse *et al.*, 2009). In beneficial interactions, the recognition patterns present different molecular patterns, activating the immune system response at a systemic level. In this response, there is a signal that travels long distances through the plant's vascular system, activating the immune system. This is commonly regulated by jasmonic acid and ethylene-dependent pathways and does not activate the PR genes of systemic acquired resistance. The activation of this signaling is mediated by beneficial soil microorganisms, rhizobacteria, and mycorrhizae, which promote plant growth (Pozo & Azcón-Aguilar, 2007; van Loon *et al.*, 1998).

Tolerance to biotic stress

The main characteristic of these beneficial endophytic organisms is the production of secondary metabolites, such as antibiotics with antifungal, antibacterial, and insecticidal properties (Gunatilaka, 2006), which inhibit the development of phytopathogens. These properties promote the search and prospecting of endophytic organisms, derived from their activity as phytopathogen controllers and their contribution to biomass production.

Some endophytic fungi, such as *Piriformospora indica*, isolated from sandy soil in the Indian desert from the shrubs *Prosopis juliflora* and *Ziziphus nummularia* (Verma *et al.*, 1998), induce resistance to saline stress in barley (*Hordeum vulgare*) plants and resistance to pathogens like *Fusarium culmorum* and *Cochliobolus sativas* (Kumar *et al.*, 2002). In addition to these examples of resistance to these types of stress, it also stimulated biomass production. At the root level, the roots showed greater antioxidant capacity, increasing the concentrations of ascorbic acid and dehydroascorbate reductase, reducing cell death (Waller *et al.*, 2005).

In another study, a non-pathogenic strain Fo47 of *Fusarium oxysporum* reduced the symptoms of the strain *Fusarium oxysporum* f. sp. *radicis lycopersici* in tomato cultivation (Bolwerk *et al.*, 2005). These symptoms were root and stem collar production. For this, a concentration 50 times higher of biocontrol spores is needed. The control mediated by this strain is due to the occupation and reduction of fixation sites, resulting in fewer symptomatic lesions. The interaction of the strain used as biocontrol had a direct effect on the acquired resistance mechanism, as it increased the levels of PR-1, β-1-3-glucanase, and β-1-4-glucanase (Duijff *et al.*, 1998; Fuchs *et al.*, 1997), indicating that this beneficial fungus acts similarly to the acquired resistance system, *i.e.*, it conserves the patterns that, like pathogenic organisms, trigger the plant's defense systems with a positive symbiosis.

The most studied entomopathogenic fungi are *Lecanicillium* spp., *Metarhizium* spp., *Hirsutella* spp., *Isaria* spp., *Nomuraea* spp., *Sporothrix* spp., *Aschersonia* spp., *Paecilomyces* spp., *Tolypocladium* spp., and *B. bassiana*. These fungi have been shown to produce secondary metabolites with antagonistic activities against mammals, microorganisms, insects, and even plant cells (Vidal & Jaber, 2015). Studies on *Metarhizium* spp. have shown that it causes susceptibility in *Drosophila* spp. to infection by other bacterial entomopathogens (Vega *et al.*, 2009).

Beauveria bassiana is one of the fungi considered as an entomopathogen and is the most used in the control of insect pest populations. Synergistic applications of *M. brunneum* on alfalfa, tomato, and melon plants lead to endophytic colonization, causing an increase in mortality rates of *Spodoptera littoralis* larvae when they feed directly on the inoculated plants. Particularly in melon, a whitefly mortality rate of 53% was observed (Garrido-Jurado *et al.*, 2017; Jaber & Ownley, 2018).

Adverse effect of some endomycorrhizae

The function of secondary metabolites produced by endophytic fungi can be either positive, such as providing protection to plants against herbivore attacks, or negative, such as the emission of certain alkaloids. Their production has diversified due to the increased

number and diversity of herbivores, which affects even the natural enemies of plants, leaving them unprotected from other more complex organisms (Tanaka *et al.*, 2005).

The main function of volatile organic compounds is the communication of the plant with its environment and therefore, they affect other organisms in various ways. When a plant is invaded by an endophytic microorganism, this situation can disrupt such communication, depending on the species and colonizing strain.

Dual effect of endomycorrhizae

There are reported cases of fungi that exhibit this type of functionality. The fungus *Phomopsis oblonga* produces alkaloids and mycotoxins, which in turn can control the population of the insect *Physocnemum brevilinenu*, considered a non-pest. However, the interaction of this fungus with trees like the Dutch elm also decreases the spread of the pathogenic fungus *Ceratocytis ulmi*, as the insect targeted by biotechnological products based on this fungus is controlled (Dutta *et al.*, 2014; Webber, 1981).

The fungus *B. bassiana* produces active metabolites that exhibit mycotoxin activity against phytopathogenic fungi under laboratory conditions, unlike in soil assays where its activity is directed against soil pathogens such as *Pythium*, *Rhizoctonia*, and *Fusarium* species. The fungus *Lecanicillium* spp. induces systemic resistance and acts as a mycoparasite against powdery mildew (Ownley *et al.*, 2010).

Endomycorrhizae in Mexico

Mexico ranks fifth globally in terms of biodiversity and endemism, hosting 10% of the world's diversity, the geographical location, topography, altitude, and climate types create an environmental diversity that promotes habitat variety (Aguirre-Acosta *et al.*, 2014; Comisión Nacional para el Conocimiento y Uso de la Biodiversidad, 1998).

In the cultivation of fava beans, 758 endophytic fungi have been identified, classified into 129 families and 66 genera. The most representative genera include *Alternaria*, *Colletotrichum*, *Curvularia*, *Fusarium*, and *Phomosis* (López-González *et al.*, 2017).

In the medicinal species *Dendropanax arboreus*, 45 endophytic fungi have been isolated from various plant organs such as leaves, bark, and roots. The predominant genera include *Fusarium*, *Alternaria*, *Colletotrichum*, as well as *Corynespora*, *Endomelanconiopsis*, and *Thozetella*, which show potential for generating antimicrobial compounds (Ramos-Garza *et al.*, 2016).

Successful commercial products based on endophytic fungi

The development of a biotechnological product generally begins with the identification of the microorganism and its properties, large-scale production, product development, efficacy testing, and finally, registration and commercialization. Below are descriptions of three filamentous fungal products with endophytic characteristics, two with insecticidal activity and one as a mycotoxin.

Strains 5 and F52 of the fungus *M. brunneum*, isolated from codling moth or apple moth, are the active ingredients in a wettable powder. They have a broad spectrum of insect targets including thrips, whitefly, aphids, beetles, weevils, mosquitoes, and mites (Ríos-Moreno *et al.*, 2016; Singh *et al.*, 2011). This biocontrol agent causes insect death through

physical obstruction, nutrient depletion, organ invasion, or paralysis, and it produces 15 toxins known as destruxins (A-E, Ed and Ed1, A2, B and B2, D2, E2, CL, DesmA, and DH-A), with destruxin A being the most abundant, mainly produced by the genus *Metarhizium* (Ríos-Moreno *et al*., 2016).

The biopesticide process is as follows: conidia germinate on the host surface and form appressoria that penetrate the exoskeleton. The infective hypha then penetrates through the host's cuticle, eventually emerging as a homothallic, where the fungus produces cells and toxins. Upon the insect's death, and under humid conditions (25-30 °C), the mycelium penetrates the insect's cuticle and produces infectious conidia outside the cadaver. Under minimal humidity conditions, the fungus survives in the mycelial phase but does not produce conidia outside the insect's body (Authority, 2012).

Application of this formulation on insects *Strophosoma melanogrammum* and *Strophosoma capitatum* under laboratory conditions showed 100% pathogenicity at 21 days, with average survival rates of 13.5% and 96.5% at 32 days, respectively, and a mean survival time of 22.6 days. In field conditions, with two applications at a 15-day interval to the soil of pine and red fir crops, a prevalence of 90% and 78% was achieved in both species after 3 years. This study demonstrated suppression of *Strophosoma* spp. populations and potential cumulative effects preventing population increases in the next generation (Nielsen *et al*., 2006).

Naturalis-L is a bioinsecticide from strain ATCC 74040 of *B. bassiana* oil-based. It was primarily sourced from the cotton boll weevil *Anthonomus grandis* in Texas, USA (Mayoral *et al*., 2006). This pesticide is recommended for controlling larvae of the *Aleyrodidae* family, including greenhouse whiteflies *Trialeurodes vaporariorum* and *Bemisia* spp. These organisms are highly reproductive, significant in crops, and have a wide range of hosts, including weeds, and they also act as vectors for plant viruses. This biopesticide, applied at different doses (125, 250, and 300 mL L^{-1}) weekly during the fruit ripening stage of tomato crops, reduced greenhouse whitefly infestations by reducing live nymphs with 72% and 82% efficacy compared to the control (Mayoral *et al*., 2006).

Lastly, the product "Tricovab," *Trichoderma stromaticum*, affects cocoa tree witches' broom disease (WBD) caused by *M. perniciosa*, a disease of great importance in Latin America. The basidiospores of the fungus infect plant growth tissues, causing a variety of symptoms depending on the infected organ, particularly in apical meristems (De Souza *et al*., 2008).

CONCLUSIONS

The biological potential and alternative use of endomycorrhizal fungi for plant disease control are huge. The biological description of these microorganisms, their ecological role, action mechanisms, and potential dual or adverse effects are aspects that biotechnological production and technology should address in the upcoming years of research. Additionally, integrating social aspects, such as the transition from traditional products to these alternatives, should be included in all biotechnological research focused on such applications.

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Colegio de Postgraduados

Assessment of Harvest Losses in Mejhoul Date Variety in Northwest Mexico

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ABSTRACT

Objective: To assess date losses during harvest and identify the factors causing them.

Design/methodology/approach: A complete randomized block design with ten repetitions was used; the different farms where data on the evaluated variables were taken were the treatments. In each treatment, 10 plants were selected from which data on average fruit weight, polar and equatorial fruit diameter, fruit weight, number of clusters per plant, and yield in tons per hectare were taken.

Results: For yield in tons per hectare of good fruits during the date harvest in the 2023 cycle, an average of 11 t ha^{-1}, 14 t ha^{-1}, 8 t ha^{-1}, and 15 t ha^{-1} was found for the plantations of El Pólvora, El Pino, Las Palmas and Cucapah respectively. In losses during the harvest, Cucapah Farm presented the highest losses with an average of 1.2 t ha^{-1}, while El Pino was the one that presented the lowest losses with an average of 0.7 t ha^{-1}. Fruits with a percentage greater than 10% of peeled skin are also considered losses during the harvest, because they cannot be marketed for fresh consumption.

Limitations on study/implications: Data were collected only from the harvest; exact dates of irrigation and fertilizer application are not available as the study was conducted with cooperating producers. This factor is crucial for the quality and yield of date palm cultivars.

Findings/conclusions: The age of the plant represents a significant difference in the yield per hectare due to the number of clusters it has; the more clusters it has, the higher the yield. The relative humidity directly affects the quality of the dates, and there are a more significant number of bulging fruits (with peeled skin). In this evaluation, we found that the rains during August and September 2023 significantly affected the losses during the date harvest in that season.

Keywords: Losses, Date Palm, Harvest, Agroclimatic Conditions.

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INTRODUCTION

The date palm (*Phoenix dactylifera* L.) is one of the oldest crops in history, with evidence of its use dating back to 4000 B.C. in southern Iraq and Mesopotamia (Propenoe, 1913

& 1973). Today, the date palm dominates value chains due to modernization, sustainable production systems, and the expansion of exports, thereby contributing to sustainable development goals (FAO, 2022).

In Mexico, date production, especially of the Mejhoul variety, has gained increasing importance, particularly in Baja California, due to its high demand in international markets. This variety of date palm was introduced to Mexico in 1968 through the importation of offshoots from the United States, establishing itself in the San Luis Río Colorado Valleys in Sonora and Mexicali, Baja California (Salomón, 2021). Today, Mejhoul, known as "the jewel of dates," accounts for 94% of the total area dedicated to date cultivation in Mexico. Its popularity is due to its attractive appearance, large size, brown color, juicy flesh, and exceptional flavor (Abdelouahhab & Abdallah, 2023).

According to the Secretariat of Rural Development (SADER), Mexico ranks third in the world in Mejhoul date production, after Israel and the United States. It is estimated that the area planted in Mexico is 3,268 hectares, producing 19,465 tons of dates, providing significant benefits to farmers and their families (SADER, 2022). In recent years, Northwest Mexico has experienced remarkable growth in the development of large commercial areas dedicated to date palm cultivation, especially in the Mexicali Valley, Baja California, and San Luis Río Colorado, Sonora. These regions have stood out for their ability to achieve excellent yields due to their favorable agroclimatic conditions. The increase in date production has significantly boosted the local economy, providing job opportunities and improving the quality of life for many rural families.

However, date production faces significant challenges. Climatic variations, such as abrupt changes in temperature and irregular precipitation, directly affect fruit growth and quality. Agricultural management practices also play a crucial role; inadequate management can lead to decreased yields and date quality, affecting competitiveness in the international market.

Moreover, the threat of climate change adds a layer of complexity to these challenges. Rising temperatures and prolonged droughts are putting additional pressure on date producers. Therefore, this research focuses on estimating losses during the harvest of the Mejhoul date variety in Northwest Mexico, with the aim of providing producers with a clear overview of the main factors contributing to these losses and how to mitigate them.

MATERIALS AND METHODS

Study Area Location

The research was conducted during the 2023 season in the Mexicali Valley, which covers an area of approximately 3,709 km^2 and is situated in a broad tectonic basin formed by sediments deposited by the Colorado River and the alluvial fans of the Sierra Cucapá (Lira, 2005). It is bounded to the east by the Colorado River, to the west by the mountain ranges (Sierra Cucapá, Sierra El Mayor, and Cerro El Centinela), and to the north by the sandy mesa on which the border with the United States is located. The Valley is part of the Sonoran Desert, specifically the Bajo Delta of the Colorado River subunit (Shreve and Wiggins, 1964), characterized by a nearly flat surface with altitudes slightly exceeding 40 meters above sea level (asl).

Treatments and Variables

Four strategic farms in the Mexicali Valley were selected, with different ages of plantations (El Pólvora at 30 years, El Pino at 11 years, Las Palmas at 9 years, and Cucapah at 12 years). In each farm, the common variety was Mejhoul. Ten plants were selected as replicates, and a randomized complete block design with 10 replicates was used, applying the statistical model for this design according to the methodology of Steel and Torrie (1980). The treatments consisted of the different farms where data on the evaluated variables were collected, maintaining the common Mejhoul date palm variety in each one. The evaluated variables were the number of clusters per plant (NCPP), Total fruit weight per cluster (TFWC) in kg, losses per cluster (LPC) in kg, average fruit weight (AFW) in grams, polar and equatorial diameter of the fruit (PDF, EDF) in centimeters, Weight of Good Fruits per Cluster, Bulging Fruits per Cluster, Crystalline Fruits per Cluster, and Waste Fruits per Cluster and per Plant (WGFC, WBFC, WCFC, and WWFC) in kg, and yield in tons per hectare of good, bulging, crystalline, and waste fruits.

The numerical data obtained in the field were organized and subjected to analysis of variance and multiple comparisons of means using the Tukey test with $\alpha \leq 0.05$ in the R statistical software package.

Field Procedure

At the four selected farms, 10 plants were sampled, and the harvest was carried out manually. Date fruits from each cluster were collected and placed in separate containers; the total weight of each cluster was recorded in kilograms, and the data were noted in the field notebook. Subsequently, the fruits in each cluster were classified, starting by separating those with damage from disease, pests or that were dried; in general, fruits that were unusable and went directly to waste were classified as waste.

Then, fruits that did not lose moisture on the cluster and remained yellowish were identified. These fruits require more sun to lose moisture and were classified as crystalline fruits. Fruits with more than 10% peeled skin are not suitable for fresh commercialization

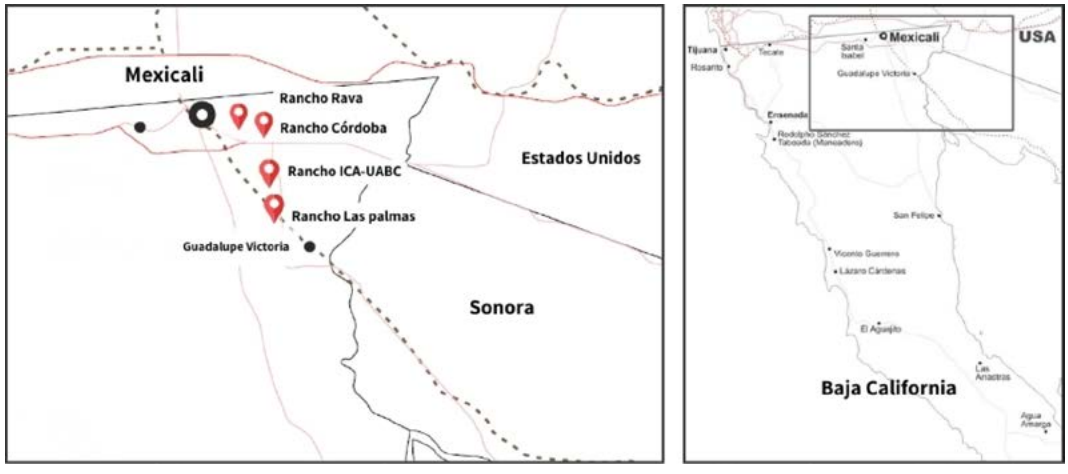

Figure 1. Location map of farms in the Mexicali Valley to assess date losses during harvest.

and are designated for processing; these were classified as bulging fruits. Finally, fruits that are ready for fresh commercialization and can be sent to a controlled atmosphere are those with less than 10% peeled skin, no damage from diseases or pests, and acceptable moisture for storage in the post-harvest area. These fruits have an average weight of 23 to 27 grams and were classified as good fruits.

RESULTS AND DISCUSSION

Hamadttu *et al.* (2022) mention that cultural practices in date palms, such as irrigation, fertilization, and fruit thinning, improve fruit quality. However, this factor can be influenced by environmental conditions prior to harvest and mechanical damage during harvest. They conducted a study using four irrigation levels: 80%, 100%, 120%, and 140% of the palm's evapotranspiration. The study demonstrated that the lowest and highest irrigation water levels significantly affect fruit quality, particularly regarding skin detachment.

Glenn (2016) mentions that date harvest begins in late August in the Mexico and United States regions, and that spontaneous rains during these months can cause losses of up to 20% during harvest, a natural factor that cannot be controlled. He also notes that each mature palm can produce between 100 to 125 kilograms per plant. Similar results were obtained in this study, where the average was 90 kg per plant, and in the 30-year-old plantation, 118 kilograms of dates per plant were achieved.

Table 1 presents the results for the number of clusters per plant. El Pólvora farm has the highest number of clusters per plant, with an average of 20 clusters, while the Cucapah plantation had the lowest number of clusters per plant, with an average of 10 clusters.

Good fruits **Domed fruits** **Crystalline fruits** **Waste fruits**

Figure 2. Classification of date fruits during harvest to assess losses.

Table 1. Date yield per cluster and plant age. Mexicali Valley, Baja California, Mexico. October 2023.

Treatment	Plant age (years)	Number of clusters per plant	Fruit weight per bunch (kg)	Clusters losses (kg)
El Pólvora	30	20.1 [a]	6.02 [b]	0.31 [b]
El Pino	11	14.8 [b]	6.25 [b]	0.32 [ab]
Las Palmas	9	11.1 [bc]	4.87 [b]	0.52 [ab]
Cucapah	12	10.2 [c]	9.57 [a]	0.64 [a]

* Means with the same letter within each column are statistically similar (Tukey, $p \leq 0.05$).

However, Cucapah achieved the highest weight of fruits per cluster, with an average of 9.5 kg. These results align with Glenn (2016), who mentions that a mature date palm can produce an average of 90 to 120 kilograms per year. For the variable of date losses per cluster, Cucapah experienced higher losses with an average of 640 grams per cluster.

The plant age is directly related to the number of clusters each plant produces. It was found that Cucapah Farm has the highest total fruit weight per cluster; however, it also registers the highest date losses during the harvest. This is because, in this plot, the thinning practice was not performed after pollination. Thinning involves removing some fruits per cluster after fruit set to leave an average of 10 to 12 fruits per strand and 45 to 50 strands per cluster, to provide more space for the fruits to develop and reach acceptable quality. According to Morales *et al.*, 2023, this activity ensures fruit quality.

These results are consistent with the information from Glenn (2016), who recommends leaving 10 to 12 fruits per strand and an average of 50 strands per cluster to ensure good quality and quantity of fruit per cluster. Morales *et al.* (2023) mention that for achieving the greatest success in establishing date palm plantations, climate is the most important factor, determining the areas where optimal growth and development of the palms can be achieved. According to various studies, areas within a geographic belt between coordinates 24° N and 34° N are considered the most suitable, as this is where the largest commercial area is established worldwide. In the United States and Mexico, date palms are between 32° and 33°N. Due to climatic factors, the date palm will grow, but the fruit will not develop properly outside the suggested geographic limits.

Table 2 shows the average yield in tons per hectare for each treatment. Pólvora Farm presents the highest number of clusters per plant, which increases the yield in tons per hectare (Figure A and B). However, the fruit size decreases in this case. On the other hand, the results indicate that the Las Palmas plantation presents larger fruits, which is reflected in higher quality and acceptance for the export market (Figure C). The quality of the date is classified according to its size and weight as follows: premium super jumbo dates of more than 27 g, premium jumbo 23-27 g, premium large 18-23 g, premium medium 15-18 g, if the peeled skin ranges from 0 to 10% (Abdelouahhab & Abdallah, 2023). In Table 2, it is shown that the weights of the dates in the evaluated treatments range from 17 to 24 grams. Regarding the variables of polar and equatorial fruit diameter, no significant differences were found, showing a single homogeneous group in the mean comparison, which indicates that this variable does not significantly influence the average date fruit yield. In Las Palmas Farm, corresponding to Treatment 3, the plantation is nine years old, which is reflected

Table 2. Average Fruit Weight per Cluster and Total Fruit Weight per Plant in Valle de Mexicali, Baja California, Mexico. October 2023.

Treatment	AFW (g)	PFD (cm)	EFD (cm)	WGFC (kg)	WBFC (kg)	WCFC (kg)	TFWP (kg)	Yield (t ha^{-1})
El Pólvora	17.54 ^{b}	4.43 ^{a}	2.43 ^{a}	3.82 ^{a}	1.27 ^{b}	0.61 ^{a}	118 ^{a}	18.43 ^{a}
El Pino	21.04 ^{ab}	4.88 ^{a}	2.61 ^{a}	3.46 ^{a}	1.28 ^{b}	1.18 ^{a}	93 ^{ab}	14.57 ^{ab}
Las Palmas	24.92 ^{a}	5.11 ^{a}	2.71 ^{a}	1.99 ^{b}	1.97 ^{b}	1.18 ^{a}	58 ^{b}	9.14 ^{b}
Cucapah	22.91 ^{a}	4.82 ^{a}	2.69 ^{a}	3.92 ^{a}	3.64 ^{a}	1.05 ^{a}	91 ^{ab}	14.29 ^{ab}

*Means with the same letter within each column are statistically equivalent (Tukey, $p \leq 0.05$).

in a lower number of clusters and, therefore, a lower yield in tons per hectare. Statistically, this treatment shows significant differences and heterogeneity, attributable solely to the age of the plantation.

In Table 2, it is observed that, for the variable of average fruit weight, there are highly significant differences. This indicates that plantations older than 30 years produce fruits of lower quality, primarily due to the number of clusters per plant and the competition among them for nutrients. On the other hand, nine-year-old palms produce fruits of greater weight and quality. As the number of clusters decreases, competition for nutrients is reduced. Additionally, environmental factors also influence fruit formation and filling. According to Zaid and de Wet (2002a), temperature has direct effects on fruit set and growth, with the average temperature needing to be around 25 °C to achieve greater success in fruit set. The ripening process of the date is another critical stage, as temperature directly affects the quality and timing of ripening. Therefore, temperatures during this stage should be above 18 °C from early April, after pollination, and continue until October, when the harvest season is ending. For the variable of weight of good fruits per cluster, two heterogeneous groups in the means are observed, with Cucapah Farm showing the highest fruit weight and Rancho Las Palmas showing the lowest fruit weight per cluster. For the variable of weight of bulging fruits per cluster, regardless of the age of the plantations, all were affected with considerable amounts in this variable, with around 4 kg of bulging fruits per cluster, which is directly related to the climatic conditions prior to the harvest. The recommended average annual relative humidity ranges from 27% to 41%, preferably during the flowering,

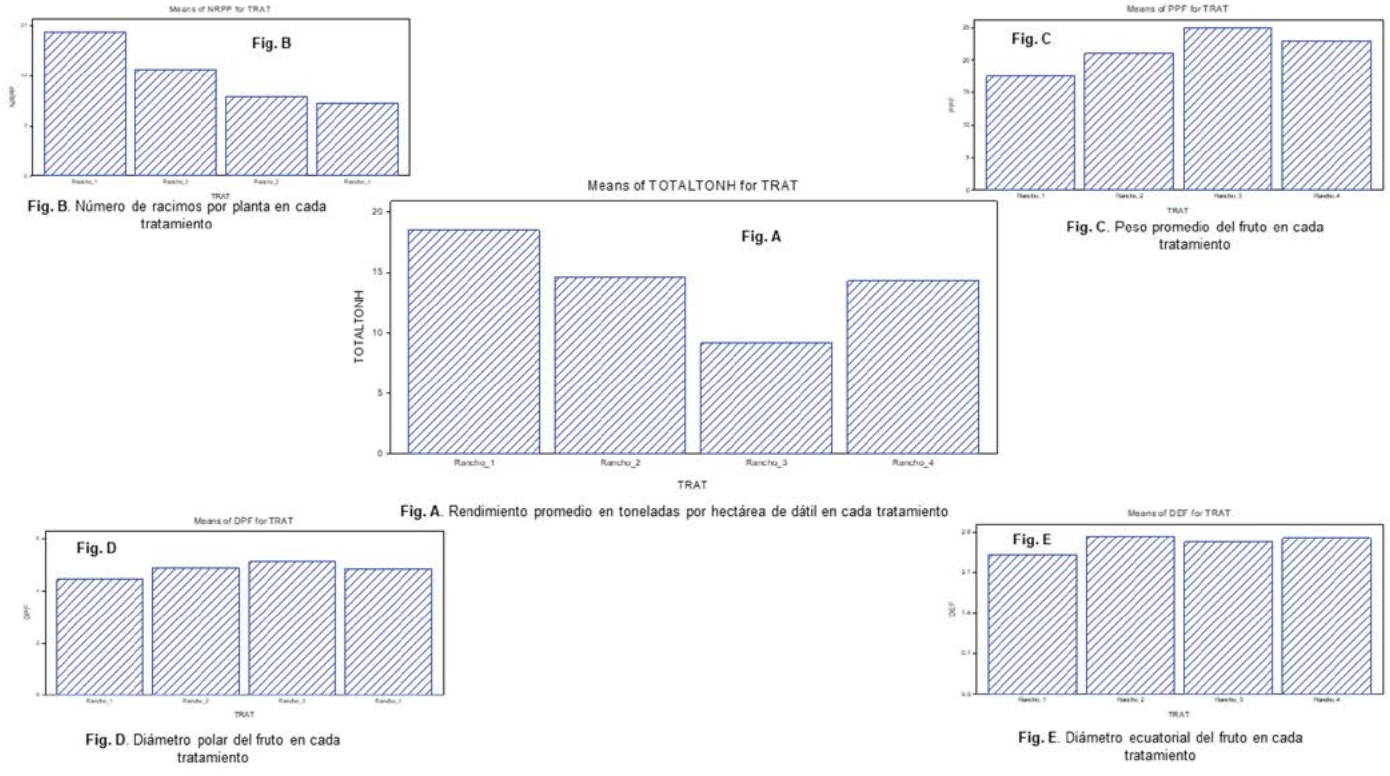

Figure 3. Comparison of date fruit yield in tons per hectare and its relationship with fruit quality and number of clusters per plant.

growth, and fruit maturation (dehydration) periods, which span from March to October (El-Sharabasy *et al.*, 2022).

In Figure 2, the average date fruit weight per cluster in the different ranches is shown. It is observed that Cucapah Farm exhibits the highest quantity of all four variables: good fruits, bulging fruits, crystalline fruits, and waste fruits.

In Table 3, the results for yield in tons per hectare of date fruits harvested from different plantations during the 2023 cycle are presented. The yield per hectare is directly related to the weight per plant, the number of clusters per plant, and the age of the plantation. The Cucapah ranch shows the highest yield in tons per hectare of waste and bulging fruits, while the El Pino and Las Palmas ranches present lower losses during harvest.

According to Morales *et al.*, 2023, air humidity affects the quality of dates, making them highly susceptible to diseases during the maturation and dehydration process. Higher humidity promotes the growth of saprophytic fungi, which appear as black mycelium with small dark hairs that, when touched, release spores that affect nearby fruits. Additionally, high humidity causes dates to become soft and sticky, while low humidity makes them very

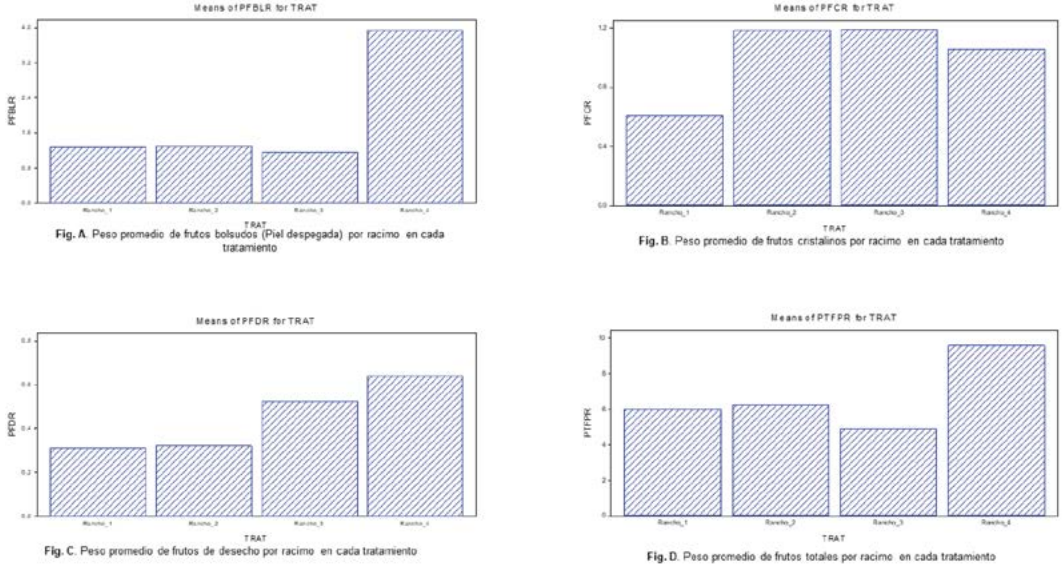

Fig. A. Peso promedio de frutos bolsudos (Piel despegada) por racimo en cada tratamiento

Fig. B. Peso promedio de frutos cristalinos por racimo en cada tratamiento

Fig. C. Peso promedio de frutos de desecho por racimo en cada tratamiento

Fig. D. Peso promedio de frutos totales por racimo en cada tratamiento

Figure 4. Average Date Fruit Yield per Cluster and Its Relationship with Different Ages of Date Palm Plantations in the Mexicali Valley, Baja California. October 2023.

Table 3. Yield in tons per hectare of date fruit (good fruits, crystalline fruits, bulging fruits, and waste fruits) in different plantations in the Mexicali Valley, Baja California, Mexico. October 2023.

Treatment	WGFC (kg)	WCFC (kg)	WBFC (kg)	WWFC (kg)	THAGF (t ha^{-1})	THACF (t ha^{-1})	THABF (t ha^{-1})	THAWF (t ha^{-1})
El Pólvora	75.2 ^{ab}	12.16 ^{a}	24.5 ^{ab}	6.28 ^{a}	11.74 ^{ab}	1.89 ^{a}	3.82 ^{ab}	0.98 ^{a}
El Pino	92.61 ^{a}	17.47 ^{a}	19.1 ^{b}	4.91 ^{a}	14.45 ^{a}	2.73 ^{a}	2.99 ^{b}	0.76 ^{a}
Palmas	55.29 ^{b}	13.86 ^{a}	13.7 ^{b}	5.72 ^{a}	8.63 ^{b}	2.16 ^{a}	2.13 ^{b}	0.89 ^{a}
Cucapah	100.4 ^{a}	10.91 ^{a}	43.1 ^{a}	6.51 ^{a}	15.67 ^{a}	1.71 ^{a}	6.73 ^{a}	1.02 ^{a}

*Means with the same letter within each column are statistically equivalent (Tukey, $p \leq 0.05$).

dry and hard. On the other hand, dry and hot winds cause rapid maturation, leading to the drying of dates and the appearance of a yellow or white ring at the base of the fruit, rendering them unsuitable for export and resulting in significant losses during harvest.

The Cucapah farm, with 12 years of plantation age, showed higher losses during the harvest, with more than one ton of waste fruit per hectare and an average of 6.7 t ha^{-1} of fruit with more than 50% skin detached.

CONCLUSIONS

The age of the plant represents a significant difference in yield per hectare, influenced by the number of clusters present. While an increased number of clusters leads to higher yield, it also results in a decrease in fruit quality. Relative humidity in the environment and pre-harvest rainfall directly affects the quality of dates, leading to a higher number of bulging fruits (with skin separation). This evaluation found that the rainfall during August and September 2023 significantly impacted losses during the date harvest.

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Effect of saline concentrations and humidity percentage on alfalfa varieties (*Medicago sativa* L.) from the Mexicali Valley, Mexico

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ABSTRACT
Objective: The aim of this experiment was to evaluate the resistance of different varieties of alfalfa grown in the Mexicali Valley against abiotic factors such as salt stress and drought stress.
Design/methodology/approach: Four alfalfa (*M. sativa* L.) varieties were used: Cuf-101 C, FD9, Pioneer and Cuf-101 P.
Drought stress resistance was evaluated using four different percentages of commercial peat moss substrate (100%, 50%, 25%, and 15%), and saline stress resistance was evaluated by applying four concentrations of NaCl (0 mM, 50 mM, 100 mM, and 200 Mm.The variables evaluated in each treatment were: plant height, number of leaves and root length. The data obtained were subjected to an analysis of variance using the SAS statistical package version 9.0 with an $\alpha=0.5$.
Results: Alfalfa variety Cuf-101C had a good adaptation to both stresses with an average height of 11.28 cm and an average number of leaves of 3.54. Seed germination of alfalfa varieties subjected to salt stress were affected even at the lowest NaCl concentration. Conclusions: The alfalfa variety with the highest germination percentage in all NaCl concentrations was Cuf-101 C with 91.17%.

Keywords: adaptation, alfalfa, salinity, drought.

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INTRODUCTION

Alfalfa (*Medicago sativa* L.) is one of the most important perennial forage legumes worldwide, due to the qualities it presents in terms of performance, its nutritional value, and the ability to resist various abiotic stress factors (Wang *et al.*, 2021). In the Mexicali Valley, Baja California, it is the third most important crop by established and harvested area and is the most used forage for cattle in the arid and semi-arid regions of Mexico (Martínez-Varela *et al.*, 2015; SADER, 2020; Sánchez-Santillán *et al.*, 2019). Salinity and

drought stress are critical environmental factors limiting plants' growth, development, and agricultural productivity, such as alfalfa (Li *et al.*, 2022; Khodayari, & Abedini, 2022). Studies indicate that plants' responses to different stressors reveal they can detect subtle environmental changes and respond immediately to complex stress conditions, minimizing damage and conserving valuable resources for growth and reproduction (Martínez *et al.*, 2016). There are also reports detailing alfalfa varieties' growth capacity when under various stress conditions, and their ability to develop different resistance mechanisms has been demonstrated (Quan *et al.*, 2016). Further to this, current information reflects the effects of water or saline stress or a combination of both on alfalfa yield. The development, growth, quality, and yield of alfalfa are significantly inhibited and decrease drastically under salt stress conditions (Ling *et al.*, 2022). However, it is necessary to evaluate the physiological response mechanisms exerted by alfalfa when subjected to both factors (Hou *et al.*, 2022). Studies indicate that plants' responses to different stressors reveal they can detect subtle environmental changes and respond immediately to complex stress conditions, minimizing damage and conserving valuable resources for growth and reproduction (Martínez *et al.*, 2016). There are also reports detailing alfalfa varieties' growth capacity when under various stress conditions, and their ability to develop different resistance mechanisms has been demonstrated (Quan *et al.*, 2016). Further to this, current information reflects the effects of water or saline stress or a combination of both on alfalfa yield. The development, growth, quality, and yield of alfalfa are significantly inhibited and decrease drastically under salt stress conditions (Ling *et al.*, 2022). However, it is necessary to evaluate the physiological response mechanisms exerted by alfalfa when subjected to both factors (Hou *et al.*, 2022).

In this sense, it is necessary to understand the physiological responses of plants to different stress conditions to ensure a good harvest in future climatic conditions that may arise. Therefore, this work focuses on identifying the best alfalfa varieties resistant to saline and drought stress in the Mexicali Valley, Mexico.

MATERIAL AND METHODS

Study site

This research was carried out in the Phytopathology Laboratory and in the greenhouse Area of the Instituto de Ciencias Agrícolas (ICA) of the Universidad Autónoma of Baja California (UABC) located in the Mexicali Valley in the extreme northeast of the state of Baja California, between 114° 45' to 115° 40' west longitude and 31° 40' to 32° 40' north latitude.

Obtaining seeds and sowing

Four varieties of seeds were used, three provided by the seed marketer Stell: Cuf-101 C, FD9, Pioneer, and one donated by an alfalfa farmer from the Mexicali Valley, produced by himself Cuf-101 P. Three alfalfa seeds were sown per experimental unit using Styrofoam cups with a capacity of 1 L. Peat moss was used as the substrate, which was previously sterilized and had its pH adjusted to 7.17. The glasses were placed in the ICA greenhouse. Fertilization was based on Miracle-Gro Brand granulated inorganic fertilizer and following the manufacturer's instructions (Echeverria *et al.*, 2021).

To evaluate the effect on germination, a completely randomized design with a factorial arrangement of two factors at four levels each was used: Factor A: variety (Cuf-101 P, Cuf-101 C, FD9 and Pioneer).

Height (H), number of leaves (LN), and root length were evaluated for both cases. Data for H and LN was taken once a week, and root length was taken at the end of the experiment.

Treatments salinity and drought *in vivo*

NaCl concentrations, and in the case of drought stress, the different percentages of substrate humidity were recorded.

In the case of salt stress, 48 experimental units were evaluated (a Styrofoam glass with two alfalfa plants of one of the varieties. Four concentrations of NaCl were applied: 0 mM, 50 mM, 100 mM, and 200 mM, for 16 treatments with three repetitions (Lastiri-Hernández *et al.*, 2017; Pacheco *et al.*, 2022). Each experimental unit (a 9 cm diameter petri dish) with 10 seeds of each of the varieties had four percentage of humidity (100, 50, 25, and 15%) applied, for a total of 16 treatments, and each treatment had three repetitions, for a final total of 48 experimental units. Each experimental unit was a Styrofoam cup, which contained two alfalfa plants of one of the varieties, and was subjected to four different irrigation distributions: 1 day of irrigation and 2 days without irrigation (1R/2SR), 1 day of irrigation and 5 days without irrigation (1R/5SR), 1 day of irrigation and 10 days without irrigation (1R/10SR), and 1 day of irrigation and 15 days without irrigation (1R/15SR), for a total of 16 treatments with three repetitions, for a final total of 48 experimental units (Hou *et al.*, 2022).

Germination tests for salinity and drought

A standard "between paper" germination test was performed for both effects. 25 seeds were sown and distributed in five columns and five rows, in two towels previously moistened with the corresponding treatment on a flat surface and subsequently covered with two other wet towels to then be then rolled into the shape of a "taco", which were introduced into a VWR Scientific Inc. brand incubator Model VWR 1550 C at ±20 °C. The initial (GP %) data was taken on the third day, and the final percentage was taken on the seventh day (Martínez-Solis *et al.*, 2010). The seed was germinated when the radicle was 5 mm long (Abril-Saltos *et al.*, 2017). The germination test moistened the substrate at four different percentages (100, 50, 25 and 15%). A Lutron humidity sensor model, PMS 714, was used to measure the percentage of humidity. Once the humidity was adjusted, petri dishes were filled, and 10 seeds per box were sown. This procedure was the same for all treatments.

Treatments salinity and drought

The treatments were applied when the plants reached an approximate height of 10 cm. Watering was applied manually three times a week. Once the approximate height of 10 cm was obtained, each experimental unit was brought to field capacity and placed in two environmental chambers (Lab-Line Instruments Inc. Biotronette Mark III Model) at ±40

°C to begin the application of the treatments. The variables evaluated in each treatment were: H, LN, and root length. Data for H and LN were taken once a week and root length was determined at the end of the experiment.

Statistical analysis

The data obtained was subjected to an analysis of variance using the SAS statistical package version 9.0 with an $\alpha = 0.5$

RESULTS AND DISCUSSION

Germination percentage (GP %) It was observed during the two data collection dates that the interaction between the factors did not influence the (GP %). However, their influence was observed among the four varieties analyzed (Table 1). These results coincide with what Castroluna and collaborators (2014) reported, who evaluated three varieties of alfalfa, which were affected by the incidence of both factors. Concerning the factor humidity percentage, a highly significant influence on the germination percentage (%) was observed. On the first date, there was very significant evidence of the influence of the factors separately on germination, and on the second date, there was much notable evidence due to the variety factor, and highly significant due to the NaCl concentration, both on germination. On both dates, the best variety was CUF-101 C at a concentration of 0 mM NaCl. In Table 1, it is observed in data collection 1 that the interaction between the factors had no influence on the germination percentage with stress in alfalfa due to drought; however, on both dates, the best percentage of substrate humidity was 15%, with an average germination of 98.33%, which was obtained from the initial germination data collection and was maintained up to the final one.

For date one, in the variables H and LN highly significant evidence was observed with respect to the variety factor. The best variety in both cases was the commercial seed variety Cuf-101 C, with an average height of 11.28 cm and an average number of leaves of 3.54. In week two, the data for the variables H and LN showed significant and highly significant evidence due to the variety factor. The best variety was the Cuf-101 C, with an average height of 12.72 cm and an average number of leaves of 4.42. In week three, the data for the H variable showed highly significant evidence due to the variety factor. Significant evidence was found in the LN variable due to an interaction between the factors. The variety that presented the most significant height was Cuf-101 C with 18.81 cm. In the case of the variable number of leaves, the best treatment was that corresponding to the variety Cuf-101 C seed with a concentration of 100 mM NaCl, with 8.17 average leaves. In week four, significant evidence was found in the height variable due to the variety factor. In the case of the LN it was found that the interaction between the factors influenced said variable the best variety corresponds to the variety Cuf-101 C seed with an average height of 18.81 cm. In the case of the LN, the best treatment corresponded to the variety Cuf-101 C seed with a concentration of 100 mM, with an average Cuf-101 C of 12.50. In week five, highly significant evidence was found for the H variable due to the variety factor. In the case of the LN, very highly significant evidence was found due to separate factors. The variety with the highest height was the

Table 1. Percentage of initial and final germination of alfalfa at different concentrations of NaCl.

Date	Variety	[NaCl]	Germination (%)
1	1 ('Cuf 101' P)	1	91.33
		2	91
		3	87
		4	78
	2 ('Cuf 101' C)	1	91.67
		2	89.33
		3	92
		4	84.33
	3 (FD9)	1	84.67
		2	80.33
		3	85
		4	72.33
	4 (Pioneer)	1	84.67
		2	85.33
		3	80.33
2	1 ('Cuf 101' P)	1	93.33
		2	92.67
		3	88
		4	88.33
	2 ('Cuf 101' C)	1	93
		2	90.67
		3	92.67
		4	88.33
	3 (FD9)	1	85.33
		2	81.33
		3	85.33
		4	76
	4 (Pioneer)	1	86.33
		2	89.67
	1 ('Cuf 101' P)	3	81.33
		4	81.67
		Variety	***
		NaCl	**
		Variety*NaCl	NS

* ≤0.05 Significant.
** ≤0.01 Highly significant.
*** ≤0.001 Very highly significant.

Cuf-101 C with an average height of 18.27 cm, and the variety with the highest LN with an average of 13.10. The NaCl concentration, was the best, corresponding to 50 mM, with an average of 13.05 leaves. The experimental data did not show significant

evidence that the interaction of the factors, or the factors separately, affected the root length variable. All treatments were the same (Figure 1).

Effect of salt stress on alfalfa development

For date one, highly significant evidence was observed in the variables H and LN due to the variety factor. The best variety in both cases was the Cuf-101 C, with an average H of 11.28 cm and an average LN of 3.54. In week two, the data for the variables H and LN showed significant and very highly significant evidence due to the variety factor. The best variety was the Cuf-101 C with an average height of 12.72 cm and an average LN of 4.42. Some studies show that alfalfa has salinity tolerance at all growth stages (Pacheco *et al.*, 2022). In week three, the data for the height variable showed highly significant evidence due to the variety factor, and in the case of the number of leaves variable, significant evidence was found due to the interaction between the factors. The variety that presented the most significant height was the Cuf-101 C with 18.81 cm. In the case of the variable number of leaves, the best treatment was that corresponding to the Cuf-101 C with a concentration of 100 mM NaCl, with 8.17 average leaves. In week four, significant evidence was found in the height variable due to the variety factor. In the case of the LN, it was found that the interaction between the factors had the more significant influence. In the case of height, the best variety was the one corresponding to the Cuf-101 C with an average height of 18.81 cm, and in the case of the LN, the best treatment was the one corresponding to the Cuf-101 C with a concentration of 100 mM, with an average LN of 12.50. In week five, significant evidence was found for the height variable due to the variety factor. In the case of the LN, highly significant evidence was found with the factors separately. The variety with the highest height was the Cuf-101 C with an average height of 18.27 cm, and the variety with the highest LN with an average of 13.10. The best concentration for the NaCl concentration was 50 mM, with an average of 13.05 leaves. The experimental data did not show significant evidence that the interaction of the factors, or the factors separately, affected the root length variable. All treatments were the same.

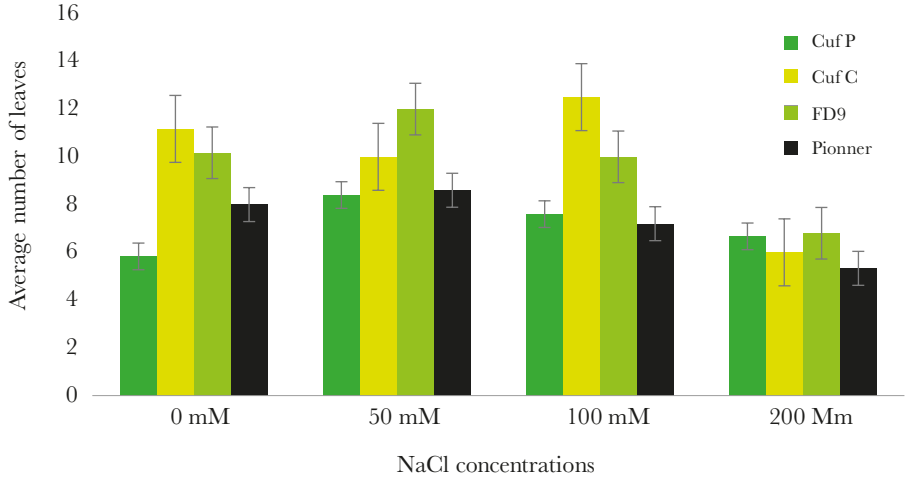

Figure 1. Average number of leaves of the different treatments in week four.

Effect of drought stress on alfalfa development

For date one, it was found that the variables H and LN were influenced by the factors separately. In the case of height, there was significant evidence for the variety factor and very highly significant evidence for the irrigation distribution factor. In the case of the LN, there was significant evidence for the variety factor and highly significant evidence for the irrigation distribution factor. The best variety according to height was the Cuf-101 C, with an average height of 9.25 cm, and the highest LN, with an average of 2.96 leaves. The best irrigation distributions corresponded to (1R/5SR) for both variables, and (1R/15SR). For date two, the experimental data did not show significant evidence that the interaction of the factors or the factors separately influenced the height variable. All treatments were the same. For the variable LN, the data showed significant evidence that irrigation distribution influenced said variable. The best irrigation distribution corresponds to (1R/5SR) with an average LN of 4.88. For date three, the experimental data showed significant evidence due to the interaction between the factors on the H variable and significant evidence due to the irrigation distribution factor for the LN, variable. The best treatment was the one corresponding to the Pioneer variety with an irrigation distribution of (1R/5SR) with an average height of 23.80 cm. For the LN variable, the best irrigation distribution corresponded to (1R/5SR) with an average LN of 6.42. For date four, the experiment data showed highly significant evidence due to the interaction between the factors on the height variable. In the case of the leaf number variable, the data show highly significant evidence due to the irrigation factor. The best treatment was the one corresponding to the Pioneer variety with an irrigation distribution of (1R/5SR) with an average height of 29.22 cm. For the variable LN, the best irrigation distribution was that of (1R/5SR), with an average of 13.35 leaves.

For date five, the experiment data on the height variable did not show significant evidence due to the interaction of the factors or the factors separately. All treatments were the same. In the case of the variable LN significant evidence was found due to the irrigation distribution factor. The best irrigation distribution was that corresponding to (1R/5SR), with an average of 13.24 leaves. The experimental data did not show significant evidence that the interaction of the factors, or the factors separately, affected the root length variable. All treatments were the same. The dry weight of the shoots of five alfalfa cultivars was significantly reduced by water deficit and showed a significant difference between the cultivars. Similar results were also observed in previous studies on drought response of alfalfa (Guo *et al.*, 2019). However, drought itself is a stress for plants. The positive influence of drought on subsequent salt stress can be counteracted by the negative effect of drought itself. Therefore, the ameliorative effect of light drought was more pronounced than severe drought's. In contrast, if drought occurred simultaneously with salinity, it reinforced salinity-induced damage, according to multiple studies.

CONCLUSIONS

All alfalfa varieties (*M. sativa* L.) subjected to salt stress were affected in their germination even at the lowest NaCl concentration. The highest average total (GP %) variety in all NaCl concentrations was Cuf-101 C with 91.17%. The (GP %) in the case of drought stress was

higher when the substrate had 15% humidity. All varieties had a good (GP %), but this is conditioned by the moisture content in the seed. The variety presented a better response to salt stress was the Cuf-101 C variety because it presented greater height and average total number of leaves even at concentrations of 100 mM NaCl.

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Molecular identification of fungal isolates from different tissues samples of Blueberry (*Vaccinum* sp.) in Baja California

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ABSTRACT

Objective: Molecular identification of fungal isolates presents in fruits and leaf samples of *Vaccinium* with pathogenic or agro-industrial potential.

Design/methodology/approach: Plant material (fruits and leaves) was collected in blueberry commercial plantations of San Quintin, Baja California, México. The samples were placed in humid chambers for fungal growth and then in culture plates with Potato-Dextrose-Agar alone or with lactic acid for purification. The resulting fungal isolates were cultured in liquid media, the total DNA was extracted and quantified, afterwards the ITS region was amplified by PCR, the fragments were purified and sequenced. Finally, the resulting sequences were compared in the NCBI database with the BLAST algorithm, the phylogenetic reconstruction was performed with the MEGA (v.10.0) software.

Results: A total of 22 isolates from *Vaccinium* were obtained from leaves and fruits. These isolates showed high identity percentages (96-100 %) with *Botrytis*, *Didymella*, *Phoma*, *Alternaria* and *Cladosporium* genera. The fruit isolates were closely related with *B. cinerea* Group I, whereas the leaf samples grouped with other complexes such as the *C. cladosporoides*, *A. muriae*, *Dydimella bervipilosa* and *Phoma*.

Limitations on study/implications: The use of the ITS region provides only a partial characterization in some types of fungi, the use of other molecular markers are required to fully characterize some isolates.

Findings/conclusions: The molecular characterization of the fungal isolates showed that most of the genera were saprophytes with phytopathogenic members reported. The reported genera could have an impact in post-harvest due fruit spoilage or by the presence of cytotoxic compounds. The presence of fungal genera (*Cladosporium*) with reported potential antagonistic and growth promoting capabilities was identified.

Keywords: Agrobiotechnology, Bioinformatics, Molecular characterization, Phylogeny, Phytopathogens, *Vaccinium*.

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INTRODUCTION

The state of Baja California, located at Mexico northwest, is among the main producers of several fruit crops, such as the blueberry (*Vaccinium* sp). This crop is produced in the coast region and in recent years has shown a steady increase in the production surface,

with incomes of 1,417 million pesos in 2022 being the exportation the main destiny of this product (Figueroa *et al.*, 2010, SIAP, 2023, SADER, 2023).

One of the main factors that impacts severely the crop production is the presence of pathogens, which in turn causes yield reductions and quality losses. In dependence of the causal agent the losses that can be generated would oscillate from 30-50% for *Vaccinium* (Figueroa *et al.*, 2010; Mondragón *et al.*, 2013). The control of phytopathogens is performed by the use of chemical agents, which have a high economic cost, could present low specificity, environmental risk and, depending on the type of chemical, his use in fruits for exportation could be restricted due its residuality [Altieri *et al.*, 2004; Zadehdabag *et al.*, 2010; Samaniego-Gámez *et al.*, 2012).

Also, the chemical management of phytopathogens alters the composition of the microorganisms present, both in the plant and in the environment, due the reduction of the population of potential suppressive agents for some types of pathogens (Weller *et al.*, 2002; Lugtenber *et al.*, 2009). Recent studies have shown that microorganisms influence several aspects plant processes such as health, development, nutrition and production (Hartmann *et al.*, 2009; Philippot *et al.*, 2009; Caro-Quintero *et al.*, 2015; Samaniego-Gámez *et al.*, 2017). Previous studies in different species of berries have shown that the microbial communities are strongly dependent of the culture conditions and several agents for the growth promotion could be isolated (Salhi *et al.*, 2022; Thimmappa *et al.*, 2023).

The isolation and molecular study of microorganisms allows an effective management of isolated phytopathogenic genera, and also allows the identification of those that may be agents of biocontrol, growth promotion and agro-industrial interest. In the present study, the molecular identification of several fungal isolates in different blueberry tissues was performed, in order to identify fungal pathogens and fungi with agro-industrial potential.

MATERIALS AND METHODS

Collection of samples from Blueberries (*Vaccinum* sp.) plantations

Sample collection was performed during November-February, 2022 with growers of San Quintin, Baja California. During the collection, leaf and fruits samples were taken from plants with symptoms from pathogens, as well as from asymptomatic plants. Both types of samples were placed individually in closed labeled plastic bags and placed in a cooler (4 °C) for subsequent analysis in the UABC Physiology and Postharvest Management laboratory.

Processing of blueberry samples

The superficial sample disinfection was made according to the Samaniego-Gámez and Cervantes-Diaz, (2012). The samples were placed in humid chambers (20±2 °C) in photoperiod (12 h) for 7 d, with daily observations. The growths were cultured in Potato-Dextrose-Agar (PDA) medium and PDA with lactic acid (0.1% v/v) in Petri dishes by central stinging. The plates were sealed with Parafilm (Sigma-Aldrich) and incubated (20±1 °C, in darkness) for 6 d with daily observations. The resulting fungal growths were purified by the monosporic culture method on PDA plates (Li *et al.*, 2012).

Molecular identification of fungal isolates

The cultured fungal isolates were sectioned with a sterile scalpel and placed in Yeast Extract-Peptone-Glucose broth, 10 d (25±2 °C), the culture media was filtered and the samples were stored in tubes (−20 °C) until further use. Total DNA was extracted with the protocol of Al-Sammarrai and Schmidt, (2020). DNA was observed in 1% (w v^{-1}) agarose gels, subsequently the samples were treated with RNase (Ambion) and quantified with a spectrophotometer.

The amplification of the inter-transcribed spacer (ITS) was performed using the ITS1(TCCGTAGGTGAACCTGCGG) and ITS4(TCCTCCGCTTATTGATATGC) in a reaction mixture that consisted in: 250 nM of each primer, 1.5 mM of $MgCl_2$, 1.5 U of Taq-Polymerase in a final volume of 20 μL. The reaction mixture was placed in a thermocycler (BioRad) with the following conditions: 95 °C (3 min), 30 cycles of 95 °C (1 min), 55 °C (1 min) and 72 °C (1.5 min) with a final extension of 72 °C (10 min).

Bioinformatic analyses and processing

The amplification products (420-825 bp) were visualized in agarose gels (1% w v^{-1}), the amplicons were purified with the Wizard SV PCR Clean-Up System (Promega) according to the manufacturer's instructions. The purified fragments were sequenced in Macrogen (Korea).

The obtained sequences were introduced in the GenBank for his identification using the BLASTn algorithm, with the Megablast option, the sequence search was made using the "ITS Fungi for Type-Material" database.

The BLAST search results were retrieved in fasta format and introduced in the MEGA (v10.0) software for the phylogenetic analyses. The fungal sequences were aligned with the CLUSTALW algorithm, the phylogenetic reconstruction was made with the Maximum Parsimony (MP) model with the following parameters: BooStrap=1000 replicates, Number of Parsimony tres=10 and the option Tree Bisection Reconnection. The Tree Length (TL), Consistency Index (CI), Retention Index (RI) and Composite Index (Comp-Indx) were also calculated.

RESULTS AND DISCUSION

In the blueberry producing areas located in San Quintin, Baja California it was observed symptoms associated with *Bortytis* sp., the symptoms developed under the ideal conditions for the pathogen (low temperature and high humidity). The symptoms observed where: mummified brown-colored fruits (Figure 1B), leaf with necrotic spots (Figure 1C) and brown-colored flowers with reduced size, which agrees with the reported symptoms for *Botrytis* in berries (Figueroa *et al.*, 2010; Mondragón-Flores *et al.*, 2012).

A total of 22 isolates from *Vaccinium* were obtained from leaves and fruits, the asymptomatic samples did not show the presence of fungal colonies related to the macroscopic characteristics associated with *Botrytis*.

The fruit isolates showed at 72 h of growth the formation of mycelium with a dark-grey coloration in the front of the plate (Figure 2A) and a light-grey coloration at the back of the plate (Figure 2B).

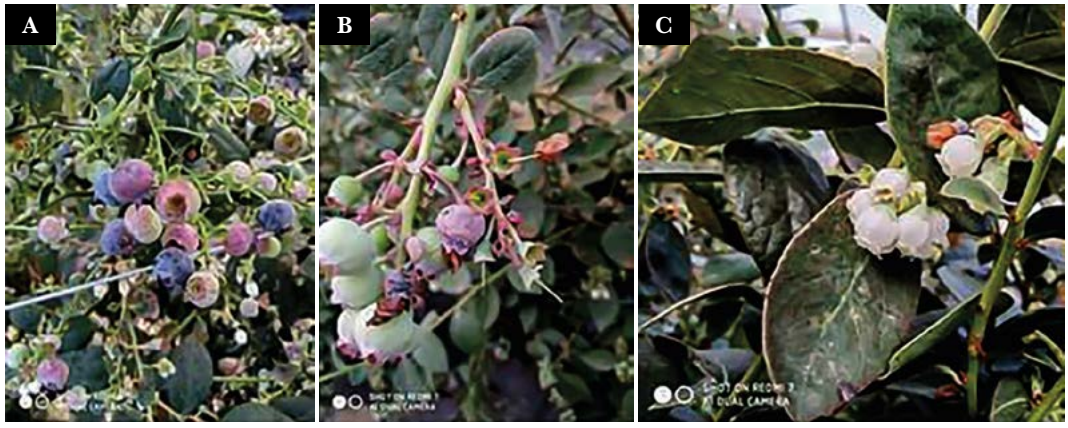

Figure 1. Observed symptomatology in Blueberries (*Vaccinium*) in San Quintín, Baja California. A) Asymptomatic plant, B) Brown-colored fruits and mummification; C) Necrosis and leaf yellowing and brown-spotted flowers.

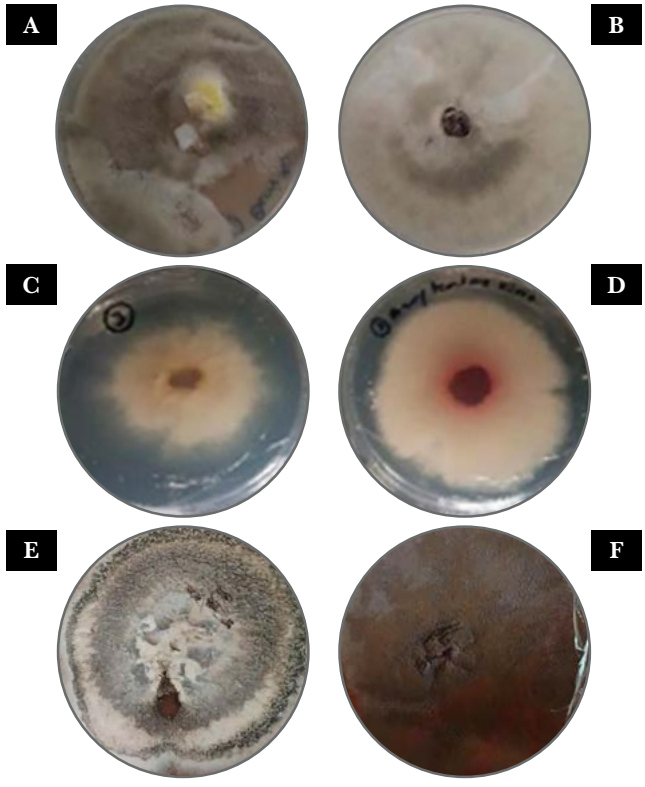

Figure 2. Fungal isolates from *Vaccinum* sp. with 72 h of incubation. A) Gray mycelial growth (front), B) Gray mycelial growth (back), C) White mycelium (front) from leaf tissue, D) White colored mycelium with a pink halo (back), E) Leaf isolate with olive-green mycelium with white borders, F) Green-gray mycelial growth from leaf samples.

Leaf samples showed white-colored mycelium at 72 h, with irregular borders at the front of the plate (Figure 2C) and a pale-pink coloring at the back of the plate (Figure 2D). Other isolates from leaves formed woolly mycelium, with regular borders, a green-olive coloration at the front of the plate y white-colored borders (Figure 2E).

The isolates were sequenced and showed high identity percentages with the *Botrytis*, *Didymella*, *Phoma*, *Alternaria* and *Cladosporium* genera (Table 1).

The gray isolates from fruit samples showed identity percentages from 99.6-100% with different strains of *Botrytis cinerea*. The white isolates from leaves, showed 99.6-99.8% identity with Phoma and 97.1-99.1% identity with two species of *Didymella* (*D. keratinophila*, *D. brevipilosa*). In this sense, the olive-green isolates showed high percentages of identity with two *Cladosporium* species: *C. ramonetellum* (99%) and *C. cladosporoides* (96-99.2%).

The phylogenetic analyses showed that all the fruit isolates of this study grouped close to *Botrytis* sp. EX2019-m34 (Figure 3A). Also, they formed a paraphyletic group with *B. cinerea* and *B. californica* (Figure 3B).

The presence of *Botrytis* in *Vaccinium* fruits have been reported previously, this genus affects more than 200 plant species and is considered an important postharvest pathogen in several types of berries [Samaniego-Gámez *et al.*, 2012; Saito *et al.*, 2016; Terrones-Salgado *et al.*, 2019; Garay-Serrano *et al.*, 2021; Esterio *et al.*, 2020; Saito *et al.*, 2020; Amed and Abed, 2023). The use of molecular markers, such as the ITS region, have been used previously for the characterization of the *Botrytis* complex, which has been difficult to characterize because its high morphological variability (Esterio *et al.*, 2020; Saito *et al.*, 2020; Amed and Abed, 2023).

In the present study the use of the ITS region grouped members of the *B. cinerea* Group I (*B. cinerea*, *B. californica*, *B. pelargoni*, *B. pseudocinerea*) in a single clade as reported previously (Esterio *et al.*, 2020; Saito *et al.*, 2020). The *Botrytis* isolates obtained in the present study did not form a group with members of *B. cinerea* Group I. The use of additional markers would be necessary in order to fully characterize the isolates from the present study. The use of additional molecular markers has been previously reported in *Botrytis* with a substantial improvement in the allocation of isolates in the different *Botrytis* complexes (Saito *et al.*, 2016; Terrones-Salgado *et al.*, 2019; Garay-Serrano *et al.*, 2021; Delong *et al.*, 2020).

Table 1. BLAST search results of sequences from fungal isolates in fruits and leaves of *Vaccinum* in the NCBI database.

Description	Accession	Identity (%)	E-value
Botrytis cinerea	MH665643.1 OR544948.1 OR544951.1 OR237160.1	99.6-100	0.0
Botrytis sp.	MT912776.1	99.6	0.0
Phoma sp.	MT420629.1 MT912578.1	99.8-99.6	0.0
Didymella keratinophila	NR_158275.1	99.1	0.0
Didymella brevipilosa	NR_158236.1	97.1	0.0
Alternaria destruens	NR_137143.1	99.4-99.2	0.0
Cladosporium ramontellum	MZ301265.1	99.2	0.0
Cladosporium cladosporoides	OP006753.1 LC325159.1 OP006753.1	96.4-99.2	0.0

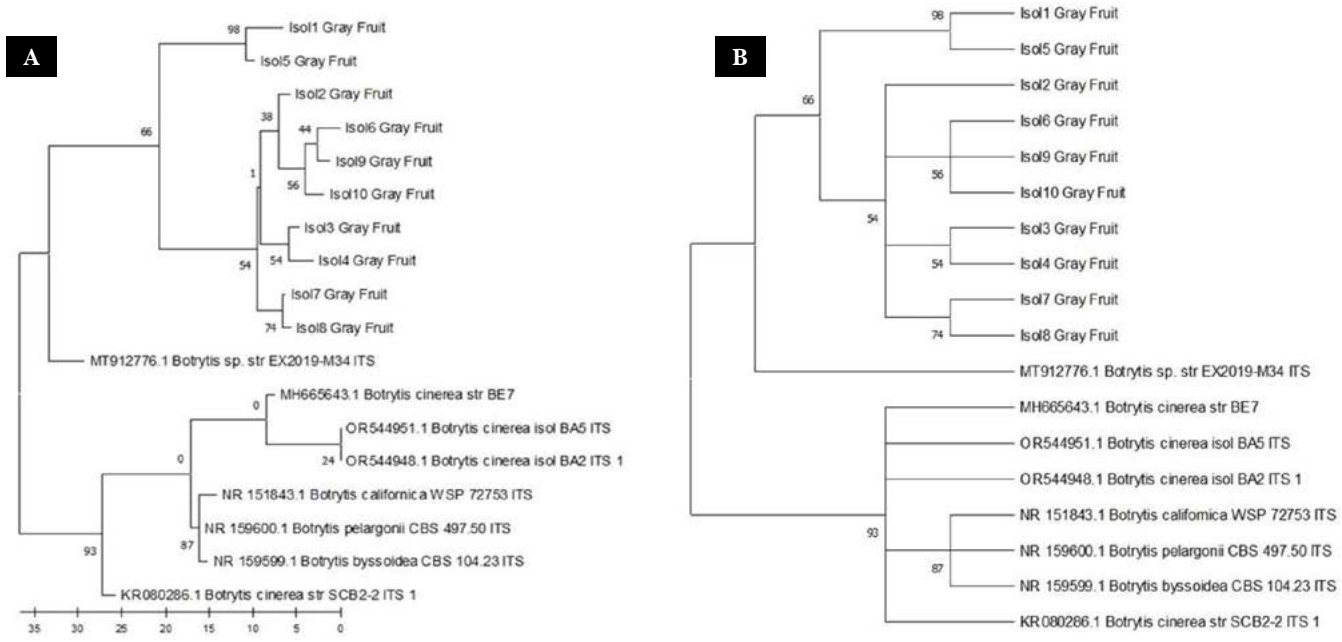

Figure 3. Phylogenetic generated by Maximum Parsimony of fungal isolates from Vaccinium fruits. A) Tree in terms of the number of changes in the entire sequence, B) Condensed tree with a minimum branch support cut-off=50%. TL=116, CI=0.876289, RI=0.897436, Comp-Indx=0.804598 (0.786413).

White fungal isolates with pink halos (Figure 4A) formed two monophyletic groups with the two strains of *Phoma* sp. (isolates 1 and 2) and with *Didymella brevipilosa* (isolates 3 and 4). The gray isolates formed a monophyletic group with *Alternaria murispora* (Figure 4B). Olive green mycelial isolates from *Vaccinium* leaves, clustered closely with *Cladosporium cladosporoides* complex (Figure 4C).

It was observed that most of the isolated genera in leaves (*Phoma*, *Didymella*, *Alternaria*, *Cladosporium*) have been listed in previous studies as saprophytes with phytopathogenic potential (Woundenberg *et al.*, 2009; Aveskamp *et al.*, 2010; Bennett *et al.*, 2018, Derviş *et al.*, 2024). The presence of this type of fungi it could be associated to the presence of necrotic spots in the collected samples.

The presence of *Phoma* and *Didymella* (*Didymellaceae*) were identified in isolates with similar morphology, previous studies considers the *Dydimellaceae* as a polyphyletic family, with members which have a high morphological and molecular variability making his identification complicated, it's also been reported to find Phoma and Dydimella in complexes (Aveskamp *et al.*, 2010; Bennett *et al.*, 2018, Derviş *et al.*, 2024), a similar behavior was observed in the present study.

Didymella and *Phoma* can colonize different surfaces, such as: water, soil and other inorganic substrates, thus being considered as cosmopolites (Stranska *et al.*, 2022; Luo *et al.*, 2024; Magaña-Dueñas *et al.*, 2021). Regarding the phytopathogenicity of these two genera, previous reports considers that *Phoma* is an opportunistic pathogen, with a wide host range and can cause respiratory and dermal symptoms in humans (Bennett *et al.*, 2018; Lugauskas *et al.*, 2006). In the case of *Didymella*, two species (*D. glomerata* and *D.*

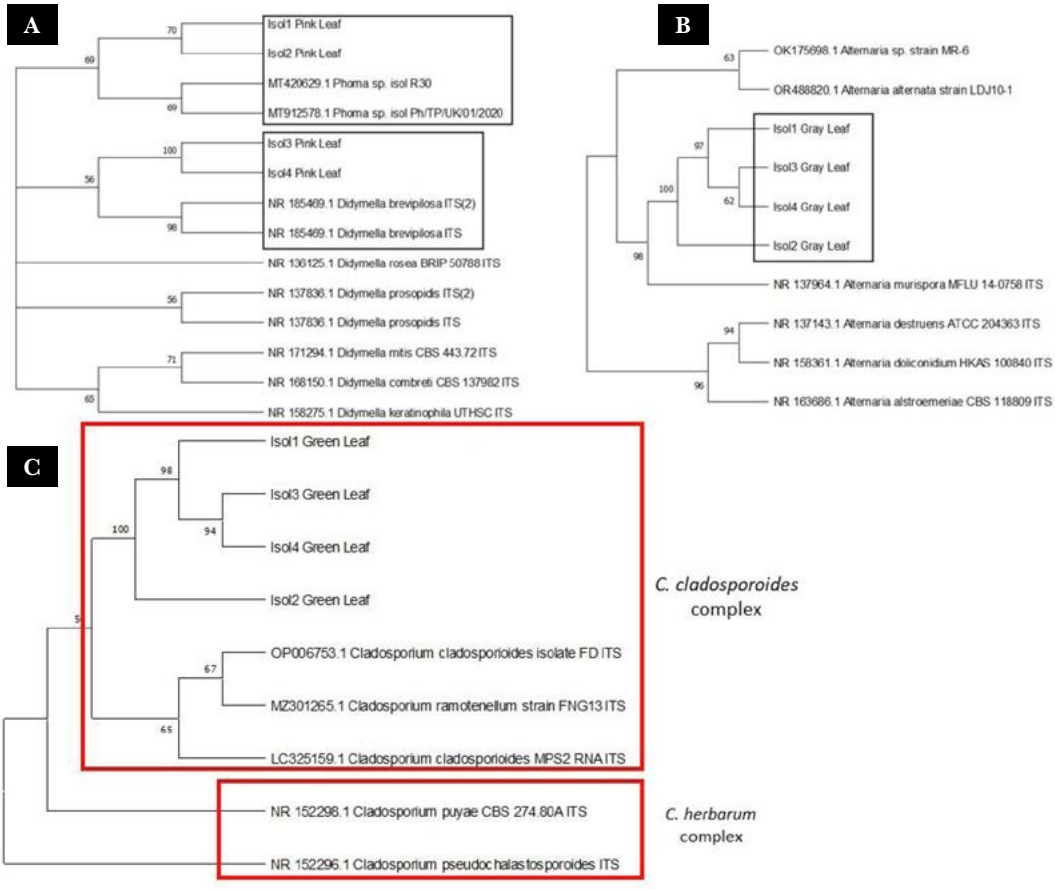

Figure 4. Phylogeny generated by MP from fungal isolates of *Vaccinum* leaves, condensed trees with a minimum branch support cut-off=50%. A) White fungal isolates. TL=391. CI=0.985591, RI= 0.985915 and Comp-Indx=0.973308 (971709). B) Gray fungal isolates, TL=419, CI=0.990291, RI =0.996507, Comp-Indx=0.986993 (0.986832). C) Green fungal isolates. TL=383, CI=0.979042, RI = 0.991716, Comp-Indx=0.973591 (0.970932).

aplanata) have been reported as causal agents of necrotic leaf spots in blackberries (*Rubus fruticosus*) (Woundenberg *et al.*, 2009; Derviş *et al.*, 2024), which agrees with the symptoms of the collected samples.

Previous studies performed in berries mentions that the presence of *Alternaria* and *Cladosporium* as cosmopolite species and are part of the fungi associated with fruit spoilage (Torres *et al.*, 2017; Raynaldo *et al.*, 2024; Sinkevičienė *et al.*, 2023; Woundenberg *et al.*, 2013; Ariyawansa *et al.*, 2015; El-Dawy *et al.*, 2021; Iturrieta-González *et al.*, 2021). In the present study the presence of these two genera were observed in leaf isolates with the presence of necrotic spots, which also has been reported previously (Ariyawansa *et al.*, 2015; El-Dawy *et al.*, 2021; Iturrieta-González *et al.*, 2021; Martin-Feliux *et al.*, 2017).

The *Cladosporium* genera has been divided in three complexes: *C. cladosporoides*, *C. herbarum* and *C. sphaerospermum* (Iturrieta-González *et al.*, 2021; Martin-Feliux *et al.*, 2017; Sandoval-Denis *et al.*, 2016), in the present study the molecular characterization of the isolates formed a monophyletic group related to *C. cladosporoides* complex.

The presence of *Phoma*, *Cladosporium* and *Alternaria* genera are important because different species of the aforementioned genera are able to generate cytotoxic compounds such as: gliotoxin, tenuazonic acid and prenylated alkaloids (Bennett *et al*., 2018; Stranska *et al*., 2022; Klapec *et al*., 2022), which pose a risk for the food safety and the commercialization of these products in the international markets. On the other hand, previous studies were able to identify *Cladosporium* strains with antagonistic activity against phytopathogens and as plant growth promoters through the nutrient solubilization (Torres *et al*., 2017; Raynaldo *et al*., 2024; Zalewska *et al*., 2022; Patriarca *et al*., 2019; Mantzoukas *et al*., 2023; Räut *et al*., 2021).

CONCLUSIONS

In the present study isolates from leaf and fruit samples from *Vaccinium* were identified by the ITS region sequencing, where differences in the fungal composition were observed in dependence of the type of tissue sample. In this sense, several isolates with similar morphology could be associated, through his molecular identity in different genera. Most of the isolates were associated with saprophytic genera such as: *Phoma*, *Didymella*, *Alternaria* and *Botrytis*, where phytopathogenic members have been reported. These genera have been reported to have an impact in postharvest due fruit spoilage or by the presence of cytotoxic compounds that may pose a food-safety risk.

Fungal isolates identified as members of the *C. cladosporoides* complex were obtained, previous reports have found *Cladosporium* strains with antagonistic capabilities against pathogens and as plant growth promoters. Further research should be focused in the use of additional molecular markers in order to elucidate more accurately the fungal isolates. In this sense, the antagonistic and the growth promoting capability of certain isolates should be determined

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Consumptive water use in pecan trees in the Hermosillo Coast

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ABSTRACT
Objective: To estimate the consumptive use of water for pecan trees (*Carya illinoinensis*).
Design/methodology/approach: In this study, the consumptive use of water for pecan trees was estimated by applying the water balance equation ($Irrigation + rainfall - ET_c = 0$).
Results: The results obtained from the 2020 to 2023 cycles show that an average irrigation depth of 1,365 mm should be irrigate per cycle, similar to the ET_c estimated in situ by the Eddy Covariance Method.
Limitations on study/implications: Regional scope.
Findings/conclusions: The water balance is positive and shown a water surplus of 369 mm per cycle. This means that, considering only irrigation, savings of 147 mm per season could be achieved, representing an average of 1,470 m^3 ha^{-1} per agricultural cycle, without causing water stress for pecan trees.

Keywords: Water-balance, Soil-Water-depletion, Pecan.

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INTRODUCTION

The pecan tree (*Carya illinoinensis* K.) is one of the most profitable crops in the Hermosillo Coast. It currently occupies 51.2% (10,878 ha) of the total production in Sonora, with an average yield of 2.0 t ha^{-1} (Retes *et al.*, 2021; SIAP, 2023). The pecan tree has adapted well to this region, as it prefers arid and semi-arid climates. However, it has a long phenological cycle and high canopy coverage (Rodríguez *et al.*, 2022), which leads to high annual evapotranspiration (Brown, 2010; Rodríguez *et al.*, 2018; Rodríguez *et al.*, 2022).

Sammis *et al.* (2004), Brown (2010), and Valdez *et al.* (2010) report a seasonal and annual evapotranspiration (ETc) value of 1,200 to 1,450 mm in pecan orchards. Rodríguez *et al.* (2022) have measured ETc in pecan trees on the Hermosillo Coast using the Eddy Covariance (EC) method and report an average ETc of 1,469 mm. They have also recorded irrigation and rainfall, obtaining an annual average of 1,718 mm.

Valdez *et al.* (2010) recommended annual irrigation depths ranging from 1,360 to 2,100 mm for mature pecan trees. These irrigation depths were estimated using the reference evapotranspiration (ETo) recorded at agroclimatological stations located on the Hermosillo Coast. Rodríguez *et al.* (2010) reported an annual irrigation depth of 2,020 mm applied in a mature orchard on the Hermosillo Coast. Additionally, Rodríguez *et al.* (2022) observed a reduction in irrigation from 1,898 mm to 1,536 mm $year^{-1}$. This decrease in irrigation depths is attributed to the practice of burying irrigation lines.

Valdez *et al.* (2013) and Vieira *et al.* (2013) indicate that irrigation in pecan orchards in the region exceeds actual demand by 30%. Valdez *et al.* (2010 and 2018) mention that applying irrigation depths of 1,360 to 1,430 mm has resulted in average yields of 2.02 to 2.7 t ha^{-1} of high-quality pecans. Furthermore, daily irrigation depths applied in the region range from 8 to 10 mm day^{-1}, which is similar to atmospheric demand (Brown, 2010). Valdez *et al.* (2013) also point out that monthly requirements during the summer months can reach approximately 220 mm.

The differences between applied and evapotranspired irrigation depths indicate a potential reduction of approximately 200 mm per cycle for pecan trees. Therefore, it can be speculated that the annual irrigation depths for mature pecan orchards should range from 1,200 to 1,300 mm. However, there is still ongoing debate regarding the actual annual irrigation requirements.

Furthermore, the common practice in pecan irrigation management is to replenish soil water to field capacity (FC) after the available moisture, defined by FC and the critical irrigation point, has been depleted. However, the water content at FC is rarely determined in situ for each agricultural cycle. This often leads to a misperception of the water available for crops and, consequently, to poor irrigation management. Many agricultural soils are not uniform and may include horizons that restrict internal flow and drainage. Therefore, it is difficult to estimate the water retained in the soil that needs to be replenished through irrigation.

The latter necessitates the search for strategies to improve irrigation efficiency, such as drip irrigation, as well as determining the correct irrigation scheduling by estimating crop water requirements using precise *in situ* techniques like the Eddy Covariance method (turbulent techniques) and soil moisture monitoring, among others. Therefore, the objective of this study was to determine the monthly and annual irrigation depth required for pecan trees on the Hermosillo Coast by applying the water balance concept during the 2020 to 2023 cycles.

MATERIALS AND METHODS

The study has been conducted in a mature pecan orchard covering 108 ha in the Viñas de la Costa de Hermosillo plot (28° 55' 25", −111° 17' 59"). The pecan trees are planted with a spacing of 6 m between plants and 12 m between rows (139 plants ha^{-1}), and the orchard was established between 1999 and 2000. A 21-meter-tall micrometeorological tower was installed at the site. At the top of the tower, sensors are installed to measure net radiation (Rn), temperature (T), precipitation (P), and relative humidity (Rh). Additionally, an Eddy Covariance System (LI7550RS and WindMaster Pro Sonic Anemometer) is

installed. The data recorded by the micrometeorological tower are used to calculate the daily reference evapotranspiration (ET_o) and hourly *in situ* evapotranspiration (ET_c) of the pecan trees.

Time domain reflectometry sensors (TDR 315L, ACClima) were installed in the soil at 0.30, 0.60, 0.90 and 1.2 m, at 0.20 m from the irrigation line to measure the moisture content. These sensors were connected to a Datalogger DataSnap SDI-12 (Table 1). Data logging is done at 10 min intervals, and average values are stored every 30 min.

The soil moisture content at field capacity (FC) for the 0.3 and 0.6 m strata was determined according to the standard method, using the Richards' pressure extractor (Soil moisture Corp, USA) and drying the samples at 105 °C for 24 hours in a convection oven. The bulk density was measured using the Uhland cylinder method (Gabriels and Lobo, 2006).

Irrigation is applied through a drip system using RAM-type irrigation pipes, with a dripper flow rate of 1.6 L h^{-1} spaced 0.6 m apart. Two irrigation lines were placed on each side of the pecan tree row. The first irrigation line is located 1.5 m from the trunk, and the second line is positioned 1.5 m from the first, resulting in an average of 40 drippers per tree. Beneath one of the drippers, a rain gauge (Texas Electronics) was installed to record the irrigation depth applied and the duration of each irrigation event.

Evapotranspiration

Reference evapotranspiration (ET_o) was calculated using daily meteorological data recorded and the FAO56 approach (Allen et al., 1998) (Equation 1).

$$ET_o = \frac{0.408\Delta\left(R_n - G\right) + \gamma \frac{900}{T+273} u_2 \left(e_s - e_a\right)}{\Delta + \gamma\left(1 + 0.34u_2\right)} \quad (1)$$

where: ET_o is the reference evapotranspiration (mm·d^{-1}), R_n is net radiation (MJ m^{-2} d^{-1}), G is soil heat flux (MJ m^{-2} d^{-1}), T is the average daily air temperature (°C), Δ is the slope of the saturation vapor pressure curve at T (kPa $°C^{-1}$), γ is the psychrometric constant (kPa $°C^{-1}$), e_s is saturation vapor pressure at T (kPa), e_a is average daily vapor pressure (kPa), and u is average daily wind speed (m s^{-1}).

Meanwhile, the calculation of ET_c was performed using the general equation (2) (Burba, 2022) with the EddyPro ver. 6.0 software (LI-COR), utilizing the averaged data recorded at 30-minute intervals with the Eddy Covariance System equipment.

$$F \approx \underline{\rho_\alpha}\,\underline{W'S'} \quad (2)$$

where: F represents the latent heat flux (LE; W m^{-2}), sensible heat (H; W m^{-2}), and CO (mg m^{-2} s^{-1}); ρ_α is the air density (kg m^{-3}), W' is the vertical wind velocity (m s^{-1}), and S' represents the covariance of fluctuations in water, heat, carbon dioxide, methane, etc., respectively.

By expressing equation 2 in terms of the energy balance (equation 3), equation 4 is obtained.

$$LE + H = R_n - G \tag{3}$$

where R_n represents net radiation (W m^{-2}), and G represents the soil heat flux (W m^{-2}). From equation 3, LE is expressed in equivalent energy $ET\lambda$, and then the corrected $ET\lambda$ in the Eddy Covariance System is converted into an equivalent water depth ET (mm h^{-1}) (Wang *et al.*, 2020), expressed as:

$$ET = \frac{3600 * \lambda ET}{\lambda * \rho_W} \tag{4}$$

where the value of λ is 2.501−0.00236 *Ta* (*Ta*=air temperature, °C), and ρ_W is the density of water vapor (103 kg·m^{-3}), with 3600 being the conversion factor from hours to seconds.

Water balance

Pereira *et al.* (2010) recommend using the concept of water balance (WB) (Equation 5) to estimate the water inputs and outputs in the soil over a time interval Δt, considering a soil layer of thickness Z, bounded at the top by the soil surface and at the bottom by the depth Zn, as previously defined.

$$\theta_i = \theta_{i-1} + \frac{\left(P_i - Q_{ri}\right) + I_{ni} - ET_{ci} - DP_i + GW_i}{1000\, z_{ri}} \tag{5}$$

where: θ_i is the soil water content in the root zone (mm·mm^{-1}) on day i; θ_i-1, is the soil water content in the root zone (mm·mm^{-1}) on day i^{-1}; P_i, is the precipitation on day i (mm); Q_{ri}, is the surface runoff on day i (mm); I_{ni} is the irrigation depth on day i (mm), or the amount of irrigation water that actually infiltrates for storage in the root zone; ET_{ci} is the crop evapotranspiration on day i (mm); DP_i is the percolation on day i (mm); and GW_i is the accumulated capillary rise flow on day i (mm).

However, Equation 5 considers the variables Q_{ri}, DP_i, and GW_i, which are difficult to record, measure, or estimate on a daily basis. Therefore, in this study, the water balance (WB) was calculated considering the input variables (irrigation and rainfall) and the output variable (ET_c), ($WB=Irrigation+Rainfall-ET_c$) for a daily time interval for the cycles from 2020 to 2023.

RESULTS AND DISCUSSION

Under the concept of the water balance ($WB=Irrigation+Rainfall-ET_c=0$), it was determined that an excess of water exceeding 300 mm per cycle is applied in the pecan

Table 1. Sensors installed in the micrometeorological tower of the pecan walnut orchard in Viñas de la Costa de Hermosillo, Sonora.

Measured factors	Sensor	Measurement height (m)
Sensitive heat flux (H, W m^{-2})	3D Sonic anemometer (CSAT3, Campbell Scientific Ltd. USA)	21.0
Latent heat flux (LE, W m^{-2})	IRGA 7500Rs (LICOR, USA) and 3D Sonic anemometer	21.0
Soil heat flux (G, W m^{-2})	Plate HFP01SC (Hukseflux)	−0.1
Carbon flux (CO_2, μmol m^{-2} s^{-1})	IRGA 7500Rs (LICOR, USA) and 3D Sonic anemometer	21.0
Temperature and air humidity (°C and %)	HMP60 (Vaisala, Finland)	15.0
Wind speed and direction (m s^{-1}, degrees)	3 D Sonic anemometer (CSAT3, Campbell Scientific Ltd. USA)	21.0
Soil temperature (°C)	TDR315L (Acclima, USA)	−0.30, −0.60, −0.90, − 1.20
Soil moisture (m^3 m^{-3})	TDR315L (Acclima, USA)	−0.30, −0.60, −0.90, − 1.20
Precipitation (mm)	Rain gauge (Texas Electronics, USA)	12.0
Irrigation (mm)	Rain gauge (Texas Electronics, USA)	0.5
Net radiation (W m^{-2})	Net Radiometer (Kipp & Zonen, Netherlands)	19.2
Incident solar radiation (W m^{-2})	Albedometer (Kipp & Zonen, Netherlands)	19.2
Reflected solar radiation (W m^{-2})	Albedometer (Kipp & Zonen, Netherlands)	19.2

walnut orchard at the study site (Table 2). If rainfall is disregarded, this excess due to irrigation is between 60 to 235 mm, considering that an annual irrigation application of over 1500 mm is applied (Table 2).

This indicates that appropriate adjustments are not made to the applied irrigation amounts during the rainy season (July and August). Table 2 also shows that the average actual evapotranspiration of the pecan (ET_c) is around 1365 mm per cycle. The ET_c does not show significant differences between cycles, and the trend is similar across all four cycles (Figure 3).

This allows us to indicate that average irrigation amounts of 1,365 mm per cycle should be applied, similar to the ET_c determined *in situ*. If rainfall is not considered, this would represent a reduction of 147 mm in the annual irrigation amount applied, which means that savings of approximately 2000 m^3 ha^{-1} per cycle (2.0 thousand m^3 ha^{-1}) could be achieved (Table 2). These results support the proposals by Valdez *et al.* (2010, 2018) and Vieira *et al.* (2013), which indicate that by applying irrigation amounts of 1360 to 1430 mm to mature trees, average yields of 2.02 to 2.7 t ha^{-1} of high-quality pecans can be achieved. Furthermore, this aligns with what Sammis *et al.* (2004) and Rodríguez *et al.* (2010) mention,

Table 2. Water Balance in Pecan Orchard in the Costa de Hermosillo, Sonora.

Crop Season	Water balance					Thousand m^3 ha^{-1}
	Irrigation (mm)	Rain (mm)	ETc (mm $año^{-1}$)	R+Ll−ETc	m^3 ha^{-1}	
2020	1594	153	1445	(+) 300	1446	1.45
2021	1528	267	1293	(+) 500	2410	2.41
2022	1415	246	1356	(+) 305	1463	1.46
2023	1370	140	1356	(+) 150	736	0.74
Average	1476	201	1362	(+) 315	1514	2.0

that a pecan orchard in production can consume an amount of approximately 1.4 m of water per year.

The annual ET_c is also consistent with the values of 1200 to 1450 mm reported by Sammis *et al.* (2004), Brown (2010), Valdez *et al.* (2010), and Rodríguez *et al.* (2022). Furthermore, Figure 3 shows that the maximum daily ET_c values between 8 and 9 mm during the spring-summer season are similar to those observed by Sammis *et al.* (2004) and Djaman *et al.* (2018) for arid and semi-arid climates. On the other hand, analyzing the soil moisture content (θv) and its variation due to the frequencies and amounts of irrigation applied (Figure 1), values close to field capacity (FC) are observed. The figure shows that in some periods, the moisture remained above FC, even in the layers of 60, 90, and 120 cm. This means that the applied amounts exceed the soil's retention capacity and the plants' demand through evapotranspiration.

Soil moisture exhibited the same behavior across the four cycles. This moisture reaches values of 28 to 31% (θv) from March to September. It then shows a decrease to between 22 and 27% (θv) from September to November, due to the reduced frequency of irrigation. It is also observed that in all four layers, there is movement of water and soil moisture, indicating a redistribution towards deeper layers, as well as extraction by the roots of the pecan tree and possibly some percolation.

As previously mentioned, the common practice in irrigation management for pecan trees is to replenish soil water to field capacity (FC) after the readily available moisture has been consumed. However, FC is not necessarily achieved in the field, as shown in Figure 1. This leads to a misperception of the irrigation requirements, as well as the available water for the crop, and consequently results in poor irrigation management.

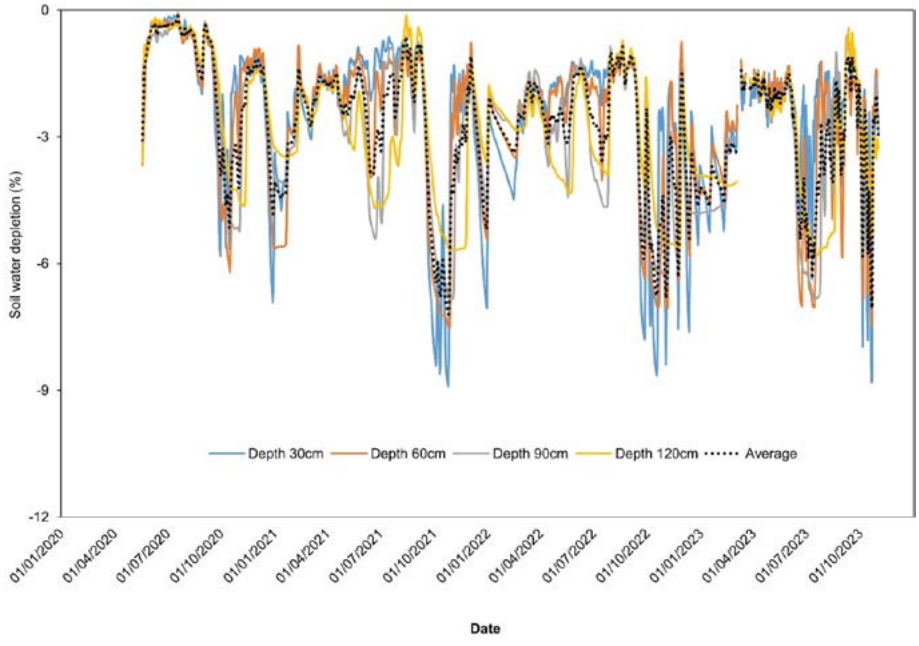

Figure 1. Irrigation depths applied in the pecan orchard in the Costa de Hermosillo, Mexico, and variation of soil moisture content (θv) in the soil profile.

To reduce ambiguity regarding the available water in the soil profile, the concept of "soil water depletion" proposed by Evett *et al.* (2019) was used. The maximum soil water content was utilized as a reference point, and depletion was determined for each stratum. The water content values were converted into equivalent values for each depth to minimize ambiguity concerning the soil's water storage and retention capacity (Figure 2).

This approach reveals a more consistent representation of the soil's response to the application and removal of water. In Figure 2, it is observed that in the 30, 60, and 90 cm strata, there was greater depletion, between 5 to 8.5% (θv), equivalent to 65% of the total soil moisture. It is also noted that the greatest depletions of water in the soil profile occur from the second week of September to the third week of November across all four cycles. This coincides with the decrease in irrigation frequency, as this stage corresponds to the opening of the husk, harvesting, and leaf drop of the pecan trees in the area.

The use of sensors, such as the TDR 315L, and the implementation of the concept of "soil water depletion" allow for a more precise estimation of the amount of water that needs to be replenished during each irrigation event. This approach eliminates the influence of factors such as texture, bulk density, and organic matter content on the recorded values. Furthermore, the TDR 315L sensors have a margin of error of about 2% of θv (Acclima, Inc. 2017).

If the depletion of water in the soil profile is expressed in terms of depth (mm d^{-1}), the retention and removal capacity of water is further clarified (Figure 3). This figure clearly shows the applied irrigation depths (mm d^{-1}) and the amount of water stored and removed in the soil profile (mm d^{-1}) from 30 to 120 cm. The figure also displays the amount of water transpired (mm d^{-1}). In this figure, it is observed that the soil can retain up to 12

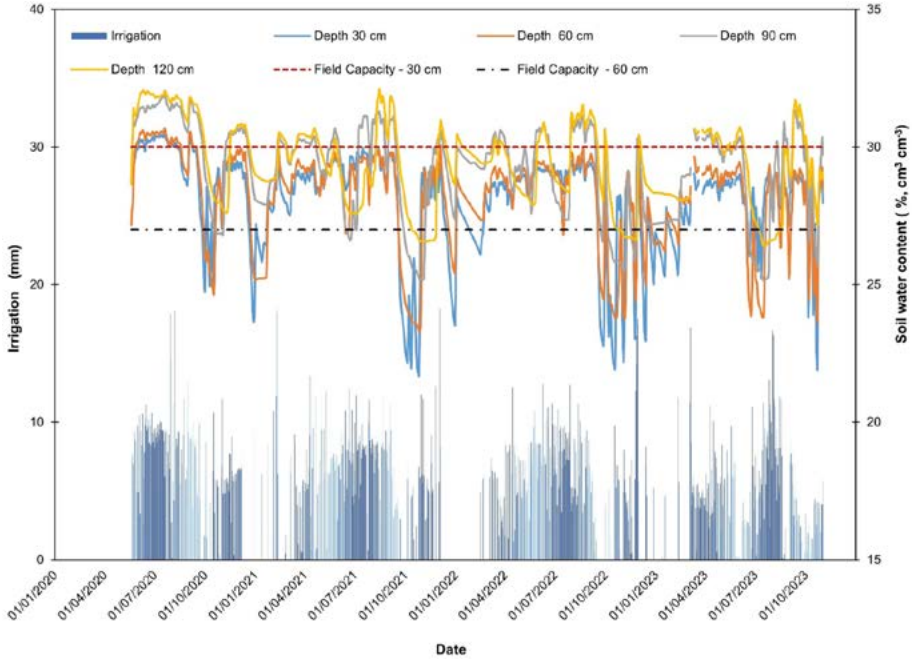

Figure 2. Maximum soil water content and depletion by strata, equivalent values, applying the concept of "soil water depletion".

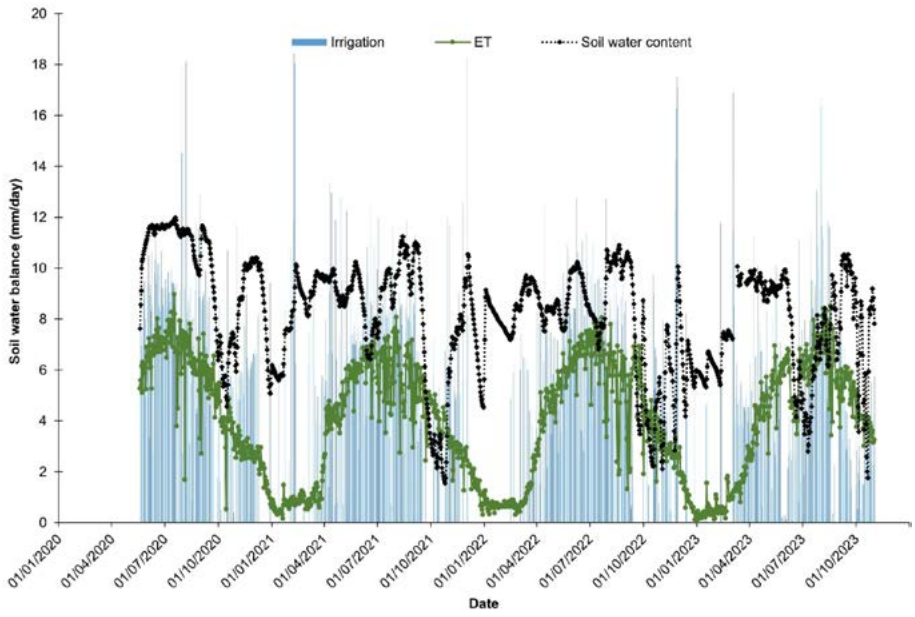

Figure 3. Irrigation, crop evapotranspiration, and available water in the soil profile in a pecan orchard in the Costa de Hermosillo, Sonora.

mm d^{-1}. It is also noted that the amount of water removed from the soil profile can reach up to 9.5 mm (from 12 to 2.5 mm), indicating that total depletion is not reached. This removed amount, during certain periods of the pecan cycle, is equivalent to the maximum ET_c observed in the crop. Conversely, the applied irrigation depths per event are always greater than the ET_c, and in some cases, they reach up to 18 mm per day (Figure 3).

This causes the soil to have available moisture for most of the pecan cycle. Only during the period from the second week of September to the third week of November does the greatest depletion occur. However, with the decrease in the evapotranspirative rate due to the senescence of the pecan tree, and subsequent irrigations, the available moisture is restored to between 5 and 8 mm for the next cycle. This reinforces the recommendation that irrigation depths of 8 to 10 mm per event should be applied, as previously indicated. These irrigation depths are related to the water retention capacity in the soil profile and the evapotranspirative rate of the pecan tree in the study site (Figure 3).

With the results shown in Figure 3, it is possible to generate and recommend an irrigation schedule. In this regard, a calendar with monthly irrigation amounts for pecans is proposed in Table 3. These monthly amounts can be applied according to the crop demand (ET_c) and the suggested irrigation amounts of 8 to 10 mm per event. Generally, irrigation calendars for most crops are reported on a monthly basis.

This calendar considers applying at least one irrigation in January to complement the residual soil moisture of around 5.5 mm (Figure 3). In February, it is proposed to apply two irrigations. In the following months, the frequency of irrigation increases due to the sprouting of the pecan tree foliage, fruit formation, and dry matter accumulation. By implementing this irrigation calendar, the consumptive use of the pecan tree would be around 1368 mm by the end of the production cycle (Table 3). This annual calendarization

Table 3. Recommended irrigation amounts for pecan trees at the Viñas de la Costa site, Hermosillo, Sonora.

Month	Crop season				Monthly irrigation (cm)	Standard deviation
	2020	2021	2022	2023		
January	1.0	1.5	1.5	1.5	1.4	0.3
February	3.1	1.4	1.6	3.1	2.0	0.9
March	4.5	4.4	4.7	4.5	4.5	0.2
April	12.3	9.4	13.4	12.3	11.7	2.1
May	16.8	17.0	17.5	16.8	17.1	0.4
June	20.6	18.5	20.3	16.8	19.8	1.1
July	22.9	18.8	20.1	18.8	20.6	2.1
August	18.6	17.8	17.6	20.5	18.0	0.5
September	17.2	14.9	14.3	16.5	15.5	1.5
October	11.9	12.4	12.5	12.1	12.3	0.3
November	8.6	8.5	9.1	8.2	8.7	0.3
December	6.8	5.4	3.5	5.0	5.2	1.7
Total	144.2	130.0	136.0	136.0	136.8	

and consumptive use is similar to that recommended by Valdez (2010, 2015, and 2018). Although the same author mentions that the irrigation amount for March includes a moisture reservoir corresponding to an additional amount of 42 mm. However, as shown in Figure 3, the soil has available moisture for the crop during that period.

CONCLUSIONS

The results presented in this study indicate that the consumptive use of the pecan tree for the study site is approximately 1365 mm per year. Therefore, it is feasible to reduce the current irrigation amounts by 150 mm without causing water stress to the pecan tree, reinforcing the proposal by Rodríguez *et al.* (2022). This reduction represents a savings of around 1470 m^3 ha^{-1} per cycle per hectare (1.47 thousand m^3 ha−1). This strategy will allow for efficient water use without significantly impacting yields, considering that, in most cases, as shown in this work, there is a general trend toward over-irrigation.

On the other hand, the use of the water balance, soil moisture sensors, and the "soil water depletion" concept allows for a more accurate estimation of the amount of water that should be replenished in each irrigation event.

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Response to chemical and organic fertilization in date palm (*Phoenix dactylifera* L.) Mejhoul variety in Northwestern Mexico

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ABSTRACT

This research was conducted to determine the effect of chemical, organic, and combined fertilization on the fruit yield of the Mejhoul date palm variety in Northwestern of Mexico. The variables measured were polar diameter, equatorial diameter of the fruit, roundness index, number of clusters, number of leaves per palm, number of leaves per cluster, fruits with separated skin, dried fruits and commercial yield. The design used was complete randomized blocks with five replicates, where each palm tree was the experimental unit. The results indicate that chemical fertilization 262N-138P_2O_5-540K_2O alone or in combination with compost application (three or six tons per hectare) increases polar and equatorial diameter, promoting the oval growth of dates. Fruits with separated skin continue to occur regardless of the origin of the fertilizers. The number of fruits increases with chemical fertilization, but the total yield of dates remains consistent across the treatments evaluated. In conclusion, date palms respond favorably to the combination of chemical and organic fertilization, but date yields did not increase.

Keywords: Dates, chemical fertilizers, organic fertilizer, yield.

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INTRODUCTION

The date palm (*Phoenix dactylifera* L.) is one of the oldest cultivated fruits and is increasing its cultivation area worldwide. It is known as the "tree of life" due to its resilience, its ability to adapt to limited water supply, its long-term productivity, and its multipurpose anthropocentric qualities. Additionally, it is the most common fruit tree grown in warm, semi-arid, and arid regions (Marzouk, 2011). Dates are rich in numerous therapeutic, bioactive, and functional compounds such as polyphenols, dietary fiber, carotenoids, vitamins, amino acids, carbohydrates, and minerals, making them one of the most nutritious natural foods (Ibrahim *et al.*, 2021; Noutfia and Ropelewska, 2022).

Currently, around 9.61 million tons of dates are produced worldwide on 1.25 million hectares of land (FAO, 2024). From 2021 to 2022, the cultivated area of date palm in Mexico grew by 8.12 percent, reaching 3,268 hectares that produced 19,465 tons of this fruit. Mexico ranks third in the world as a producer, after Israel and the United States, of the Mejhoul date, which is recognized for its high nutritional quality and its contribution to territorial development in these regions of the country, with social inclusion and justice. The main date producers are San Luis Río Colorado, Sonora, with 55 percent of the total national volume, followed by Mexicali, Baja California, with 41 percent. In 2022, these regions exported just over seven thousand tons.

The yield and quality of date palm fruit largely depend on the cultivar, nutrition, water relations, soil type, type of fertilizers, and the fertilization program, which determine the efficiency of the production system in relation to the previous year's yield and the quality of the dates. It is common to supplement the nutritional requirements of date palms mostly with chemical or synthetic fertilizers. However, the continuous use of chemical products leads to the deterioration of soil characteristics and fertility (Shimbo *et al.*, 2001).

Organic fertilizers have been used in agriculture to improve soil fertility, promote plant growth and protection, which enhances crop productivity. According to Ghouili *et al.* (2022), the application of organic amendments such as compost could be considered a good strategy for environmentally sustainable management. Cultivating date palms in organic systems represents the optimal solution for sustainable production through the use of organic fertilizers (compost), biofertilizers (nitrogen-fixing bacteria, phosphate-solubilizing microorganisms, and potassium-solubilizing biological microorganisms), as well as biocontrol agents (Safwat, M.S.A. 2007).

Magdoff (1998) suggests that organic matter from manure is an excellent source of macro and micronutrients such as nitrogen, phosphorus, and potassium (N, P, and K), and its incorporation into the soil increases the availability of P and exchangeable contents of K, calcium, and magnesium. The application of shredded organic matter or compost in date palms provides additional benefits by reducing water evaporation from the soil surface, helping to control weed invasion, suppressing dust, preventing soil erosion from wind or water, and providing thermal stabilization by keeping the soil cooler in hot climates and warmer in cold climates (EL-Mously *et al.*, 2023).

Similarly, the application of organic fertilizers improves soil structural stability and decreases bulk density, increasing moisture retention, water infiltration rate, and soil hydraulic conductivity (Tisdale *et al.*, 1990; Young, 1997).

The recommended doses of N, P, and K conventionally applied to date palms vary widely among farmers in the region, possibly due to limited knowledge of the actual nutritional needs of date palms, soil variations, climatic conditions, irrigation systems, tree age, and the lack of research related to fertilization. In this regard, research conducted on Iraqi and United Arab Emirates date palms recommends approximately 2.3 kg of N, 1.2 kg of P, and 1.4 kg of K per tree (Al-Rawi, 1998).

On the other hand, the FAO calculated a global average application of 0.65 kg of N $tree^{-1}$ 20 years ago (Klein and Zaid, 1999). However, more recent research suggests that the optimal application rate of N could range from 0.4 to 3.6 kg $tree^{-1}$ $year^{-1}$,

depending primarily on the type of fertilizer, application method, and the presence of other micronutrients (Al-Qurashi *et al.*, 2015; Hesami *et al.*, 2017; Ibrahim *et al.*, 2013; Saleh, 2009). In Israel, nitrogen fertilization recommendations range from 1.5 kg of N tree^{-1} year^{-1} for three-year-old trees, up to 3.6 kg of N tree^{-1} year^{-1} by the sixth year, for palms grown in sandy soil with drip irrigation (Wright, 2012). This is because research in Israel has shown that the estimated annual nitrogen consumption of a mature date palm is around 2 kg of N (MOAG, 2019).

However, the most recent fertilization recommendations suggest a concentration of 35 mg N L^{-1} of fertilizer in fertigation (fertilization and irrigation) throughout a year (MOAG, 2015). As a result, this would lead to a triple dose of 6 kg of N mature tree^{-1}, and with 123 trees planted per hectare, a total fertilization of 732 kg N ha^{-1} year^{-1} would be achieved.

According to Kassem (2012), in Zaghloul date palms, fertilization with 1000 grams per palm of ammonium sulfate increased fruit yield, weight, length, and color compared to ammonium nitrate or urea. Additionally, potassium and sulfur fertilization improved the physicochemical characteristics of the fruit. Poultry manure (PM) and cow manure (CM) resulted in the best fruit weight, pulp weight, and fruit length in twenty-six-year-old Zaghloul palms grown in clay loam soil (Kassem and Marzouk, 2010).

Plants require nutrients such as N, P, K, Ca, Mg, S, Fe, Mn, Zn, and other trace elements for their growth and development, which can be supplied through organic and inorganic sources. Nutrients from organic sources decompose more slowly, and plants take longer to absorb them, whereas inorganic fertilizer sources are readily available (Nyamangara, 2020; Chen, 2023). Inorganic mineral fertilizers combined with organic manures have been shown to have a positive impact on global food production and are an essential part of many agricultural production systems (Hernández, 2019).

The use of fertilization, based on the application of both compost and chemical fertilizers, can improve the yield of date palms and increase farmers' income, while also restoring soil fertility. This research was conducted to determine the effect of applying chemical, organic, and combined fertilization on the fruit yield of the Mejhoul date palm variety in Northwestern Mexico.

MATERIALS AND METHODS

The experiment was conducted during the 2023 season in a 26-year-old plantation located in Ejido Tula, Mexicali Valley, Baja California, Mexico (32.56453167449391, −115.20770875361818). The variety was Mejhoul. The trees were planted at 8 meters between palms and 8 meters between rows. The palms were grown in sandy loam soil with a localized irrigation system using tubing and gravity irrigation. Pollination was done manually by obtaining pollen from male flowers and subsequently placing it on female flowers. After five weeks, fruit thinning was performed at the "Hababouk" stage, with a spacing of 2.5 cm between fruits and 8 to 10 fruits per bunch.

The physical and chemical characteristics of the soil are as follows: loam texture, bulk density of 1.04 g/cm^3, hydraulic conductivity of 4.80 cm hr^{-1}, pH of 8.07, carbohydrates at 9.36%, 3.66 dS m^{-1}, 0.92% organic matter, 19.6 ppm N, 23.1 ppm P_2O_5, 192 ppm K_2O,

3323 ppm Ca, 463 ppm Mg, 239 ppm S, 442 ppm Na, 5.16 ppm Fe, 0.42 ppm Zn, 5.48 ppm Mn, 0.63 ppm Cu, and 1.76 ppm B.

The palms were fertilized with inorganic and organic products either alone or in combination, according to the treatments. The inorganic fertilizer used was ammonium nitrate (33.5% N)+phosphoric acid (54% P_2O_5)+potassium nitrate (12.66% N+43.3% K_2O+3.05% S) and heptahydrated magnesium sulfate (13.8% S+10% Mg).

The organic fertilizer was compost made from bovine manure, with 21.91% organic matter, pH 8.89, EC less than 1.4 dS m^{-1}, CEC less than 40 cmol l^{-1}, 1.08% N, 0.02% P_2O_5, 1.40% K_2O, 0.26% Ca, 0.78% sulfate, 0.5% humic acids, 0.5% fulvic acids, beneficial microorganisms, a C/N ratio of 11.5, moisture content less than 25%, and a physical appearance of granulated powder less than 10 mm.

The treatments were as follows: T1-COMPOST (38.46 kg of compost per palm), T2-FERQUIM with chemical fertilization (262 N-138 P_2O_5-540 K_2O), T3-CP50%+FQ50% (chemical fertilization 131 N-69 P_2O_5-270 K_2O plus 19.23 kg of compost per palm), T4-CP100%+FQ100% (chemical fertilization 262 N-138 P_2O_5-540 K_2O plus 38.46 kg of compost per palm), and T5-FERCONV (conventional fertilization 456 N-162 P_2O_5-252 K_2O). The application of the fertilization doses was distributed across the months as follows: February (5%), March (10%), April (12%), May (20%), June (21%), July (14%), August (8%), September (5%), and October (5%).

Yield Estimation: The fruits were harvested at the Tamer stage, characterized by less than 25% fruit moisture. Harvesting began in September and continued until the end of October. The average yield and weight of the bunches were recorded in kilograms. Samples of 50 fruits from each palm were randomly taken (as a sample for each replicate) to determine fruit quality characteristics.

Fruit Weight (g): From the 50 fruits, samples of 20 fruits from one bunch per palm were taken. The weight was obtained using a digital electronic scale (Scout Pro SP 602, Ohaus®, USA), with a capacity of 0.6 kg and a sensitivity of 0.01 g.

Polar and Equatorial Diameter (mm): Samples of 20 fruits from one bunch per palm were taken. Measurements were made with a caliper (CAL-6MP, Truper®, Mexico) along the polar and equatorial planes of the fruit.

Roundness Index (dimensionless): Using the polar and equatorial diameter data, this index was calculated using Mohsenin's (1986) formula, where Ra is the roundness or sphericity, Ap is the projected area of the fruit corresponding to the polar diameter in mm, and Ac is the projected area of the fruit corresponding to the equatorial diameter in mm.

$$Ra = \frac{Ap}{Ac}$$

Fruits with separated skin and dry: Samples of 20 fruits from a bunch per palm were taken and classified by comparing the total area of fruits with more than 10% of separated skin, known as "pouchy" fruits.

Experimental design: The design used was a completely randomized block design with five replications, with one palm as the experimental unit.

Statistical analysis: All data were tested to determine the effects of the treatments using analysis of variance (ANOVA) and multiple mean comparisons using Tukey's test ($P \leq 0.05$), with the assistance of Statistical Analysis Software (SAS Institute, 2002).

RESULTS AND DISCUSSION

Polar diameter and equatorial diameter of the fruit

In this study, the application of compost alone for date palm nutrition showed significant differences ($P \leq 0.05$) in polar diameter and equatorial diameter, with values of 25.99 mm and 39.44 mm, respectively. This is in comparison to treatments involving chemical fertilization or a combination of chemical and organic fertilizers (Table 1).

In general, it is observed that fertilizing with chemical sources and their combinations with organic fertilizers increases both the polar diameter and the equatorial diameter of the fruit, with the polar diameter always being greater. This results in the fruit maintaining an elliptical or elongated shape. These results regarding the polar and equatorial diameter of the fruit are consistent with general opinions and trends reported in various cultivars (Idris *et al.*, 2014; Mahawar *et al.*, 2017; Metwally *et al.*, 2019).

Roundess index

The relationship between the equatorial and polar diameter, expressed as the roundness index, was significant ($P \leq 0.05$) for T1-COMPOSTA, with a value of 1.76. In general, the fruits that had a larger equatorial diameter were from T1-COMPOSTA (25.99 mm), but with a smaller polar diameter (39.44 mm), resulting in a higher roundness index (1.76). However, in the other treatments, the fruits were elongated, which is a characteristic of the Mejhoul variety (Table 1), with the characteristic "elongated" pattern, meaning those fruits had a greater polar diameter than equatorial diameter (Montoya-Holguin *et al.*, 2014).

Although for tomatoes (*Solanum lycopersicum*) Becvort-Azcurra *et al.* (2012) mention that this characteristic shows a stronger correlation with genotype than with environmental and agronomic management factors. However, in the case of the present study, it was found that for date fruits, this characteristic is related to the number of fruits per bunch, the number of bunches per palm, and the total number of fruits per bunch per palm. This suggests a source-demand relationship where a greater number of fruits leads to more rounded growth.

Table 1. Equatorial diameter, polar diameter, and roundness index in date fruits with chemical, organic, and combined fertilization.

Treatment	Repetitions	Equatorial diameter of the fruit (mm)	Polar diameter of the fruit (mm)	Roundness index
T1-COMPOST	5	25.99 a	39.44 b	1.76 b
T2-FERQUIM	5	21.84 b	44.69 a	2.07 a
T3-CP50%+FQ50%	5	21.27 b	43.55 a	2.06 a
T4- CP100%+FQ100%	5	22.02 b	44.52 a	2.04 a
T5-FERCONV	5	20.95 b	43.67 a	2.09 a

*Means with the same letter within each column are statistically equal (Tukey, $p \leq 0.05$).

The above is corroborated by the research of Mahawar *et al.* (2017), who found that in dates c.v. Khadrawy and Medjool or Mejhoul, the roundness or sphericity of c.v. Khadrawy and Medjool were 0.60±0.03 and 0.71±0.024, respectively, determining that the fruits of date palm c.v. Medjool, being larger, achieved greater roundness, which resulted in higher sphericity values. However, according to Noutfia and Ropelewska (2022), this is due to the determination of the elliptical shape factor (W1) of 1.000 and the circular shape factor (W2) of 0.110, which confirmed the elliptical shape of the "Mejhoul" fruit.

Number of clusters, number of leaves, and number of leaves per cluster

No statistically significant differences were found for the number of clusters and the ratio of the number of leaves per cluster among the treatments evaluated. However, for the number of leaves, there were significant differences in the combinations of chemical fertilization with organic fertilizer, with values ranging from 72.8 to 74.8 leaves per palm. The highest values were observed in T2-FERQUIM (84.0 leaves per palm), T1-COMPOSTA (83.6 leaves per palm), and T5-FERCONV (74.8 leaves per palm), as shown in Table 2.

The leaves are very important for maintaining a balance between source and demand in date palms; according to Said *et al.* (2002), the distance from the fiber at the base of the leaf to the base of the leaflets is 28% of the entire leaf, where only the spines occupy around 4%, and the leaflets around 62%. Al-Sekhan (2009) suggests that maintaining a higher number of clusters may affect the source-demand balance in palms, ultimately negatively impacting fruit size and quality, as well as reducing the plant's growth and yield in subsequent years.

Panchal *et al.* (2021) found that increasing the number of leaves per cluster proportionally increases the final yield of the palm, with a leaf-to-cluster ratio of 4:1 compared to an 8:1 ratio, the latter resulting in the highest yield. Similarly, fruit characteristics also improved with a higher leaf-to-cluster ratio. Hegazi *et al.* (2008) found that retaining 9-11 leaves per cluster is adequate for the date cultivar Oreabi, while a ratio of 5 leaves per cluster resulted in lower yield and poorer fruit quality under the conditions of the Lower Delta region of Egypt.

In general, the production capacity and fruit quality of the Mejhoul date palm variety are proportional to the number of leaves and, consequently, to the leaf area. An excess of fruit relative to the leaf area of the palm reduces fruit size and quality and also promotes alternate production.

Table 2. Number of bunches, number of leaves, and the leaf-to-bunch ratio in the Mejhoul variety. Mexicali Valley, Baja California, Mexico. October 2023.

Treatment	Repetitions	Number of clusters	Number of leaves per palm	Ratio of the number of leaves per bunch
T1-COMPOST	5	15.8 a	83.6 a	5.34 a
T2-FERQUIM	5	16.2 a	84.0 a	5.26 a
T3-CP50%+FQ50%	5	17.6 a	72.8 b	4.26 a
T4- CP100%+FQ100%	5	16.1 a	71.6 b	4.55 a
T5-FERCONV	5	14.4 a	74.8 ab	5.23 a

*Means with the same letter within each column are statistically similar (Tukey, $p \leq 0.05$).

Yield

According to Table 3, there were no statistically significant differences in fruit weight and average fruit weight. However, the number of marketable fruits was higher with conventional fertilization (T5-FERCONV, 338.8 fruits), but these were smaller in size. It was observed that when analyzing other variables, the total weight of marketable fruits was statistically similar in T3-CP50%+FQ50% (1924.8 g), T4-CP100%+FQ100% (2563.2 g), and T5-FERCONV (4138.3 g).

This is consistent with the findings of Mahawar *et al*. (2017), who observed that in dates of the c.v. Khadrawy and Medjool, the weight of 100 fruits was 860.45±4.64 g for Khadrawy and 2199.6±13.30 g for Medjool. They noted an approximate 155% increase in the weight of 100 fruits for the Medjool variety compared to the Khadrawy variety, attributed to the proportional increase in fruit size in Medjool compared to Khadrawy. The responses to this combination were better than the independent application of each of them.

In this regard, Elamin *et al*. (2017) observed similar responses in the Khenazi cultivar with the application of N, P, K combined with organic matter concerning fruit pulp weight, pulp-to-seed ratio, fruit length, fruit width, fruit volume, ripeness, number of fruits per strand, number of fruits per cluster, and fruit yield. This is due to the fact that the application of macro and micronutrients increases date production and improves fruit quality (Al-Rawi, 1998; Yahia and Kader, 2011).

Fruits with Skin Separation and Dryness

Skin separation (appearance of swelling or peeling of the fruit's skin) in date palm fruits is a critical physiological disorder that significantly reduces the visual appeal and quality of the fruit. Skin separation (swelling) typically occurs when the date's skin is dry, hard, and brittle. According to Table 4, there were no statistically significant differences in the number, weight, and average weight of fruits with separated skin, nor in the number of dry fruits. Physiological disorders associated with skin separation potentially reduced the visual quality of the fruit and therefore caused economic losses for the producer by lowering its commercial value. The price range for fruit with 30-50% skin separation (swelling) is approximately 80% lower than that of fruit with intact and normal skin (without swelling).

Table 3. Weight of fruits, average fruit weight, number of commercial fruits, and total weight of commercial fruits with chemical, organic, and combined fertilization in Mejhoul date palm in the Mexicali Valley, Baja California, Mexico. October 2023.

Treatment	Fruit weight (g)	Average fruit weight (g)	Number of commercial fruits	Weight of commercial fruits (g)
T1-COMPOST	263.18 a	13.16 a	94.4 b	1270.4 b
T2-FERQUIM	263.14 a	13.15 a	49.75 b	712.1 b
T3-CP50%+FQ50%	292.60 a	14.63 a	163.60 b	1924.8 ab
T4- CP100%+FQ100%	319.10 a	15.96 a	160.80 b	2563.2 ab
T5-FERCONV	296.20 a	14.81 a	338.80 a	4138.3 a

* Means with the same letter within each column are statistically similar (Tukey, $p \leq 0.05$).

Table 4. Number, total weight, average weight of fruits with separated skin, and number of non-commercial dry fruits with chemical, organic, and combined fertilization in Mejhoul date palm in the Valley of Mexicali, Baja California, Mexico. October 2023.

Treatment	Number of fruits with separated skin	Weight of fruitswith separated skin (g)	Average weight of fruit with separated skin (g)	Number of dried fruits
T1-COMPOST	31.4 a	309.4 a	11.01 a	33.02 a
T2-FERQUIM	46.5 a	1133.5 a	25.94 a	20.75 a
T3-CP50%+FQ50%	58.4 a	676.1 a	12.95 a	33.41 a
T4- CP100%+FQ100%	54.4 a	739.1 a	13.79 a	25.81 a
T5-FERCONV	82.1 a	1125.8 a	13.25 a	37.81 a

* Means with the same letter within each column are statistically similar (Tukey, $p \leq 0.05$).

Dry and total fruits

No significant statistical differences were found for dry fruit weight, average weight of a fruit, and total fruit weight. However, there were significant differences ($p \leq 0.05$) for the total number of fruits, where T5-FERCONV (458.6 fruits) and T3-CP50%+FQ50% (255.4 fruits) were superior to the other treatments. Al-Hajjaj and Ayad (2018) found that foliar boron (1600 ppm) significantly affected yield, bunch weight, fruit set, physical fruit characteristics, and fruit quality. Kassem and Marzouk (2010) found that in twenty-six-year-old Zaghloul date palms on clay-loam soil, application of organic fertilizer, whether alone or combined with mineral fertilizers, increased palm yield and improved fruit color compared to mineral fertilization alone. On the other hand, Tagelsir *et al.* (2012) found that in Barakawi date palms, the combination of N and P resulted in the most pronounced increases in foliage growth, average fruit size, and total yield. Similarly, El-Mously *et al.* (2023) concluded that using compost from date palm residues could provide a viable ecological and sustainable alternative to conventional fertilizers. According to Kassem and Marzouk (2010), micronutrient contents were significantly higher in fruits with the application of organic fertilizer alone compared to organic fertilizer combined with NPK or mineral fertilization alone. Ghazzawy *et al.* (2023) found that in Sukary date palms with 12 clusters, there were negative effects on most of the attributes of date yield and quality; however, applying potassium sulfate at doses of 5 and 7.5 kg per palm allowed the palm to retain 8 to 10 fruit clusters per palm with significantly positive results.

Table 5. Individual weight, number of dry and total fruits with chemical, organic, and combined fertilization in Medjool date palms in the Mexicali Valley, Baja California, Mexico. October 2023.

Treatment	Weight of dried fruits (g)	Average weight of a dried fruit (g)	Number of total fruits	Total fruit weight (g)
T1-COMPOST	72.6 a	2.42 a	158.8 b	1625.4 a
T2-FERQUIM	56.8 a	2.71 a	117.1 b	1402.5 a
T3-CP50%+FQ50%	117.36 a	3.71 a	255.4 ab	2718.3 a
T4- CP100%+FQ100%	165.78 a	7.21 a	241.01 b	3468.1 a
T5-FERCONV	462.32 a	12.61 a	458.6 a	5166.5 a

*Means with the same letter within each column are statistically similar (Tukey, $p \leq 0.05$).

CONCLUSIONS

Chemical fertilization with 262N-138P_2O_5-540K_2O alone or in combination with compost at three or six tons per hectare increased the polar and equatorial diameters, promoting the oval growth of dates. Application of either compost alone or chemical fertilization results in a higher number of leaves per palm compared to their combination. Fruits with separated skin continue to be present regardless of the source of the fertilizers. The number of fruits increases with chemical fertilization, but the total yield of dates remains consistent across the evaluated treatments.

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Colegio de Postgraduados

Seed Quality Assessment of the Blue Corn Hybrid Vampiro H10 (*Zea mays* L.) Through Its Parental Genotypes

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ABSTRACT

Objective: To monitor the physical and physiological quality of the trilinear corn hybrid Vampiro H10 seeds through its parental genotypes and to rank the evaluated characteristics according to their importance for germination and seedling development.

Design/methodology: Using a completely randomized design with four replications (100 seeds), in parental genotypes of the hybrid Vampiro H10 seeds, it was evaluated their physical (width, thickness, length, volume, relative density, and width/length and thickness/length ratios) and physiological characteristics (normal and abnormal seedlings, inert seeds, and dry weight and length of plumule, radicle, and total). Seed germination was estimated by the proportion of normal seedlings produced. The results underwent analysis of variance, comparison of means (Tukey's test, $P \leq 0.05$), and principal components analysis.

Results: Each cross involved in the formation of Vampiro H10 made different contributions to the seed characteristics. In the single cross, the physical dimensions and dry matter of the seedling were increased, and in the trilinear cross, the formation of normal seedlings, radicle elongation and biomass were favored. This indicated that the hybridization sequence first affected the dimensions and then the physiological quality of the seed.

Limitations of the study: No limitations were presented for the present study.

Conclusions: An increase in volume, weight and length of seed was observed in the single cross and an increase in germination and seedling development in length and biomass was observed in the trilinear cross.

Keywords: Germination, heterosis, seedling development, seed dimensions.

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INTRODUCTION

Corn is currently the most widely planted species and has the highest production volume and economic value worldwide [1]. It holds this position due to its broad adaptability to various climates, soils, production technologies, and crop purposes [2], as well as the heterogeneity of its final product, whether it be ears, grain, or forage (fresh or dry).

In the extensive genetic variability of corn [3], pigmented corns stand out due to their content of carotenoids or anthocyanins [4]. These corns have functional and nutraceutical properties in themselves, meaning without the need for post-harvest treatments, and are considered biofortified foods obtained through conventional genetic improvement. They are included alongside crops that, through genetic crosses, the addition of fertilizers, or genetic modification, have been enriched with vitamins or minerals [5,6]. Anthocyanins are phenolic compounds from the flavonoid group [7] that give corn kernels their attractive and distinctive blue color, which varies in shades and intensities [8]. These pigments are located in the pericarp and/or in the aleurone layer of the caryopsis.

Blue corn is a staple ingredient in many traditional Mexican dishes. Additionally, the numerous nutraceutical and functional properties recently reported for anthocyanins (anticancer, lipid-reducing, antidiabetic, anti-inflammatory, antimicrobial, antioxidant) [7] have increased their consumption [9], leading to a current production that does not meet the high and growing demand.

Therefore, it is pertinent to develop high-yielding hybrids and improved varieties with superior agronomic qualities, among which seed quality stands out, as it is the essential input for crop establishment.

Seed quality is determined by its health attributes (presence of pests or diseases), genetic attributes (varietal purity), physical attributes (size, weight, density), and physiological attributes (germination suitability). In agronomic terms, physiological quality [10,11] is the seed's ability to germinate and produce complete, healthy, and potentially productive seedlings [12] with which the crop is established in the production field [13].

In the Central High Valleys of Mexico (Mexico State, Puebla, Tlaxcala, and Mexico City), 150,000 hectares of blue corn are planted using traditional varieties, traditional technology, and rainfed conditions, yielding 300,000 tons of grain, which only cover 65% of the demand [14,15].

Despite its agronomic significance, there is limited knowledge about the fluctuation of seed quality components throughout the sequential stages of maize hybrid formation. Therefore, the objectives of this study were: i) To monitor the physical and physiological quality of Vampiro H10 maize hybrid seeds through its sequence of parental genotypes (lines and single and trilinear crosses) and ii) To rank the evaluated characteristics according to their contribution to germination and seedling development.

The study of the fluctuation of physical and germinative seed components, as well as their contribution to seed quality, will contribute to the understanding of how these characteristics are established through the different genotechnical stages inherent to the development of a maize hybrid.

MATERIALS Y METHODS

Genetic Material

Seeds from the parental genotypes of the blue corn trilinear hybrid Vampiro H10 (Table 1) were used. This hybrid was developed in the Blue Corn Genetic Improvement Program at the Valle de México Experimental Station of the National Institute of Forest, Agricultural, and Livestock Research (CEVAMEX, INIFAP).

Table 1. Description of the parental genotypes (treatments) of the blue corn hybrid Vampiro H10 used in this study (Valle de México Experimental Station, INIFAP, Chapingo, Mexico, 2019).

Genotype	Genealogy	Inbreeding (%)
Parental lines		
A	NXOAX-19-5-1-1-2	96.87
B	BXCC-54-11-1-1-1	96.87
C	BXCC-5-9-6	87.50
Crosses		
D	(BXCC-54-11-1-1-1)×(BXCC-5-9-6)	
E	[(BXCC-54-11-1-1-1)×(BXCC-5-9-6)]×(NXOAX-19-5-1-1-2)	

The mentioned seeds were produced in a plot that was sown annually on May 2, 2019 (spring-summer cycle), under rainfed conditions, at CEVAMEX (Chapingo, Mexico, 19° 29' N and 98° 53' W), at an altitude of 2240 m, with a temperate climate, clayey-sandy soil, and annual averages of precipitation and temperature of 643 mm and 15.1 °C, respectively [16]. The experimental plot consisted of four rows measuring 5×0.8 m, with 26 plants per row and 0.2 m spacing between plants. The population density was 65,000 plants ha^{-1}, and the fertilization dosage (kg ha^{-1}) was N (120), P (60), K (30).

Physical Characteristics of the Seeds

Seed weight (SW, mg). Seeds were weighed using an analytical balance (AE Adam P W 184, precision 0.1 mg). Seed width (SWID), thickness (ST), and length (SL) were measured (mm) with a digital caliper (Mitutoyo CD-6 CSX). The volume (SV, mm^3), relative density (SDR, g/cm^3), and the ratios SWID/SL and ST/SL of the seeds were calculated according to maize variety description guidelines [17,18,19].

Physiological Characteristics of the Seeds

Germination

The standard germination test [12] was used. Seeds were placed on moistened paper, which was then rolled up and placed vertically in plastic bags, in a germination chamber at 25±2 °C and 100% r.h. After seven days of incubation, the percentage of normal seedlings (PN) was counted, *i.e.*, those that displayed a coleoptile, plumule, seminal radicle, and at least two adventitious roots. Abnormal seedlings (PA), classified as such for lacking any of the structures, and seeds with no apparent metabolic activity (SI) [20] were also counted. Seed germination was considered equivalent to the proportion of PN produced.

Seedling Development

In normal seedlings, the lengths of the plumule (PLU), radicle (RAD), and total length (TL) were measured (mm). Additionally, these structures were dried (80 °C, 3 days) in an oven (RIOSSA H-102, USA) and their dry weight (PDW, RDW, and TDW, respectively) was determined (mg).

Statistical Analysis

The experiments were conducted using a completely randomized design with four replications of 100 seeds each. To ensure normal distribution and to homogenize the scales of the variables, percentage data were transformed using the arcsine $\left(\text{arcsine}\ \sqrt{x}\right)$ [21].

The significance of the treatments was tested using analysis of variance, and treatment means were compared using the Tukey test ($P \leq 0.05$). To determine the relative contribution of each response variable to germination and seedling development, the results were subjected to principal component analysis; for this, the data were standardized to mean=0 and variance=1 [22]. Statistical processing of the results was performed using the SAS program [23].

RESULTS AND DISCUSSION

The response variables used in this study were statistically significant in the analysis of variance (except for ST), showed high fits to the statistical model used, and had reduced coefficients of variation (data not shown); therefore, the experiments were relevant and robust.

The significances ($P \leq 0.05$) detected indicated the high heterogeneity of the genetic material used in the measurements and allowed for the estimation of its fluctuation through the sequential genotechnical stages of the development of the blue corn trilinear hybrid Vampiro H10.

Physical and Physiological Characterization

In the statistical comparison of mean values for the variables (Table 2), the physical descriptors of the seed —SW, SV, SL, SWID, and ST— were lower ($P \leq 0.05$) in treatment C, intermediate in A and B, and higher in D and E. In terms of physiological aspects, treatments C, D, and E were superior ($P \leq 0.05$) in RSD, SWID/SL, and ST/SL. Treatments A, B, D, and E had the lowest ($P \leq 0.05$) amount of IS, while treatment C had the highest.

In the seedling development variables, the minimum plumule length (PLU) was for C and the maximum for A ($P \leq 0.05$); while E achieved the highest results ($P \leq 0.05$) in RAD, TL, RDW, and TDW, and D in PDW. In RDW and TDW, the superiority of treatments D and E was again observed, while the highest PDW value was for D and the lowest for C. Treatments D (single cross) and E (trilinear cross) had greater physical dimensions (SW, SV, SL, SWID, and ST) and greater seedling dry matter (PDW, RDW, and TDW) ($P \leq 0.05$) compared to treatments A, B, and C (inbred lines) (Table 2).

The inferior performance of the lines compared to the crosses in the described variables is explained by the genetic effects of homozygosity or inbreeding, which lead to reductions in yield, plant height, seed size, and seed volume and weight [24], as these are dominant traits. In contrast, the crosses exhibited heterosis or hybrid vigor, a metabolic process where the hybrid offspring surpass the average of their parents in certain characteristics [25]. In this study, these characteristics were the physical measurements SW, SV, SL, SWID, and ST, and the physiological traits PDW, RDW, and TDW.

Table 2. Comparison of means (Tukey, P≤0.05) for the physical and physiological characteristics of the Vampiro H10 maize hybrid seeds and its parents (UPIBI, IPN, Mexico City, Mexico, 2020).

Variables	Treatments					HSD
	A	B	C	D	E	
SW (mg)	22.06 b	21.70 b	16.66 c	31.45 a	30.34 a	2.26
SV (mm^3)	367.29 c	353.16 c	290.17 d	433.76 b	493.96 a	55.69
SL (mm)	11.55 bc	10.92 c	9.79 d	12.15 ab	12.42 a	0.85
SWID (mm)	7.23 b	6.35 c	6.58 c	7.46 ab	7.91 a	0.55
ST (mm)	4.40 b	5.09 a	4.50 b	4.78 ab	5.03 a	0.43
RSD	1.07 b	1.06 b	1.37 a	1.37 a	1.22 ab	0.17
SWID/SL	0.63 ab	0.59 b	0.67 a	0.61 ab	0.65 ab	0.08
ST/SL	0.38 b	0.47 a	0.46 a	0.39 b	0.41 ab	0.06
NS (%)	50 ab	68 ab	45 b	60 ab	83 a	34.98
AS (%)	5 a	5 a	5 a	5 a	0 a	13.81
IS (%)	45 ab	27 ab	50 a	35 ab	17 b	31.64
PLU (mm)	9.59 a	7.45 ab	6.23 b	8.10 ab	8.38 ab	2.79
RAD (mm)	12.80 b	13.45 b	12.50 b	13.98 b	19.48 a	2.57
TL (mm)	22.39 b	20.91 b	18.73 b	22.08 b	27.87 a	4.22
PDW (mg)	0.17 ab	0.16 ab	0.10 b	0.24 a	0.20 ab	0.10
RDW (mg)	0.17 b	0.20 b	0.15 b	0.30 ab	0.40 a	0.16
TDW (mg)	0.34 bc	0.36 abc	0.26 c	0.54 ab	0.60 a	0.25

In rows, different letters indicated significant differences. HSD=Honest Significant Difference. SW=Seed weight, SV=Seed volume, SL=Seed length, SWID=Seed width, ST=Seed thickness, RSD=Relative seed diameter, NS=Normal seedlings, AS=Abnormal seedlings, IS=Inert seeds, PLU=Plumule length, RAD=Radicle length, TL=Total length, PDW=Plumule dry weight, RDW=Radicle dry weight, TDW=Total dry weight, SWID/SL=Seed Width/Seed Length, ST/SL=Seed Thickness/Seed Length.

Hierarchization of Variables

In the principal component analysis, components 1 (CP1) and 2 (CP2) accounted for 79% of the variation in the experiment, which was an adequate proportion for this multivariate analysis procedure [22]. In CP1, the vectors with the largest positive magnitudes corresponded to the variables SV (0.31), RDW (0.29), TDW (0.30), SW (0.28), SL (0.29), NS (0.27), and TL (0.29). In CP2, the vectors were PLU (0.36), IS (0.31), and SWID (0.26). The negative eigenvectors had marginal values in PC1 but were high in CP2, corresponding to ST/SL (−0.50), ST (−0.46), and NS (−0.30).

In the graphical representation (biplot) of this method (Figure 1), treatments A, B, and C were in quadrants II and III, along with the variables RSD, SWID/SL, IS, AS, and ST/SL. In contrast, D and E were positioned in quadrants I and IV, respectively, with the remaining variables. The vectors of each variable assumed specific slopes according to their coordinates on the Cartesian plane (eigenvectors), and their graphical behavior followed the variations of CP1 and CP2.

Each cross made different contributions to the seed characteristics of Vampiro H10; in D, there was an increase in physical dimensions and seedling dry matter, while E favored the formation of normal seedlings, root elongation, and biomass. This indicated that the

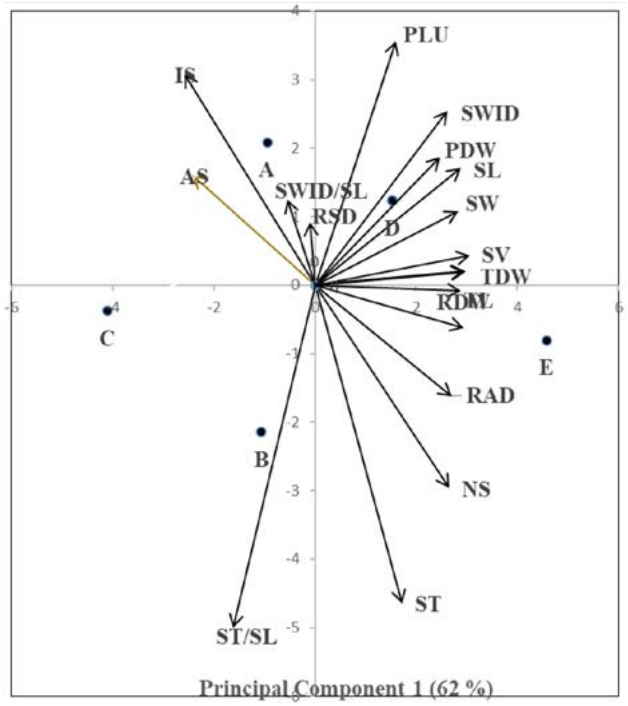

Figure 1. Biplot for the Principal Component 1 (accounts for the62% of the variation) and Principal Component 2 (accounts for the 17% of the variation) obtained for the blue maize hybrid Vampiro H10 and its parents (UPIBI, IPN. Mexico City, Mexico, 2020).
A, B, C, D, and E=Treatments (Genotypes). SW=Seed weight, SV=Seed volume, SL=Seed length, SWID=Seed width, ST=Seed thickness, RSD=Relative seed diameter, NS=Normal seedlings, AS=Abnormal seedlings, IS=Inert seeds, PLU=Plumule length, RAD=Radicle length, TL=Total length, PDW=Plumule dry weight, RDW=Radicle dry weight, TDW=Total dry weight, SWID/SL=Seed Width/Seed Length, ST/SL=Seed Thickness/Seed Length.

hybridization sequence first affected the dimensions and then the physiological quality of the seed.

It is feasible that lines B and C had phylogenetically related genetic sequences for germination, which is why there was only marginal heterosis in these events in the single cross; in contrast, the involvement of line A in the trilinear cross had a notable and favorable impact on these aspects. This can be attributed to the fact that line A was derived from a collection conducted in a different agroclimatic region (Oaxaca, Mexico) compared to the rest of the genotypes in the study (Central Mexican Highlands) (Table 1), meaning that its genetic background may have been distinct and recombined favorably [26,27], exhibiting high heterosis in essential traits for seed physiological quality, such as NS, RAD, RDW, and TDW. This strategy of using two germplasm sources to develop trilinear maize hybrids has been reported to exploit heterosis for seed production in maize [25, 28, 29].

Variable Hierarchization

In the principal component analysis, fluctuations in variables and genotypes (treatments) in response to the variation of CP1 and CP2 were visualized (Figure 1). CP1, which contributed the majority to the experimental results (62%), was characterized by

seed physical dimensions (SV, SW, and SL), seedling biomass (RDW and TDW), and the formation and total elongation of normal seedlings (TL and NS). Consistent with previous observations, seed weight and size attributes were associated with the formation and elongation of normal seedlings, as well as with the biomass accumulated in them.

In summary, the variables were predominant for the germination of the Vampiro H10 seeds and the development of seedlings. CP2, which accounted for only 17% of the results, was composed of the elongation of the plumule (PLU), seed width (SWID), and inert seeds (IS). Notably, it also included normal seedlings (NS) with a proportionally high and negative value.

The placement of A, B, and C in quadrants II and III of the Cartesian plane indicated the poor seed quality of these inbred lines, as they were positioned alongside unfavorable physiological variables such as abnormal seedlings (AS) and inert seeds (IS).

This was logical, given that the initial goal of plant breeding is to identify parental lines with desirable agronomic traits and, in later stages, to focus on seed quality. In contrast, cross D was located in quadrant I and cross E in quadrant IV, alongside traits favorable for seed physiological performance such as plumule length (PLU), dry weight of plumule (PDW), seed weight (SW), total dry weight (TDW), dry weight of radicle (RDW), and especially close to normal seedlings (NS); *i.e.*, through hybridization, the aforementioned seed quality attributes were optimized in the single cross, and this effect was even more pronounced in the trilinear cross with the involvement of line A.

The variables located in quadrant I, already referred to as favorable for germination and seedling development, responded directly to the variation in both principal components. In contrast, those placed in quadrant IV reacted directly to CP1 but inversely to CP2, such as normal seedlings (NS) and radicle length (RAD and TL) and dry weight of radicle (RDW). This indicates an interaction among all the analyzed variables, with the resulting effect being specific to each genotype.

The previous arguments indicated that in the genotechnical phases of the Vampiro H10 development, a series of interdependent events occurred. This process began with the single cross between lines A and B, which increased the physical capacity of the seeds to store reserves (SV, SW, and SL), and continued with the trilinear cross involving line A, which enhanced the genetic and physiological potential for germination (NS) and seedling development in terms of length (TL) and biomass accumulation (RDW and TDW).

CONCLUSIONS

The establishment of seed quality for Vampiro H10 began with the increase in physical dimensions (volume, weight, and length of the seed) in the single cross (between lines B and C). This process continued in the trilinear cross with the effects of line A, which enhanced the genetic and physiological potential for germination (normal seedlings) and seedling development in terms of length and biomass accumulation.

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Nutritional potential value of *Sesbania grandiflora* (L.) Pers., *Lablab purpureus* (L.) Sweet, and *Vigna radiata* (L.) Wilczek in two sowing methods

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ABSTRACT

Objective: To evaluate the agronomic performance and nutritional potential of *Sesbania grandiflora*, *Lablab purpureus*, and *Vigna radiata* in two sowing methods.

Design/methodology/approach: The morphological and bromatological parameters of leaves and stems of *S. grandiflora*, *L. purpureus*, and V. radiata sown flat and in beds were determined. The forages of leaves and stems of the three species were classified according to their quality parameters. Data for morphological and bromatological parameters were analyzed with a completely randomized design with a 3×2 factorial arrangement and least squares means were compared with Tukey ($p<0.05$).

Results: *Sesbania grandiflora* in flat and bed sowing presented greater height, higher percentage of leaf dry matter, and percentage of stem dry matter. While *V. radiata* in flat sowing presented greater plant weight, leaf weight and stem weight. *Sesbania grandiflora* leaf forages in flat and bed sowing obtained a higher percentage of crude protein, while *V. radiata* in flat and bed sowing obtained a lower percentage of neutral detergent fiber. For stem forage, the three species in flat and bed sowing presented high percentage values of neutral detergent fiber and acid detergent fiber. The leaf forages in bed sowing were classified as excellent quality, as were the leaf forages of *S. grandiflora* and *V. radiata* in flat sowing, which also had excellent quality.

Findings/conclusions: The forage of *S. grandiflora* leaves in flat and bed sowing was of excellent quality, related to its higher percentage of dry matter and crude protein and its lower percentage of neutral detergent fiber and acid detergent fiber.

Keywords: Fabaceae, crude protein, Sesbania, mung bean

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INTRODUCTION

Alfalfa (*Medicago sativa* L.) is one of the forage species with the highest nutritional quality, considered among the main sources of protein (21% Crude Protein) in animal feed (Wayu & Atsbha, 2019). However, there are some drawbacks in its production process, as it is a crop with high water requirements and is marketed in dried conditions (Medina-García *et al.*, 2020). It consumes 1.5 times more water than its water footprint (181 $m^3\ t^{-1}$), indicating low water use efficiency (IMTA, 2020).

Due to the challenges posed by the production of *M. sativa* in the Mexicali Valley and the growing demand for proteins for human and animal consumption in developing countries, exploring alternative protein sources for animal feed is justified. These alternatives must present high nutritional value to supplement livestock's nutritional requirements and allow animals to express their maximum genetic potential (Percy, 2015). This is the case with fabaceae (formerly known as legumes), which, due to their adaptability and protein content, could yield good results (Guerra-Guzmán *et al.*, 2021).

The Fabaceae family exhibits a great diversity of plant species, with approximately 727 genera and 19,325 species (Lewis *et al.*, 2005). Among these are plants with potential uses in food, forage, medicine, timber, and ornamentation (Al-Sghair & Mahklouf, 2020). The consumption of these species by ruminants improves fiber degradability, increases dry matter intake, and enhances the population of ruminal microorganisms (Chanthakhoun *et al.*, 2011).

Among this variety of Fabaceae with high nutritional value are *Sesbania grandiflora* (L.) Pers. (Sesbania), *Lablab purpureus* (L.) Sweet (Dolichos), and *Vigna radiata* (Mung bean) (L.) Wilczek, which exhibit crude protein content and dry matter production equivalent to 25%, 16%, and 25%, and 87%, 32%, and 90%, respectively (Gebreyowhans *et al.*, 2019; Mekkara & Bukkan, 2021; Aguerre *et al.*, 2023). These nutritional parameters demonstrate that these species possess desirable characteristics for evaluation as forages in animal nutrition. However, in the Mexicali Valley, there is a lack of scientific information regarding their agronomic performance and nutritional value. These plant species may exhibit different parameters, as the conditions in this region are arid. Additionally, to maintain productivity in the agricultural and livestock sectors, it is necessary to make adjustments in forage production by introducing species tolerant to water and salt stress (Medina-García *et al.*, 2020). For this reason, in the search for new forage alternatives, the agronomic performance and nutritional potential value of *Sesbania grandiflora*, *Lablab purpureus*, and *Vigna radiata* were evaluated under two sowing methods, with the aim of contributing alternative forage species with high nutritional quality for animal feed.

MATERIALS AND METHODS

Study Area Location

The experiment was conducted at the experimental agricultural station of the Institute of Agricultural Sciences, located on Delta Highway S/N Ejido Nuevo León, 50 km south of Mexicali, Baja California, Mexico (32° 24' 44.16" N, 115° 11' 56.87" W) at an altitude of 12 m above sea level. The average annual temperature is 22 °C, and the average annual precipitation is 75.9 mm. The climate is classified as desert, with January being the coldest month, having an average minimum temperature of −1.66 °C, and August being the hottest month, with a maximum temperature of 45 °C. Establishment took place during the first week of June 2022, and harvesting was conducted in the second week of September. The maximum temperature in June was 44 °C, while the minimum was 13 °C. In September, the maximum temperature reached 43 °C, with minimum temperatures dropping to −3 °C. Throughout the crop development period, maximum and minimum temperatures fluctuated between −3 °C and 49 °C.

Biological Material

The genetic material used was donated by the International Maize and Wheat Improvement Center (CIMMyT) - Northern Pacific Hub: Sesbania (*S. grandiflora*), Dolichos (*L. purpureus*), and Mungo (*V. radiata*).

Crop Establishment

The species *S. grandiflora*, *L. purpureus*, and *V. radiata* were established in six experimental plots (6.4 m wide×100 m long), with three plots designated for flat sowing and three for raised bed sowing (80 cm wide). This resulted in six treatments: Sesbania flat (SES-F), Sesbania raised bed (SES-B), Dolichos flat (DOL-F), Dolichos raised bed (DOL-B), Mungo flat (MUN-F), and Mungo raised bed (MUN-B).

Irrigation was applied at the time of sowing, and two supplemental irrigations at 50 and 80 days after sowing. No fertilizer was applied during the experiment. The agronomic management of the crops with Fabaceae was conducted in accordance with the Official Mexican Standard for Agricultural Activities —Use of Phytosanitary Inputs or Pesticides and Plant Nutrition Inputs or Fertilizers— Safety and Hygiene Conditions (NOM-003-STPS-1999).

Morphological variables

At the end of flowering for each genotype, 25 plants were randomly selected for each treatment, and their fresh weight and height per plant were recorded. Subsequently, the leaves and stems of each plant were separated, and the fresh weight of each morphological component was determined.

The percentage of dry matter for each component was determined by selecting four samples of 200 g of leaves and stems from each treatment, which were dried in an oven at 65 °C for 48 h. The dry weight of each component was then recorded. Finally, the percentage of dry matter was calculated by relating the fresh weight and the dry weight of the sample using Equation 1.

$$\%DM = \frac{Dry\ weight\ of\ the\ sample}{Fresh\ weight\ of\ the\ sample} \times 100 \qquad \text{Equation 1}$$

Bromatological variables

Bromatological characterization was performed for the leaves and stems of the three species. The samples were dried in an oven at 65 °C for 48 h. The dried samples were then ground in a Willey mill using a 1 mm sieve. Subsequently, the forage samples were dried for 24 h at 105 °C in a forced-air oven to determine dry matter (DM) content, and then incinerated for six h at 600 °C in a muffle furnace.

The determination of the percentage of crude protein was performed using the Kjeldahl method and calculated as %N×6.25. The quantification of ash percentage (%ASH) and crude protein percentage (%CP) was conducted according to AOAC (2000) procedures. The percentages of acid detergent fiber (%ADF) and neutral detergent fiber (%NDF)

were determined using the procedure reported by Van Soest *et al.* (1991) employing the ANKOM-Fiber Analyzer (Ankom Technology, Fairport, NY).

Forage quality

The forage samples obtained in this study were classified according to their quality parameters based on the relative forage value (RFV) proposed by Rohweder *et al.* (1978), with the scale presented in Table 1.

Table 1. Quality standards for forages (legumes, grasses and their ixtures in the U.S.) assigned by the Hay Market Task Force of the American Forage and Grassland Council.

Quality	CP (%)	ADF (%)	NDF (%)	RFV
Excellent	>19	<31	<40	>151
1	17-19	31-40	40-46	151-125
2	14-16	36-40	47-53	124-103
3	11-13	41-42	54-60	102-87
4	8-10	43-45	61-65	86-75
5	<8	>45	>65	<75

Percentage of crude protein (%CP), percentage of acid detergent fiber (%ADF), percentage of neutral detergent fiber (%NDF), relative forage value (RFV).

The RFV Index relates %NDF and %ADF, which is determined using Equation 2:

$$RFV = \frac{[88.9 - (0.779 \times \%ADF)] \times \left[\frac{120}{\%NDF}\right]}{1.29} \qquad \text{Equation 2}$$

Statistical Analysis

The data for plant weight, plant height, leaf weight, and stem weight were analyzed using a completely randomized design with a 3×2 factorial arrangement and 25 replications. The least squares means were compared using Tukey's test ($p<0.05$) (SAS, 2001). The data for the percentage of dry matter in leaves and stems were analyzed using a completely randomized design with a 3×2 factorial arrangement and 4 replications, and the least squares means were compared using Tukey's test ($p<0.05$).

The data for dry matter, ash, crude protein, acid detergent fiber, neutral detergent fiber, lignin, and ether extract were analyzed using a completely randomized design with a 3×2 factorial arrangement and 2 replications. The least squares means were compared using Tukey's test ($p<0.05$) (SAS, 2001).

RESULTS AND DISCUSSION

Morphological variables

The effect of the interaction (genotype and planting method) was significant for plant height ($F=497.12$; df=2; $p=0.0001$), root length ($F=10.85$; df=2; $p=0.0001$), total plant

weight (F=10.25; df=2; p=0.0001), leaf weight (F=25.71; df=2; p=0.0001), stem weight (F=33.31; df=2; p=0.0001), percentage of dry matter in leaves (F=3.75; df=2; p=0.0435), and percentage of dry matter in stems (F=4.00; df=2; p=0.0366).

SES-F and SES-B exhibited greater height compared to MUN-F, MUN-B, DOL-F, and DOL-B, with greater plant height observed in the flat planting method (F=85.27; df=1; p=0.001), regardless of the plant species (Table 2). The treatment MUN-F exhibited greater root length (F=5.89; df=2; p=0.0035), total plant weight (F=7.53; df=2; p=0.008), leaf weight (F=18.54; df=2; p=0.001), and stem weight (F=19.85; df=2; p=0.0001), indicating that MUN-F produced higher biomass, with 5.32, 4.10, 3.72, 3.57, and 1.57 times more fresh weight in the whole plant compared to MUN-B, SES-B, DOL-B, SES-F, and DOL-F, respectively (Table 2). A similar behavior was observed for leaf weight and stem weight, thus highlighting Mungo bean in flat planting as having high forage potential.

The greater height exhibited by Sesbania compared to Mungo and Dolichos is related to the growth habit of these species. Sesbania is considered a small perennial tree with a high growth rate, reaching heights of 4 to 6 meters within 6 months (Ella *et al*., 1989; Prajapati *et al*., 2003; Joshi, 2008). In contrast, Mungo is an annual shrub with a maximum height of 1.25 meters (Lambrides and Godwin, 2006), while Dolichos is a perennial shrub but is typically cultivated as an annual, with an average height of 1.27 meters (Chaudhari *et al*., 2013).

Mogotsi (2006) reports that *V. radiata* is a species with good root development, which is consistent with the findings of this research, where Mungo bean in flat planting exhibited greater root development than Sesbania and Dolichos.

SES-B and SES-F exhibited a higher %DM compared to DOL-B, DOL-F, MUN-B, and MUN-F, regardless of the type of morphological component. Overall, a greater %DM production was observed in flat planting, and it was also noted that there was similar %DM production in both leaf and stem across all treatments. The higher %DM content of SES-B and SES-F (over 24% DM) compared to the other treatments indicates that

Table 2. Effect of the interaction between genotype and planting method on the morphological variables of *Sesbania grandiflora*, *Lablab purpureus*, and *Vigna radiata*.

Treaments	Morphological variables						
	ALT (cm)	**LR (cm)**	**PTP (g)**	**PH (g)**	**PT (g)**	**%MSH**	**%MST**
DOL-C	46.36±2.96e	22.04±0.85b	56.43±3.09c	25.63±2.12c	24.68±2.17c	19.96±3.08c	19.61±3.77c
DOL-P	73.12±1.88c	23.84±2.04b	133.49±8.47b	33.99±2.51b	53.71±3.01b	21.44±3.42bc	23.16±6.42b
MUN-C	29.72±1.56f	15.36±1.09c	39.44±2.72d	16.23±2.10c	13.35±1.79c	14.72±6.94d	19.78±5.79c
MUN-P	56.10±1.27d	28.28±2.52a	210.00±11.48a	90.75±7.22a	136.75±6.64a	22.19±1.61bc	17.91±2.24c
SES-C	151.84±13.10b	18.20±1.02bc	51.55±2.97c	19.50±3.40c	26.44±3.20c	24.11±3.46ab	24.59±1.16ab
SES-P	178.96±4.20a	17.92±1.12c	58.77±3.92c	21.60±2.57c	32.80±2.58c	26.36±3.18a	27.27±1.46a

DOL-F (Dolichos in flat), DOL-B (Dolichos in bed), SES-F (Sesbania in flat), SES-B (Sesbania in bed), MUN-F (Mungo in flat), MUN-B (Mungo in bed), THE (total height), SW (stem weight), LW (leaf weight), TPW (total plant weight), RL (root length), %DML (percentage of dry matter in leaves), %DMS (percentage of dry matter in stems). Means followed by the same letter in a column do not show significant differences (Tukey's test with α=0.05).

Sesbania forages would have greater nutrient availability, as a plant species with a high %DM also has a higher protein content (Reyes-Purata *et al.*, 2009), resulting in greater nutritional contribution. Additionally, the high %DM for SES-B and SES-F indicates that this species was harvested at its optimal phenological stage to achieve maximum %DM yield (end of flowering).

In general, the flat planting method had a significant effect on all evaluated variables, regardless of the plant species. This result differs from reports indicating that raised bed planting increases yield in different crops (Majeed *et al.*, 2015).

In other studies, flat planting has been reported to outperform raised bed planting (Kendal, 2019), as occurred in this research. However, raised bed planting promotes water savings for irrigation, weed control, disease management, and reduces soil erosion (Fahong *et al.*, 2004; Govaerts *et al.*, 2005). The results obtained in this study may be related to soil type, water quality, and environmental conditions present in the Mexicali Valley (Escobosa-Garcia *et al.*, 2021).

Bromatological variables (Leaves)

The analysis of variance indicated that the effect of the interaction between genotype and planting method was significant for %CP ($F=28.43$; $df=2$; $p=0.0009$) and %NDF ($F=6.71$; $df=2$; $p=0.0295$). In contrast, no significant differences were observed for %ASH ($F=1.32$; $df=2$; $p=0.3358$), %ADF ($F=0.07$; $df=2$; $p=0.9352$), %LIG ($F=0.26$; $df=2$; $p=0.7810$), and %EE ($F=0.27$; $df=2$; $p=0.7714$).

SES-F and SES-B exhibited a higher percentage of protein in leaves compared to Dolichos and Mungo, regardless of the planting method, with 6.18, 2.53, 2.51, and 4.72 times more crude protein content in relation to DOL-B, DOL-F, MUN-B, and MUN-F, respectively. The treatments SES-F and SES-B showed similar protein content, at 34.46% and 34.86%, respectively, indicating that the planting method did not affect the protein production of this species (Table 3). The high protein content of SES-F and SES-B obtained in this study was higher than that reported by Usman *et al.* (2013), who found 20-25% crude protein for Sesbania; a similar result was obtained by Chanda *et al.* (2019), who reported 18.2%.

In the case of %NDF in leaves, DOL-F showed the highest percentage (55.64%), followed by SES-F, DOL-B, and SES-B. Finally, the MUN-F and MUN-B treatments had the lowest %NDF values at 25.49% and 24.77%, respectively (Table 3). NDF is a parameter that determines forage quality (Raffrenato *et al.*, 2019), as it affects consumption, food density, digestibility, digestibility rate, and the decrease in digestibility in relation to increased consumption (Mertens, 1997). Thus, the higher the NDF in the forage, the lower its quality and the lower the voluntary intake by ruminants (Van Soest *et al.*, 1991). Therefore, DOL-F can be considered a low-quality forage due to its high %FDN, while MUN-F and MUN-B can be regarded as high-quality forages based on their low NDF content.

Bromatological Variables (Stems)

The analysis of variance indicated that the effect of the interaction between genotype and planting method was significant for %ADF ($F=8.22$; $df=2$; $p=0.0191$) and %NDF

(F=17.77; df=2; p=0.0030). However, there were no significant differences for %ASH (F=2.49; df=2; p=0.1620), %SW (F=0.49; df=2; p=0.6358), %LIG (F=0.91; df=2; p=0.4508), and %EE (F=0.40; df=2; p=0.6897). The stems of SES-F and SES-B exhibited higher %NDF, with 74.29% and 79.35%, respectively.

In general, all treatments exhibited a high content of neutral detergent fiber (NDF) (Table 4), indicating that the forage obtained from the stems of the three Fabaceae was of low quality due to its high percentage of NDF. Similar results were observed for acid detergent fiber (ADF) in all treatments, confirming the low quality of the forage obtained from the stems. Overall, the forages obtained from the stems of the three species, regardless of the planting method, showed higher percentages of NDF and ADF compared to the forage from the leaves. In contrast, these forages exhibited a lower content of crude protein compared to the forages obtained from the leaves (Table 4). The difference between the leaves and stems of the three species evaluated for NDF and ADF could be explained by the fact that the stems of the plants have a higher content of cellulose and hemicellulose

Table 3. Effect of the interaction between genotype and planting method on the bromatological variables of leaves of *Sesbania grandiflora*, *Lablab purpureus*, and *Vigna radiate*.

Treaments	Bromatological variables					
	%CEN	%PH	%NDF	%ADF	%LIG	%EE
DOL-c	13.89±6.53a	5.64±5.548 c	43.89±10.42 b	17.93±21.50a	0.0268±0.015a	5.11±4.24a
DOL-P	16.67±4.86a	13.73±3.557b	55.64±12.88 a	19.61±4.48a	0.0314±0.015a	4.76±1.95a
MUN-C	13.72±4.20a	13.87±3.539b	24.77±9.72 d	12.93±2.08a	0.0187±0.017a	3.27±1.15a
MUN-P	14.32±1.99a	7.37±4.855 c	25.49±3.16 d	13.40±1.58a	0.0166±0.009a	3.28±1.89a
SES-C	9.62±0.09a	34.46±3.532a	30.66±9.73c	15.46±4.51a	0.0429±0.032a	3.83±0.38a
SES-P	9.59±0.19a	34.86±2.232a	45.76±4.55b	16.51±12.03a	0.0417±0.067a	4.04±2.66a

DOL-F (Dolichos in flat), DOL-B (Dolichos in bed), SES-F (Sesbania in flat), SES-B (Sesbania in bed), MUN-F (Mungo in flat), MUN-B (Mungo in bed), %ASH (Percentage of ash), %NDF (Percentage of neutral detergent fiber), %ADF (Percentage of acid detergent fiber), %LIG (Percentage of lignin), %EE (Percentage of ether extract), %PH (Percentage of protein). Means followed by the same letter in a column do not show significant differences (Tukey's test with α=0.05).

Table 4. Effect of the interaction between genotype and planting method on the bromatological variables of stems of *Sesbania grandiflora*, *Lablab purpureus* and *Vigna radiata*.

Treaments	Bromatological variables					
	%CEN	%PT	%NDF	%ADF	%LIG	%EE
DOL-c	12.81±4.55a	7.35± 0.428a	61.71±10.23c	40.98±5.43cd	0.0505±0.001a	1.61±0.347a
DOL-P	13.10±6.35a	6.51± 0.454a	62.62±9.57c	39.27±0.13cd	0.0459±0.002a	1.69±1.200a
MUN-C	12.25±3.32a	6.51± 0.454a	48.65±2.65e	37.87±2.49d	0.0413±0.001a	1.18±0.253a
MUN-P	11.97±2.49a	4.34± 0.527a	55.32±5.68d	41.82±4.29c	0.0486±0.045a	1.49±1.673a
SES-C	6.67±0.41a	6.43± 0.457a	79.35±4.10a	61.52±7.26a	0.0572±0.007a	1.23±0.537a
SES-P	6.75±1.89a	4.80± 0.529a	74.29±3.63b	56.88±13.07a	0.0590±0.051a	1.30±0.631a

DOL-F (Dolichos planted flat), DOL-B (Dolichos planted in bed), SES-F (Sesbania planted flat), SES-B (Sesbania planted in bed), MUN-F (Mungo planted flat), MUN-B (Mungo planted in bed), %ASH (Percentage of ash), %NDF (Percentage of neutral detergent fiber), %ADF (Percentage of acid detergent fiber), %LIG (Percentage of lignin), %EE (Percentage of ether extract), %PT (Percentage of protein). Means followed by the same letter in a column do not show significant differences (Tukey test with α=0.05).

than the leaves, as this plant organ requires greater cell rigidity to support the weight of the plant. Cellulose and hemicellulose are the main structural carbohydrates that constitute plant fiber; thus, the higher content of these compounds in the stems translates to higher percentages of detergent fibers (Casler *et al.*, 2002).

Forage quality

Considering the chemical composition of the leaves of *S. grandiflora*, *L. purpureus*, and *V. radiata* cultivated in two types of planting —flat and raised beds— we found that SES-F, SES-B, MUN-F, MUN-B, and DOL-B were classified as excellent quality forages according to their RFV (Table 5). Among these excellent quality forages, MUN-B, SES-F, and SES-B stood out, as they exhibited high CP content and low levels of NDF and ADF. These quality parameters indicate that MUN-B, SES-F, and SES-B would provide a high amount of nutrients, greater digestibility, and increased voluntary consumption of the forage.

Although the forages from DOL-B and MUN-F were classified as excellent quality, they are less suitable for animal feeding compared to MUN-B, SES-F, and SES-B, due to their low CP content, with 5.64% and 7.37% of CP, respectively (Table 5), indicating a low nutritional contribution. Overall, the planting method did not have a significant effect on forage quality.

None of the forages derived from the stems of *S. grandiflora*, *L. purpureus* and *V. radiata* were classified as excellent quality. SES-B and SES-F were rated quality 5, DOL-B, DOL-F, and MUN-F were rated quality 3, and MUN-B was rated quality 1. In general, better forage quality was observed in the leaves compared to the stems, as leaves are the most nutritious and digestible part of the plants. In some cases, leaves can contain around

Table 5. Forage quality of leaves and stems from *Sesbania grandiflora*, *Lablab purpureus* and *Vigna radiata* in flat and bed planting systems.

Forage	CP (%)	NDF (%)	ADF (%)	RFV	Classification
Leaves					
DOL-C	5.64	43.89	17.93	158.82	Excellent quality
DOL-P	13.73	55.64	19.61	123.09	Quality 2
MUN-C	13.87	24.77	12.93	296.04	Excellent quality
MUN-P	7.37	25.49	13.40	286.34	Excellent quality
SES-C	34.86	30.66	15.46	233.19	Excellent quality
SES-P	34.86	45.76	16.51	154.58	Excellent quality
Stems					
DOL-C	7.35	40.98	61.71	92.68	Quality 3
DOL-P	6.51	39.27	62.62	95.03	Quality 3
MUN-C	6.51	37.87	48.65	125.28	Quality 1
MUN-P	4.34	41.82	55.32	101.89	Quality 3
SES-C	6.43	61.52	79.35	40.96	Quality 5
SES-P	4.80	56.88	74.29	50.74	Quality 5

35% crude protein, while stems contain only about 16% (Rojas-García *et al*., 2019), which aligns with the findings of this research.

CONCLUSIONES

The results of this research represent the first report on the agronomic behavior and nutritional value of *Sesbania grandiflora*, *Lablab purpureus*, and *Vigna radiata* in the Mexicali Valley. It is concluded that the forage from the leaves of *S. grandiflora*, grown in both flat and raised bed systems, was of excellent quality, attributed to its higher %DM and %CP and lower %NDF and %ADF. Although these results highlight *S. grandiflora* as having high nutritional value, it is important to confirm this through *in vitro* and *in vivo* studies of digestibility and voluntary dry matter intake of the forage.

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Colegio de Postgraduados

Economic Viability of Organic Fertilization in Forage Oats (*Avena sativa* L.) in the Mexicali Valley

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ABSTRACT

Objective: To economically evaluate the use of chemical, organic and organic-mineral fertilizers in of forage oat production in the Mexicali Valley; and to determine the profitability of each fertilization type.

Design/methodology/approach: Three treatments were established, one chemically fertilized (T1), one with organic fertilization (T2), and one with organic-mineral fertilization (T3) with two replications under a completely randomized design. Cash flow, financial costs and economic costs were calculated.

Results: Fertilization costs accounted for between 37% and 52% of the cost structure. Treatments T2 and T3 did not cover production costs. Only treatment T1 treatment demonstrated the ability to cover both production and financial costs. None of the treatments covered economic costs.

Limitations on study/implications: It is suggested to replicate the economic viability analyses in consecutive productive cycles, as other studies have shown positive impacts on soil fertility.

Findings/conclusions: The organic and organic-mineral fertilization systems (T2 and T3) were not profitable in the short term. The chemical fertilization system (T1) demonstrated the ability to cover, in addition to production costs, the depreciation costs of fixed assets. However, none of the treatments showed the ability to compensate the risk of investing in the activity.

Keywords: profitability, production costs, financial costs, organic production.

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INTRODUCTION

Oats (*Avena sativa* L.) is a widely cultivated grass with the purpose of producing grain for human nutrition or forage for animal feed. In 2021, it ranked sixth in global cereal production, following maize (40.3%), rice (26.26%), wheat (25.7%), barley (4.8%), and sorghum (2%) (FAOSTAT, 2023). In Mexico, it stands out as a key input for livestock feed production; additionally, due to its wide range of adaptability in different production areas, it is considered a strategic crop (SAGARPA, 2017). In 2022, Chihuahua ranked

as the leading producer with 2.57 million tons (16%), followed by Durango (16.1%) and Zacatecas (18.1%); these states accounted for 50.3% of the national production (SIAP, 2024). Regarding oat crop yields, Baja California, Sonora, and Coahuila have shown the highest average yields in recent production cycles, with 38, 32, and 28 t ha^{-1}, respectively (SIAP, 2024).

On the other hand, the fertilizer market has experienced particular dynamics due to global events. One of the effects of the war between Russia and Ukraine has been the increase in the prices of cereals, energy, and fertilizers. An analysis conducted by the OECD and FAO (2022) estimates that for every 1% increase in fertilizer prices, agricultural commodity prices would rise by 0.2%. This increase in one of the most important components within the cost structure of most agricultural crops, combined with the inverse relationship between fertilizer use and its price (García-Salazar, Borja Bravo and Rodríguez-Licea, 2018), exacerbates concerns about agricultural productivity in the short term (OECD and FAO, 2022; United Nations, 2022). In the national context, the effects have been similar; in 2022, the value of Mexico's fertilizer imports increased by 75%, amounting to 3.513 billion dollars, while exports totaled 368.87 million dollars (International Trade Centre, 2023).

In addition to the above, there is an increasing challenge for agriculture to reduce its environmental footprint and increase food availability for the coming decades. In this regard, the use of organic fertilizers has demonstrated improvements in various soil fertility indicators, and combined fertilizations with chemical and organic fertilizers have proven to be a viable alternative for achieving optimal production levels (Montaño-Carrasco *et al.*, 2017). However, despite the growing importance of diversifying fertilization sources for agricultural crops, few studies have evaluated the economic profitability of using organic fertilization as an alternative for farmers. Consequently, the objective of this research was to economically evaluate the use of an organic fertilizer, a chemical fertilizer, an organic fertilizer, and an organic-mineral fertilizer in forage oat production in the Mexicali Valley, to determine the profitability of each type of fertilization.

MATERIALS AND METHODS

Location of the experiment

The experiment was conducted during the fall-winter cycle of 2021-2022 at the experimental station of the Institute of Agricultural Sciences of the Autonomous University of Baja California, located in Ejido Nuevo León, Mexicali, Baja California, Mexico, between the geographic coordinates 32° 24' north latitude and 115° 11' west longitude, at an altitude of 15 meters, in clay-textured soil. The climate in this area is classified as very dry and very hot to hot, with an average annual temperature of 22.3 °C, a maximum of 50 °C, and a minimum of 0 °C, and an average annual precipitation of 77.8 mm (CONAGUA, 2024; INEGI, 2017).

Treatments

Three treatments were established with two replicates under a completely randomized design, with the characteristics mentioned in Table 1.

Table 1. Characteristics of the treatments.

Treatments	Replicates	Area (ha)	Type of fertilization
T1	1	0.5	Chemical
T1	2	0.5	Chemical
T2	1	0.5	Organic
T2	2	0.5	Organic
T3	1	0.5	Organic and mineral
T3	2	0.5	Organic and mineral

Agronomic management of the crop

Soil preparation involved breaking and crossing with a disk plow, followed by leveling with a medium harrow. Planting was then carried out in November 2021 using a conservation tillage planter (model 205-13-2), equipped with 13 seed dispensing systems, with a row spacing of 17 cm. The oat variety sown was Bachíniva, with a seeding density of 120 kg ha^{-1}. Chemical fertilization was applied at the time of planting with a dose of 200 kg ha^{-1} of urea (46-00-00) and 50 kg ha^{-1} of MAP (11-52-00).

Organic and organic-mineral fertilization was applied using Mar y Tierra® products and involved the application of liquid fertilizers dissolved in 400 L of water, applied during the first irrigation with the following doses: organic 20 L ha^{-1} (10-20-1) and 20 L ha^{-1} (20-1-1), and organic-mineral 15 L ha^{-1} (4-6-1) and 15 L ha^{-1} (5-1-1). Four irrigations were carried out with an average interval of 29 days between them. The oat was harvested 120 days after sowing, when the grain was in the milk-dough stage (Servin *et al.*, 2018). Subsequently, a tractor with harrows was used to pile the product and allow it to lose moisture before being baled, with approximately 45 kg of dry oat straw per bale. The harvested oats were sold at the field for 180 MXN per bale, thus no distribution or marketing costs were incurred.

Economic Analysis

For each treatment, technical and economic information about the production cycle was recorded in an Excel® 2011 file, including dates and tasks for sowing, irrigation, fertilization, harvesting, input costs, labor, infrastructure, machinery and equipment, as well as their respective maintenance. With the technical parameters and production costs, the following variables were calculated and analyzed (AAEA, 2000; Sagarnaga Villegas, Salas González, and Aguilar Ávila, 2018):

$$TOC = FC + VC$$
$$NCF = FC + VC + Loans + With$$
$$FinC = FC + VC + Depreciation$$
$$ECc = FC + CV + Depreciation + OPc$$
$$TI = Q * P$$
$$NI = TI - ECc$$

Where: *TOC*=total operating costs; *FC*=fixed costs; *VC*=variable costs; *NCF*=net cash flow, *Loans*=payments on loans, *With*=withdrawals; *FinC*=financial costs; *ECc*=economic costs; *OPc*=opportunity cost. *TI*=Total Income; *Q*=quantity of bales produced; *SP*=Sale price per bale; *NI*=net income.

Since the cultural practices were carried out using the Institute's tractor and rented agricultural implements, the cash flow analysis included the rental cost of the implements, while the financial cost analysis considered the tractor depreciation as a fixed cost.

In addition, a cost for technical consulting was assigned to treatments T2 and T3; since, under organic fertilization conditions, the producer would need professional advice at least during the initial production cycles. In calculating the opportunity costs of the producer's work, the cost of normal labor was considered, and in the case of business management of the production unit, the cost of specialized labor was considered, which for the production process is the irrigation worker.

With the above information, the target yields were determined as described below:

Target Yield 1 (Y1): yield required to cover variable costs

$$S1 = \frac{VC}{P}$$

Where: *VC*=unit variable cost and *P*=sale price.

Thus, if S1>Y1, the company will be able to cover its variable costs; on the other hand, if S1<Y1, the company will not be able to cover its variable costs. Subsequent target yields are calculated similarly, adding the cost detailed below:

Target Yield 2 (Y2): yield required to cover Y1 plus fixed operating costs.

Target Yield 3 (Y3): yield required to cover Y2 plus depreciation.

Target Yield 4 (Y4): yield required to cover Y3 plus producer labor and business management.

Target Yield 5 (Y5): yield required to cover Y4 plus cost of capital.

Target Yield 6 (Y6): yield required to cover Y5 plus opportunity cost of the production factors.

Target Yield 7 (Y7): yield required to cover Y6 and obtain a return for the risk of investing in the activity.

RESULTS AND DISCUSSION

The treatment with the highest yield was the conventionally fertilized one, followed by the organic mineral and, finally, the organic fertilization, with averages of 4.05, 1.44, and 1.21 t ha^{-1}, respectively (Table 2). The yield of T1 was consistent with those obtained in previous studies (Espitia Rangel, Villaseñor Mir, Tovar Gómez, de la O Olán, and Limón Ortega, 2012; Gil Gil, Martínez Rueda, and Estrada Campuzano, 2014; Sosa-Montes *et al*., 2020), where yields between 2.5 and 8 t of dry matter ha^{-1} were reported with different

Table 2. Obtained yields.

Treatments	Replicates	Type of fertilization	Yield	
			(oat bales ha^{-1})	(t ha^{-1})
T1	1	Chemical	88	3.96
T1	2	Chemical	92	4.14
T2	1	Organic	18	0.81
T2	2	Organic	36	1.62
T3	1	Organic and mineral	32	1.44
T3	2	Organic and mineral	32	1.44

varieties, chemical fertilization rates, and production systems. Meanwhile, SIAP (2024) reported a national average yield of 3.63 t of dry matter ha^{-1} for 2022.

On the other hand, the yields of treatments T2 and T3 compared to T1 are similar to studies such as Montaño-Carrasco *et al.* (2017), who reported higher yields in treatments fertilized with chemical sources (12.3 t of dry matter ha^{-1}) compared to those fertilized with organic sources (6.3 and 2.5 t of dry matter ha^{-1}). It is worth mentioning that the authors found that the best response in leaf nutrient content was obtained with chemical applications and their combination with an organic fertilizer. Additionally, the incorporation of organic fertilizers or their combination with chemical fertilizers positively impacts soil fertility parameters, results that may not necessarily be reflected in the yield of the first production cycle (Ibarra-Villarreal *et al.*, 2020). In this regard, Rodríguez-Herrera *et al.* (2020) found that the yield of grain oats planted for two consecutive cycles under organic fertilization increased by 14.4% in the second year.

Economic Analysis

Agronomic management showed no differences between replicates, so Table 3 presents the production cost structure by treatment. It can be observed that fertilization was the most significant item, representing between 22% and 52%, followed by planting and irrigation.

Table 3. Production Cost Structure (MXN ha^{-1}).

Concept	T1	T2	T3
Variable costs	11,898	9,119	8,286
Soil preparation	960	960	960
Sowing	2,376	2,376	2,376
Irrigation	1,500	1,500	1,500
Fertilization	6,300	2,732	1,899
Harvest	763	763	763
Technical assistant	0	789	789
Fixed costs	329	329	329
Agricultural machinery maintenance	329	329	329
Total operating costs	12,227	9,448	8,615

For the calculation of the cash flow in this research, only fixed and variable costs were considered, as no credit payments or cash withdrawals were made. These costs are those that a producer typically incurs during the production cycle and are generally the only items considered to determine whether their activity is profitable or not (Table 4).

From the cash flow analysis, only treatment T1 proved to be profitable in the short term, as reported by most studies on production costs in the agricultural sector (Aguilar Ávila, Sagarnaga Villegas, Salas González, & Arroyo Pozos, 2019, 2022; Delgadillo-Ruiz, Leos-Rodríguez, Valdez-Cepeda, Ramírez-Moreno, & Salas-González, 2016; Domínguez-García, Granados-Sánchez, Sagarnaga-Villegas, Salas-González, & Aguilar-Ávila, 2017). In this regard, producers who wish to implement organic fertilization under the conditions of this study will face liquidity problems in the short term; that is, within the same production cycle, they will not be able to cover production costs with the expected income.

In addition to the out-of-pocket costs, other costs must be considered to determine if the activity is profitable in the medium term. Therefore, in this study, depreciation was included as a fixed cost in the financial analysis. Under this premise, treatment T1 remains profitable (Table 4). That is, a producer applying chemical fertilization under the conditions of this experiment will be able to cover the replacement of the machinery and equipment needed for the operations.

Finally, the opportunity cost of production factors, which is reflected in the economic cost, should also be considered. The current analysis demonstrated that in the long term, none of the treatments are profitable and do not provide a return on the risk of investing in this activity (Sagarnaga Villegas *et al.*, 2018) (Table 4).

Several economic analyses demonstrate that few agricultural activities are profitable under economic analysis (Aguilar Ávila *et al.*, 2019, 2022; Domínguez-García *et al.*, 2017). Generally, these are production units with several years of experience and a certain size that allows them to benefit from economies of scale.

Table 4. Economic analysis (MXN ha^{-1}).

Concept	T1	T2	T3
Variable costs	11,898	9,119	8,286
Fixed costs	924	924	924
Opportunity cost	6,304	6,269	6,255
Opportunity cost of the land	5,000	5,000	5,000
Opportunity costs of the working capital	247	212	198
Opportunity costs of the producer labor	564	564	564
Opportunity costs of the business management	493	493	493
Total income (pesos ha^{-1})	16,200	4,860	5,760
Cash flow	12,227	9,448	8,615
Financial cost	12,822	10,043	9,210
Economic cost	19,126	16,273	15,423
Net income (MXN ha^{-1})	−2,926	−11,413	−9,663
Net income (MXN oat $bale^{-1}$)	−33	−423	−302

Target Yields

Considering the selling price of 180 pesos per bale, the different treatments should achieve the yields outlined in Table 5 to cover their respective costs. Ideally, production units should aim to produce at least the Target Yield 7, as it ensures coverage of economic costs and provides a return on the risk associated with engaging in this activity.

Table 5. Target Yields (t ha^{-1}).

Target yield	Description	Treatments		
		T1	T2	T3
	Obtained yield	4.05	1.21	1.44
1	Cover variable costs	2.97	2.28	2.07
2	Cover Y1 plus fixed operating costs	3.06	2.36	2.15
3	Cover Y2 plus depreciation	3.21	2.51	2.30
4	Cover Y3 plus producer labor and business management	3.47	2.78	2.57
5	Cover Y4 plus cost of capital	3.53	2.82	2.61
6	Cover Y5 plus opportunity cost of the production factors	4.78	4.07	3.86
7	Cover Y6 and obtain a return for the risk of investing in the activity	4.83	4.11	3.90

CONCLUSIONS

The treatment with chemical fertilization (T1) showed higher yields compared to the treatments fertilized with organic and organic-mineral methods (T2 and T3). Furthermore, in the cash flow analysis, only the T1 treatment demonstrated the ability to cover production costs.

In the financial analysis, treatment T1 remained viable, indicating that this system is profitable in the medium term, as it covered not only production costs but also the depreciation costs of fixed assets. However, when the opportunity costs of production factors are also considered, none of the treatments proved to be economically viable. Since fertilization was one of the major cost components in the production cost structure (between 22% and 52%), any change in the price of this input represents a sensitive aspect for production units. Therefore, it is suggested to conduct financial and economic viability analyses in consecutive production cycles, once other studies have demonstrated positive impacts on soil fertility.

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Colegio de Postgraduados

Effect of Vermicompost and Phyto-regulator on Zucchini Fruits (*Cucurbita pepo* L.) Grown in Shade Houses

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ABSTRACT
Objective: To assess the influence of vermicompost (LC) and phytoregulator (AMP) in the cultivation of zucchini, under shade house conditions.
Design/methodology/approach: A randomized block experimental design with eight treatments and six repetitions was used.
Results: The treatments did not affect fruit diameter and pH variables. The Jacobo+LC+AMP treatment induced fruit length, and all treatments where the phytoregulator was applied increased fruit weight and commercial yield. For fruit firmness and total soluble solids, the trend was not clear due to the effect of the treatments.
Findings/conclusions: The application of the phytoregulator induced fruit weight and yield.

Keywords: Fruit growth, commercial yield, fruit firmness.

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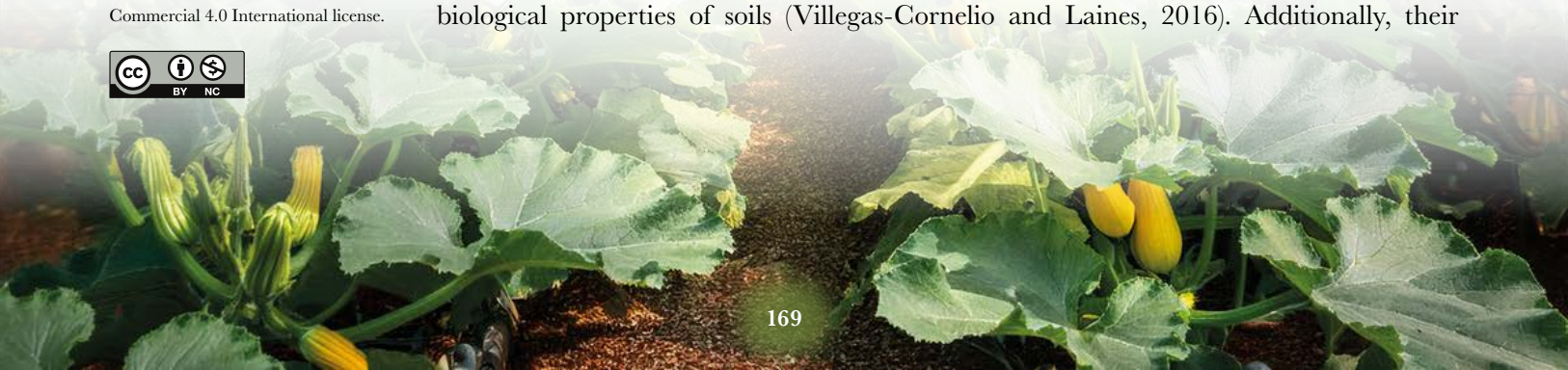
INTRODUCTION

Currently, the use of chemical fertilizers is indiscriminately applied to increase agricultural production, posing a significant threat to soil fertility and the environment, as well as altering microbial composition (Syed *et al.*, 2021). Intensive soil use leads to depletion and erosion, causing the loss of organic matter and necessitating high amounts of synthetic fertilizers (Tyagi *et al.*, 2019). An alternative to reduce the harmful effects of conventional agriculture is organic production, which has a lower negative impact on the environment and yields high-quality products (SIAP, 2019). According to Zambrano and Lima (2023), there are sustainable practices that conserve soil fertility and maintain crop productivity. Among these practices are vermicomposts, organic fertilizers that can meet the nutritional needs of plants (Moreno-Resendez *et al.*, 2019) and improve the physical, chemical, and biological properties of soils (Villegas-Cornelio and Laines, 2016). Additionally, their



derivatives, such as leachates and vermicompost teas, are also alternatives for sustainable production (Piña-Ramírez *et al.*, 2015).

Regarding the effect of vermicomposts on the soil, it is an organic fertilizer with a high percentage of humic and fulvic acids that provides assimilable nutrients (Cervantes *et al.*, 2022), which consequently promotes desirable yield enhancement (Sarmiento-Sarmiento *et al.*, 2019).

On the other hand, the application of phyto-regulators based on auxins, cytokinins, and/or gibberellins for fruit setting and yield increase has been the subject of several studies in crops such as chili, watermelon, and prickly pear (Ramírez-Luna *et al.*, 2005; Rueda-Luna *et al.*, 2015; Varela-Delgadillo *et al.*, 2018).

The objective of this research was to evaluate the effect of applying vermicompost and a complex of plant growth regulators (phyto-regulator) on the production and quality of zucchini under shade house conditions.

MATERIALS AND METHODS

The research was conducted within a crop protection structure, specifically a shade house, at the experimental field of the Faculty of Agronomy, Autonomous University of Sinaloa, located at 24° 48' 30" N, 107° 24' 30" W, and an altitude of 38.54 m. The fertility analysis conducted prior to planting indicated that the soil has a clayey texture (59% clay, 21% sand, and 20% silt), low organic matter content (1.21%), a saturation percentage of 79%, a pH of 7.85, EC of 0.58 dS m^{-1}, SAR of 1.77, and PSI of 1.33. In Culiacán, Sinaloa, the climate is BS1(h')w(w)(e): very warm semi-arid, extreme with summer rains, with less than five percent of the total annual precipitation occurring in winter (García, 2004). The average temperature (21.9 °C) and relative humidity (69.5%) recorded with thermohygrometers (DT171, Twilight®) were within the optimal ranges (18 to 24 °C and 65 to 80%) for zucchini cultivation (Molinar *et al.*, 1999; Cortés, 2003).

Soil preparation was done using manual agricultural tools, creating beds spaced 1.8 meters apart. Vermicompost was applied at a rate of 10 t ha-1 to half of the beds. Two lines of drip irrigation tape with emitters spaced every 0.20 meters were installed above each bed, and white/black co-extruded polyethylene mulch was applied. Sowing was carried out on November 14, 2021, in polystyrene trays with 128 cavities filled with peat (Brown 025W, Kekkila®). When the plants had two true leaves, 17 days after sowing (das), they were moved to the field for transplanting at a density of 11,820 plants per hectare.

A fertigation system (drip irrigation) was used, applying Steiner's (1984) solution at 50% concentration from transplanting until the anthesis of the first female flowers (33 days after transplanting, dat). Afterward, the full solution was applied. Irrigation was carried out when the tensiometers (2725ARL, Soil Moisture Equipment®) placed at a soil depth of 30 cm indicated a moisture tension of 20 to 25 kPa.

An experimental design with a randomized block arrangement was used, featuring a factorial design of 2A×2B×2C, with eight treatments and six replications. Factor A was the zucchini variety, with two levels: 'Jacobo', a "green zucchini" type, and 'Aurora', a "gray zucchini" type. Factor B, also with two levels, corresponded to vermicompost

(LC): applied at 0 and 10 t ha^{-1} to the soil. Factor C, likewise with two levels, was Agromil®Plus (AMP): plant extracts with 83.39% active ingredients; cytokinins at 2081.9 ppm; gibberellins at 31.0 ppm; auxins at 30.5 ppm; vitamins at 947.95 ppb; applied at doses of 0 and 2.5 mL L^{-1}.

Zucchini fruits were harvested between five and seven days after anthesis to evaluate fruit growth: fruit diameter (FD) was measured with a digital caliper (6MP, Truper®); fruit length (FL) was measured with a tape measure; and fruit weight (FW) was recorded with a precision scale (CP622, Sartorius®), along with yield (Molinar *et al.*, 1999; USDA, 2016).

To assess fruit quality, five fruits per replication or treatment were evaluated according to the methodology proposed by AOAC (1998). For pH, 10 g of fruit were weighed on a precision scale (PR802, Mettler Toledo) and mixed with 50 mL of distilled water adjusted to a pH of 7, blended in a blender (85554, Osterizer), and then filtered through organza fabric. A 10 mL aliquot of the filtrate was analyzed with a pH meter (HI98130, Hanna®). Firmness (N) was evaluated using a penetrometer (GY-4, Yuchengtech®) with an 8 mm steel tip, and total soluble solids (SST: °Brix) were determined by adding three drops of fruit juice to a digital refractometer (RHW040, Yieryi®).

The results obtained from the evaluated variables were subjected to analysis of variance and Tukey's multiple comparisons test ($P \leq 0.05$) using STATISTICA version 7.0 software (StatSoft, 2004).

RESULTS AND DISCUSSION

Yield Components

The variable fruit diameter of zucchini did not show differences due to the treatments (Table 1). However, Román-Román *et al.* (2023) observed an increase in zucchini fruit diameter due to the application of naphthaleneacetic acid auxin. Moreno-Resendez *et al.* (2019) reported a fruit diameter of 55.4 mm for the variety 'Mona Lisa F1' of the "gray zucchini" type.

The greatest fruit length was promoted by the Jacobo+LC+AMP treatment, which was 81.1% higher than that obtained with the Aurora treatment, but without differences compared to the other treatments. Plants of the 'Jacobo' variety had the longest fruit, surpassing those of the 'Aurora' variety, which can be attributed to the variety effect, as Moreno-Resendez *et al.* (2019) reported fruit lengths of 123.3 to 138.9 mm, consistent with the observations for the 'Aurora' variety in this study.

Fruit weight was significantly affected by the plant growth regulator factor (Table 1), such that all treatments that included the plant growth regulator (Jacobo+AMP, Aurora+LC+AMP, Jacobo+LC+AMP, and Aurora+AMP) induced increases in fruit weight, which were statistically higher ($p \leq 0.05$) than the effect of the other treatments, ranging from 24.7% (Jacobo+LC) to 56.8% (Aurora).

Moreno-Resendez *et al.* (2019) reported zucchini fruit weights (180.4 to 274.6 g) higher than those observed in the present study due to the treatments. This increase in fruit weight induced by the plant growth regulator aligns with findings by Román-Román *et al.* (2023) for zucchini fruits, as well as with observations in mango fruits (Pérez-Barraza *et al.*, 2009).

Table 1. Effect of vermicompost (LC) and plant growth regulator (AMP) on fruit diameter (FD), fruit length (FL), fruit weight (FW), and yield of zucchini, varieties Jacobo and Aurora, under shade house conditions. Culiacán, Sinaloa, Mexico.

Treatment	DF (mm)	LF (mm)	PF (g)	Yield (t ha^{-1})
Jacobo+LC+AMP	47.9 a[§]	228.6 a	134.8 a	40.8 abc
Jacobo+LC	42.7 a	211.5 ab	108.1 b	34.5 cd
Jacobo+AMP	42.1 a	203.1 ab	145.0 a	45.1 a
Jacobo	41.3 a	205.7 ab	96.0 b	31.9 d
Aurora+LC+AMP	45.9 a	138.9 ab	139.7 a	48.1 a
Aurora+LC	45.2 a	141.3 ab	93.2 b	35.7 bcd
Aurora+AMP	53.0 a	159.6 ab	137.6 a	43.5 ab
Aurora	40.4 a	126.2 b	92.5 b	33.0 cd
DMSH	19.4	84.4	20.1	7.7
Significance				
VARIETY	ns	***	ns	ns
LC	ns	ns	ns	ns
AMP	ns	ns	***	***
VARIETY × LC	ns	ns	ns	ns
VARIETY × AMP	ns	ns	ns	ns
LC × AMP	ns	ns	ns	ns

[§] Medias con letras iguales no son estadísticamente diferentes (Tukey≤0.05). DMSH=diferencia mínima significativa honesta; ns, *, **, ***: no significativo a p≤0.05, significativo a p≤0.05, p≤0.01 y p≤0.001.

The application of the plant growth regulator (AMP) promoted an increase in zucchini yield for both varieties, with up to a 31.4% increase in the four treatments where it was applied. The treatments Aurora+LC+AMP and Jacobo+AMP resulted in higher yields (48.1 and 45.1 t ha^{-1}), statistically similar to those obtained with Aurora+AMP and Jacobo+LC+AMP (Table 1), but higher than the yields achieved with Aurora+LC, Jacobo+LC, Aurora, and Jacobo, ranging from 26.3% to 50.8%. The yield obtained is consistent with the 45.5 t ha^{-1} of zucchini reported by Nogueira *et al.* (2011) for the 'Caserta' variety. This indicates that the application of plant growth regulators in zucchini fruits promotes commercial yield (Ayala-Tafoya *et al.*, 2020; Román-Román *et al.*, 2023).

Fruit quality parameters

The treatments did not influence the pH of the zucchini fruits (Table 2), which ranged from 6.3 to 6.6. Soriano-Melgar *et al.* (2020) reported a pH of 7.2 in fruits of the 'Grey Zucchini' variety, which differs from the values observed in the present study. The total soluble solids content was significantly affected by the variety factor, as well as by the interaction between the variety and plant growth regulator factors.

The total soluble solids content of the fruits harvested from the 'Jacobo' variety exceeded that of the 'Aurora' variety by 11.1%. However, the fruits obtained from

Table 2. Effect of vermicompost (LC) and plant growth regulator (AMP) on pH, firmness, and total soluble solids (°Brix) of zucchini fruits, varieties Jacobo and Aurora, under shade house conditions. Culiacán, Sinaloa, Mexico.

Treatment	pH	Firmness (N)	SST (°Brix)
Jacobo+LC+AMP	6.5 a[§]	69.4 ab	4.1 a
Jacobo+LC	6.3 a	79.1 a	3.7 ab
Jacobo+AMP	6.5 a	68.1 ab	3.9 a
Jacobo	6.4 a	76.6 ab	4.0 a
Aurora+LC+AMP	6.6 a	68.2 ab	3.3 b
Aurora+LC	6.6 a	64.4 ab	3.7 ab
Aurora+AMP	6.6 a	63.1 b	3.3 b
Aurora	6.6 a	64.8 ab	3.7 ab
DMSH	0.4	14.5	0.5
Significance			
VARIETY	*	**	***
LC	ns	ns	ns
AMP	ns	ns	ns
VARIETY × LC	ns	ns	ns
VARIETY × AMP	ns	*	**
LC × AMP	ns	ns	ns

[§] Medias con letras iguales no son estadísticamente diferentes (Tukey≤0.05). DMSH=diferencia mínima significativa honesta; ns, *, **, ***: no significativo a p≤0.05, significativo a p≤0.05, p≤0.01 y p≤0.001.

the Jacobo+LC+AMP, Jacobo, and Jacobo+AMP treatments showed the highest °Brix values, with significant differences (p≤0.05) compared to those from the Aurora+LC+AMP and Aurora+AMP treatments, surpassing them by 18.2% and 24.2%, respectively (Table 2).

These values are consistent with the 3.9 °Brix reported by Soriano-Melgar *et al.* (2020). However, Moreno-Resendez *et al.* (2019) reported TSS values ranging from 4.61 to 6.79 °Brix, which differ from those observed in this study.

CONCLUSIONS

Fruit diameter was not affected by the treatments, while fruit length was promoted by the combination of the 'Jacobo' variety with vermicompost (LC) and plant growth regulator (AMP). The application of the plant growth regulator (AMP), in both varieties, induced greater fruit weight and yield. Therefore, the application of plant growth regulators can be an alternative to increase zucchini yield under shade house conditions.

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Tomato (*Solanum lycopersicum* L.) yield and quality depending on the osmotic potential and the number of stems

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ABSTRACT

Objective: To evaluate the biochemical components, physical characteristics (number of stems and fruit), and yield of Saladette tomato (*Solanum lycopersicum* L.), Var. Cid F1, grown under greenhouse conditions and its response to a constant increase (from 1 to 2.5 dS m^{-1}) of the osmotic potential of a nutrient solution, during three phenological stages (transplantation, 2nd cluster fruiting, and 6th cluster fruiting) of plants subjected to a single- and two-stem training system.

Design/Methodology/Approach: A sampling was carried out in the 5th cluster to determine the physical characteristics (firmness, size, number, color) and biochemical components (total soluble solids, titratable acidity, vitamin C, lycopene, pH, electrical conductivity, and ripening index) of the fruits. The experiment was established in 2018, under a greenhouse hydroponic system at Colegio de Postgraduados. The experiment was set as randomized complete block design with four replicates.

Results: The increase of osmotic potential and pruning had a positive effect on yield and number of fruits without affecting the biochemical components. Regarding the physical characteristics, T1 had 76% large fruits, 19% medium-sized fruits, 4% small fruits, and 1% tiny fruits.

Study Limitations/Implications: This methodology should be evaluated to other tomato varieties using different substrate mixtures and rates of chemical and organic fertilizers to evaluate water response and crop yield.

Findings/Conclusions: Increasing the osmotic potential of the nutrient solution, during phenological stages of maximum water and nutrient demand and the removal of old leaves had a positive response by increasing fruit number, size, and yield.

Keywords: physical characteristics, biochemical components, number of stems, yield.

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INTRODUCTION

The osmotic potential (Ψo) of nutrient solution (NS) can influence crop growth and production. However, the effect depends on the Ψo magnitude and nutrient uptake capacity of each specie (Chamú-Juárez *et al.*, 2020). Several studies have focused on the

improvement of crop production (Mundo *et al.*, 2020) and fruit quality (Pérez-Díaz *et al.*, 2020). Nevertheless, there are no studies relating the response of tomato to the Ψo of nutrient solution under greenhouse conditions that could influence higher production compared with the commonly used growing systems.

In Mexico, about 50,900 ha are used for protected agriculture, greenhouses, and crops grown under a shade mesh (SAGARPA, 2020). Tomato (*Solanum lycopersicum* L.) is one of most popular produce worldwide, and mainly grown in greenhouses (SAGARPA, 2020). The main crops produced under this system are: tomato (71%), pepper (17%), and cucumber (11%) (SAGARPA, 2020). Production systems change depending on varieties, growth habits, planting density, number of stems, substrates, irrigation, nutrition, and pest and disease control (Mendoza-Pérez *et al.*, 2022). The production technique based on the number of stems of plants grown in Mexican greenhouses is relatively new and it has impacted fruit productivity, profitability and quality during the last few years (Mendoza-Pérez *et al.*, 2018a; Flores-Velázquez *et al.*, 2022). Protected agriculture is a production system developed to provide plants with the ideal conditions for their development, encouraging their maximum yield potential (Vargas-Canales *et al.*, 2018).

Meanwhile, a balanced nutrient solution can help to achieve an optimal plant growth and development. In addition, it decreases the excessive use of chemical fertilizers to avoid the pollution of aquifers and soil salinization (Villarreal *et al.*, 2006). Tomatoes are a source of vitamins, minerals, carbohydrates, and bioactive compounds that benefit human health. Fresh tomatoes can be consumed in many ways and they are also an important raw material for the processing industry (Martínez-Rodríguez *et al.*, 2017). Ripeness is fundamental to determine the right harvesting moment of this fruit (Martínez-González *et al.*, 2017). Klee and Tieman (2013) have shown that several changes in fruit chemical composition occur during this process that determines organoleptic properties such as texture, scent, taste, and color. The typical red color of these fruits indicates their freshness and lycopene content (Farneti, 2014). A two-step ripening process takes place during the final stages of fruit growth and development: physiological maturity occurs when the fruit reaches its maximum size and seed vigor; while consumption stage occurs with changes in fruit color, texture, sugar, organic acids and volatile compounds and become more sensitive to the attack of pathogens associated with the loss of cell wall integrity (Seymour *et al.*, 2013; Dos Santos *et al.*, 2015).

Physical quality is based on the fruit appearance (size, shape, color, brightness, firmness, and lack of defects and damages). However, biochemical components such as total soluble solids, titratable acidity, vitamin C content and pH are key parameters in fruits used by the agroindustry (Flores-Velázquez *et al.*, 2022). Increasing the osmotic potential of nutrient solution during stages of peak demand also increases tomato yield and fruit quality.

The number of stems per plant is one of the agronomical variables associated with tomato productivity. A higher yield is expected from a greater number of stems. However, other response variables that determine tomato quality can be impacted. Therefore, the objective of this study was to evaluate the biochemical components, physical characteristics (number of stems and fruits), and yield of Saladette tomato, var. Cid F1, grown under greenhouse conditions and its response to a constant increase (from 1 to 2.5 dS m^{-1}) in the

osmotic potential of nutrient solution during three phenological stages (transplantation, 2nd cluster fruiting, and 6th cluster fruiting) of plants subjected to a single- and two-stem pruning system.

MATERIALS AND METHODS

Crop management

The experiment was conducted in Colegio de Postgraduados at Campus Montecillo, (19° 27' 58" North latitude, 98° 54' 58" West longitude) and 2431 m above sea level during the spring-summer season 2017. Saladette tomato seeds (Var. Cid F1) were germinated in expanded polystyrene seedling trays with 200 cavities. The planting was realized March 14, the transplant on May 15; while, the process ended on November 30, 2017.

The plant material was grown under hydroponic greenhouse conditions in 12 L polystyrene bags with red tezontle as substrate. The transplanting method consisted of the triangular system (tresbolillo) with 40 cm apart from each plant, twin rows (20 m long), 40 cm between rows, and a density of three plants per m^2 for both treatments.

Experimental design and treatments

The dimension of each experimental unit was 5 m^2 with 15 plants, in a randomized complete block design with four replicates. Two treatments were established as a function of the stem number: T1 (single-stem) and T2 (two stems per plant). The osmotic potential of the nutrient solution was increased in three stages: transplant-2th cluster with osmotic potential of −0.036 MPa; 2th-6th cluster with −0.072 MPa, and 2th-9th cluster with −0.096 MPa for both treatments.

Composition and osmotic potential of the nutrient solution in treatments

T1 and T2 were subjected to three subsequent applications of osmotic potential in the nutrient solution during three phenological stages as recommended by Mendoza-Pérez *et al.* (2018b).

The procedure to obtain and modify the nutrient concentration as well as the osmotic potential was proposed by Steiner (1984). The nutrient solutions were prepared with chemical fertilizers and pH was adjusted to 6.5 using H_2SO_4 1N. Micronutrients (ppm) Mn^{2+} (2.30), Zn^{2+} (0.6), Fe^{2+} (2.0), Cu^{2+} (0.06), and B (0.6) were added to the three nutrient solutions (Steiner, 1984). The irrigation water used in both treatments had 6.69

Table 1. Composition and osmotic potential of nutrient solution used on treatments.

Ψs (Mpa)	pH	EC dS m^{-1}	TIC mg	Ca^{2+}	K^+	Mg^{2+}	$NH4^+$	NO_3^-	$H_2PO_4^-$	SO_4^{2-}	ST
				meq L^{-1}							
−0.036	6.5	1	15	4.5	3.5	2	0	6	0.5	3.5	T-2nd C
−0.072	6.5	2	30	9	7	4	0	12	1	7	2nd-6th C
−0.096	6.5	2.5	40	11.2	8.75	5	0	15	1.25	8.75	6th-9th C

Ψs=Osmotic potential, EC=electrical conductivity, pH, TIC=total ionic concentration; T-2nd C=Transplant-2th cluster, 2th-6th C=2th cluster-6th cluster; 6th-9th C=6th cluster-9th cluster; ST phenological stage.

pH and 0.34 dS m^{-1} electrical conductivity. The ion concentrations (meq L^{-1}) Ca^{2+}, K^{+}, Mg^{2+}, $NH4^{+}$, NO_3^{-}, $H_2PO_4^{-}$, SO_4^{2-}, and HCO^{3-} were 0.8, 0.1, 1.2, 0.0, 0.0, 0.0, 0.1, and 3.2 respectively.

The irrigation was applied along with the nutrient solution through the drip system. EC and pH were monitored every 10 days. Six irrigations of 0.18 L were applied during the first 30 days after the transplant (dat); subsequently, eight irrigations of 0.480 L were applied during vegetative development. Afterwards, 11 irrigations of 1.650 L were applied at the beginning of the harvest (maximum peak demand). Finally, eight daily irrigations of 1.350 L per plant were applied during the final stage (Mendoza-Pérez *et al.*, 2018b).

Evaluated variables

In order to determine yield and number of fruits, eight plants per treatment were selected. Subsequently, the fruits were harvested as they ripened and weighted in a digital scale. Fruit size was determined based on their equatorial diameter and was measured with a digital caliper (RFAIKA model). The fruits were divided into five categories: extra-large (>71 mm), large (61-71 mm), medium-sized (51-60 mm), small (38-50 mm), and tiny (26-37 mm) according to the Mexican standard for tomato diameter (NMX-FF-031-1997).

Fruit firmness and color are two of the physical characteristics analyzed and was measured with a FDV30 texturometer (Greenwich, CT 06836, USA), which is equipped with an 8mm threaded pin. Two readings were taken from opposite sides of the equatorial region of the fruit and recording the values in Newtons (N). Fruit color was measured in the skin of the equatorial area using a colorimeter "HunterLab D25A®" (HunterLab Virginia, USA). Meanwhile, tomato biochemical components were also evaluated. Total soluble solids (°Brix) of tomato juice were measured with a digital refractometer Pr-100 (ATAGO, Guang-zhou, China).

In addition, EC and pH of tomato juice were measured with a potentiometer (Corning 12 Scientific Instruments, USA). Titratable acidity (TA) was measured by homogenizing 10 g of pulp in 50 mL of deionized water. Subsequently, a 10 mL aliquot was taken and neutralized with NaOH at 0.1 N using phenolphthalein as an indicator (AOAC, 2010); the results were recorded as a percentage of citric acid.

In order to estimate vitamin C (ascorbic acid) concentration, 20 g of fresh pulp was homogenized in 30 mL of oxalic acid solution (0.5%). Subsequently, a 5 mL aliquot was taken and it was titrated in a 2,6-dichloroindophenol standard solution (0.05 g/100 mL). In addition, ascorbic acid was used as pattern and the result was expressed as mg of ascorbic acid in 100 g of sample (AOAC, 2010). The maturity index was obtained from the ratio of total soluble solids (TSS) and the titratable acidity (TA).

Lycopene was estimated with the equation proposed by Arias *et al.* (2000), which used colorimetry data, such as L, a*, and b*. A colorimeter "HunterLab D25A®" (Virginia, Sunset Hills Rd, Reston, VA, USA) was used to obtain the data; which was also calibrated to determine the L, a*, and b* color measurements reported on the International Commission on Illumination (CIE). The lycopene content of the harvested fruits was calculated with Equation 1 described by Arias *et al.* (2000).

$$Lycopene\left(mg\,100\,g-1\right)=11.848*\left(a*/b*\right)+1.5471 \qquad \text{Equation (1)}$$

Statistical analysis

Analysis of variance and Tukey mean separation test were performed for all evaluated variables using Minitab statistical software (Minitab, 2017).

RESULTS AND DISCUSSION

Yield evaluation

T1 attained a yield of 28.59 kg m^{-2}. This result showed a positive response to the increase of the osmotic potential in nutrient solution during the stage of highest water and nutrient demand. Mendoza-Pérez *et al.* (2018b) reported a yield of 20 kg m^{-2} per plant on treatments with a single stem tomato and osmotic potential of −0.072 MPa. Meanwhile, Núñez-Ramírez *et al.* (2017) found a yield of 19.3-21.2 kg m^{-2} using different nitrogen fertilization rates.

Corella *et al.* (2013) reported a yield of 23.82 kg m^{-2} on six harvested clusters conducted on treatments with single stem. Espinosa-Espinosa-Palomeque *et al.* (2019) reported yields from 4.9 to 8.5 kg m^{-2} on plants with different nutrient solution concentrations. Plants on T2 recorded a total yield of 37.74 kg m^{-2}, of which, 23.76 kg m^{-2} were obtained from the main stem with 10 clusters and 13.98 kg m^{-2} from the secondary stem with 8 clusters. This treatment also showed a positive response to the increase of the osmotic potential. However, plants with a secondary stem produced more medium-sized fruits. This result was higher from that reported by Mendoza-Pérez *et al.* (2018b) who conducted plants with two stems and attained a yield of 18 kg m^{-2} on both stems. Meanwhile, Corella *et al.* (2013) reported a yield of 21.39 kg m^{-2} harvested from six clusters in two-stem tomato plants. According to these results, the plants had a positive effect on yield and fruit quality when the concentration of nutrient solution was enhanced during the stages of higher nutrient demand. In addition, management practices such as pruning or leaf removal at senescence were carried out simultaneously.

Number of fruits per cluster

T1 produced the greatest number of medium-sized fruits in the first two clusters. However, a greater quantity of large fruits was obtained from the third to the sixth cluster. Subsequently, the plant turned to produce more medium-sized fruits. A total of 86 fruits per plant were obtained: 37 large, 37 medium-sized, 10 small and 2 tiny fruits (Figure 1). This result is higher than that reported by Mendoza-Pérez *et al.* (2018a) who harvested 62 fruits per plant. Additionally, the maximum yield potential was recorded on the seventh and eighth clusters, obtaining 11 and 12 fruits per cluster respectively. According to these results, this response is a consequence of the increase of the osmotic potential (−0.096 MPa) of the nutrient solution and leaf pruning. The response of plants on this trial is attributed to the increase in the osmotic potential of nutrient solution (−0.096 MPa) and the pruning of leaves. It is observed that water and nutrients are transported to the young leaves influencing their development and fruit filling. On the other hand, Núñez-Ramírez

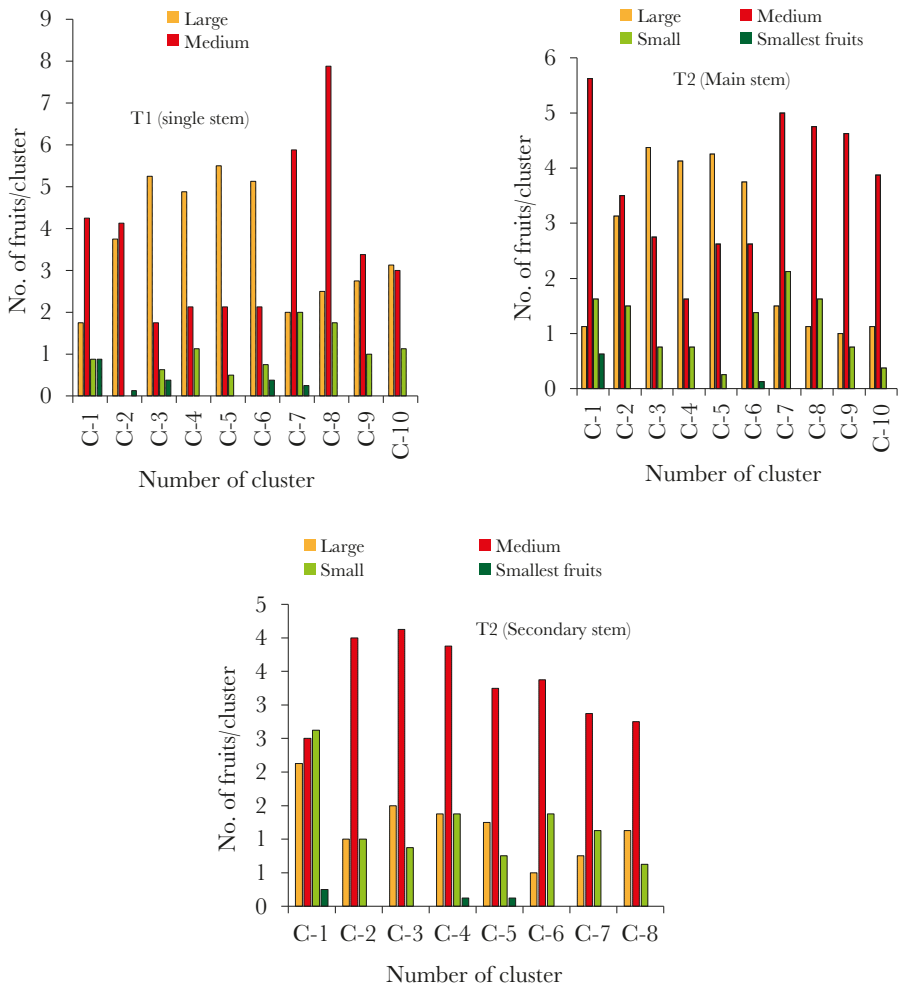

Figure 1. Number of fruits harvested from the treatments.

et al. (2017) found that the application of very high nitrogen rates on this crop increases the number of fruits, but the size of the fruits decreases.

It was found that a greater number of medium-sized fruits was harvested from the first two clusters of the main stem on T2. From the third to the sixth cluster, a greater number of large fruits were harvested and then the plant subsequently produced more medium-sized fruits. A total of 75 fruits per plants were harvested: 26 large, 37 medium-sized, 11 small, and 1 tiny. In that aspect, Mendoza-Pérez *et al.* (2018a) reported 78 fruits harvested from 10 clusters. They also observed that plants produced greater number of medium-sized fruits in all the clusters from the secondary stem of the same treatment. A total of 48 fruits were obtained: 10 large, 27 medium-sized, 10 small fruit, and 1 tiny. As previously observed, increasing the osmotic potential of the nutrient solution from −0.076 to −0.096 MPa during the fruiting stage of the sixth cluster also increased the number, size, and yield of both treatments. There was evidence that increasing the osmotic potential of the nutrient solution and removing the leaves during senescence enabled the transport of water and nutrients to the developing organs, which significantly contributed to the results

obtained in this research. In that sense, Arébalo-Madrigal *et al.* (2018) reported that plants with single stem produce fruits with better quality indexes (weight and size), while two-stem or unpruned plants produce more fruits. Nevertheless, they do not reach the right size or desirable quality due to competition for sunlight, water, and nutrients.

Color

Color is a quality attribute of food that impacts the consumer acceptance, taste, and perception (Althaus and Blanke, 2021). The fruits recorded average hue values of 62.31 (very intense red). It also recorded a value of 30.62 for brightness and 24.64 chrome value, which indicated the purity of color. According to the intervals proposed by Cantwell *et al.* (2007), hue values from 35 to 40 match an intense red color, while brightness values from 39 to 41 belong to different tomato varieties (Table 1).

Physical characteristics of the fruits

According to firmness values, T1 recorded 4.42 N, while T2 obtained 4.13 N in the main stem treatments and 4.11 N in the secondary stem treatment. Mendoza-Pérez *et al.* (2018a) reported that single stem plants intercepted a higher amount of radiation than two-stem plants. This phenomenon favored the development of a thicker and more resistant cuticle, which protects fruits from direct damage of solar radiation, increasing their shelf life. The same authors reported 4.43 N for single stem plants and 4.19 N for two-stem plants (main and secondary stems). Whereas, Navarro-López *et al.* (2012) obtained 4.21 and 4.52 N firmness values.

Fruit size

T1 recorded the best fruit size, reaching 76, 19, 4, and 1% large, medium-sized, small, and tiny fruits respectively (Figure 2). The fruits obtained on this treatment attained the size established by the NMX-FF-031-1997 rule for export products. Núñez-Ramírez *et al.* (2017) recorded 28, 31, 23, and 18% extra-large, large, medium-sized, and small fruits, respectively. Meanwhile, Mendoza-Pérez *et al.* (2018b) obtained 68, 23, 8, and 1% large, medium-sized, small, and tiny fruits, respectively. Finally, Núñez-Ramírez *et al.* (2012) reported values of 20% extra-large, 24% large, 20% medium-sized, and 36% small fruits on single-stem globe tomato plants.

Table 2. Effect of the number of stems on tomato fruit color (Cid F1).

Treatments	Number of stems	Brightness (L)	Purity (chroma)	Hue
T1 (single stem)	Main stem	30.81 a	24.19 a	61.83 a
T2 (two stems)	Main stem	30.53 a	25.68 a	61.99 a
	Secondary stem	30.53 a	24.07 a	63.13 a
SD		0.16	0.90	0.71
CV (%)		0.53	3.64	1.14

SD=Standard deviation; CV=Coefficient of variation (%). Different letters in each column indicate significant differences ($p \leq 0.05$).

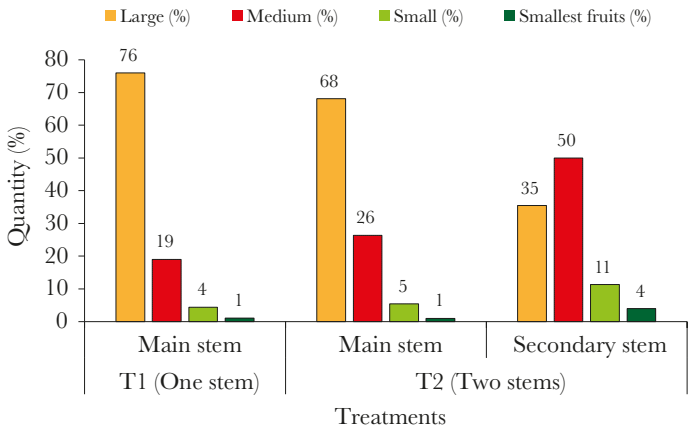

Figure 2. Quantity classification based on fruit size.

Plants with main stem on T2 recorded 68, 26, 5, and 1% large, medium-sized, small, and tiny fruits. The fruits obtained from this treatment are considered for export quality standards. Plants with secondary stem recorded 35, 50, 11, and 4% large, medium-sized, small, and tiny fruits, respectively. These fruits did not accomplish the export standards. In addition, the reduction on fruit size was mainly attributed to stem reduction, since its smaller diameter diminishes the vessel capacity to transport water and nutrients to the fruits. These results coincide with those of Mendoza-Pérez *et al.* (2018b), who reported 49, 33, 17, and 1% large, medium-sized, small, and tiny fruits. Villamán (2015) indicated that a second stem left in the plant competes for solar radiation, water, and nutrients and consequently affects the development of the main stem, causing a delay in maturity and harvest. Pruning plants with two-stems increases the number of medium-sized fruits. However, their quality is lower as compared to unpruned plants. This effect is due to the high demand for nutrients that plants require to nourish two stems and produce fruits.

Biochemical components

The total soluble solid values were of 4.33 °Brix for both treatments, without variations, despite the increase in the number of stems per plants ($P \geq 0.05$) (Table 3). Navarro-López *et al.* (2012) reported that EC of 4.5 dS m^{-1} in the nutrient solution causes a reduction in the water flux transported to the fruit, leading to salinity stress. Tomato fruits under this type of stress mainly store ions and organic molecules (increase of fructose and glucose concentration) (Kubota, 2006). Pérez *et al.* (2016) recorded 5.1 °Brix values in single stem plants and 5.0 °Brix in two-stem plants with osmotic potential of −0.072 MPa in the Steiner nutrient solution. T1 recorded a titratable acidity of 0.38%; while T2 treatments recorded 0.45% and 0.46% for main and secondary stems (Table 3). In that aspect, Pérez *et al.* (2016) reported 0.8 and 1.0% titratable acidity for single and two-stem plants respectively.

Vitamin C values of 5.54 mg, 4.49 and 4.81 mg 100 g^{-1} ascorbic acid for T1 and T2 (main and secondary stem) (Table 3). These results are lower than the range found on 30 different varieties of cherry tomato (29-73 mg 100 g^{-1}) as reported by Ceballos-Aguirre *et al.* (2012) and to the interval of 6.1 to 16.1 mg 100 g^{-1} reported by Crisanto-Juárez *et*

Table 3. Total soluble solids, titratable acidity, vitamin C, and lycopene of the fruits.

Treatments	NS	Total soluble solids (°Brix)	Tritatable acidity (%)	Vitamin C (mg 100 g^{-1})	Lycopene (mg 100 g^{-1})
T1 (single stem)	TP	4.30 a	0.38 a	5.54 a	14.61 b
T2 (two stems)	TP	4.30 a	0.45 a	4.49 a	16.40 a
T2 (two stems)	TS	4.40 a	0.46 a	4.81 a	16.10 ab
SD		0.06	0.04	0.54	0.96
CV (%)		1.33	10.14	10.88	6.10

NS=Number of stems; SD=standard deviation; CV=coefficient of variation (%). Different letters in each column indicate significant differences ($p \leq 0.05$).

al. (2010) for different wild tomato varieties. Núñez-Ramírez *et al.* (2017) reported values of 0.32 and 0.35 g 100 g^{-1} of citric acid on tomato. This study recorded higher lycopene concentrations on T2 (main stem) with 16.40 mg 100 g^{-1}. No differences were found on treatments with the secondary stem (Table 3) but it was statistically different ($p<0.05$) with respect to T1.

The lycopene accumulation is related to the number of stems: as the number of stems increases, the leaves intercept more photosynthetic active radiation with respect to single stem plants (Mendoza-Pérez *et al.*, 2018b). Pérez *et al.* (2017) found 18.5 mg 100 g^{-1} lycopene values for the Cid F1 variety grown under greenhouse conditions. While, Luna-Guevara and Delgado-Alvarado (2014) found that temperature and light intensity directly influence lycopene accumulation.

The pH values of the juice were 4.40, 4.35, and 4.43 for T1 and T2 (main and secondary stems) (Table 4). If the osmotic potential increases above 2.5 dS m^{-1}, also increases electrical conductivity due to fertilizer accumulation in the substrate. Consequently, higher values can cause negative effects in the fruit quality variables, such as a reduction in fruit size and increase in pH of the juice. Pérez *et al.* (2016) and Mendoza-Pérez *et al.* (2018a) reported pH of 4.47 and 4.30 in tomatoes harvested from single stem and two-stem plants.

Electrical conductivity (EC) recorded 3.17 dS m^{-1} from the juice of fruits harvested from T1, 3.14 dS m^{-1} from the fruits of T2 (main stem) and 2.93 dS m^{-1} from the fruits of the secondary stem. These results are within the range reported by Barrera-Puga *et al.* (2011) who found values of 0.68-3.05 dS m^{-1} EC in tomato fruits.

The maturity index was 11.29 (T1), 9.91 and 9.8 for T2 (main and secondary stem). This index is directly related to quality, taste, firmness, and post-harvest period. Al-Yahyai *et al.* (2010) recorded 13.16 and 12.60 for the ratio of total soluble solids and titratable acidity with EC 6 and 9 dS m^{-1} EC of the nutrient solution.

The maturity index values found on this study are lower than those found by Al-Yahyai *et al.* (2010) and are attributed to a lower osmotic potential of nutrient solution applied (Table 4). Brasiliano *et al.* (2006) proved that the ratio of total soluble solids and titratable acidity can increase if saline irrigation water is used or if the plants are subjected to a moderate water stress.

Table 4. pH, electrical conductivity, and ripeness index of tomatoes.

Traeatments	Number of stems	pH	EC (dS m^{-1})	Maturity index
T1 (single stem)	Main stem	4.40 a	3.17 a	11.29 a
T2 (two stems)	Main stem	4.35 a	3.14 a	9.91 a
T2 (two stems)	Secondary stem	4.43 a	2.93 a	9.88 a
SD		0.04	0.13	0.81
CV (%)		0.92	4.25	7.78

SD=Standard deviation; CV=Coefficient of variation (%). Different letters in each column indicate significant differences ($p \leq 0.05$).

CONCLUSIONS

Increasing the osmotic potential of the nutrient solution during the stages of maximum water and nutrient demand and removing leaves during the senescence of Saladette tomatoes (var. Cid F1) enhanced the number, size, and yield of fruit. The biochemical components were not affected by increasing the osmotic potential or pruning the plants.

This increase lead to a reduction of fruit size, preventing tomatoes from reaching the right export size. It is advisable to adopt the practice of a single stem plants in order to produce larger tomatoes with quality export.

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Bioprospecting of rhizobacteria with antagonistic activity against *Fusarium* spp., a parasite of cucumber (*Cucumis sativus*)

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ABSTRACT

Objective: To identify bacteria of genus *Bacillus* which, isolated from the tomato and cucumber rhizosphere, have an antagonistic effect against *Fusarium* spp. isolated from cucumber plants in Culiacan, Sinaloa.

Design/Methodology/Approach: Both the *in vitro* and *in vivo* antagonisms of rhizobacterial isolates against *Fusarium* spp. on cucumber plants were evaluated. Bacteria with the highest antagonistic effect were identified based on their morphological and molecular characteristics.

Results: Isolates FA15 and FA16 showed the highest *in vitro* biological efficacy against *Fusarium* spp., with 50.0% and 61.36% inhibition of mycelial growth, respectively. Rhizobacterium FA15 achieved the highest biological efficacy (88.89%) against *Fusarium* spp. in cucumber plants, while rhizobacterium FA16 recorded a 59.27% efficacy. The morphological and molecular characterization of isolates FA15 and FA16 confirmed a 100% molecular identity between FA15 and Bacillus velezensis and FA16 and *B. subtilis*.

Study Limitations/Implications: The rhizobacteria identified in this study inhibited the mycelial growth of the phytoparasite. Therefore, further studies about these rhizobacteria should be carried out to determine the potential antibiosis that may cause the inhibitory effect.

Findings/Conclusions: During the search for native beneficial rhizobacteria, two bacteria that exercise a biologically-effective control over *Fusarium* spp were identified in Culiacan: *Bacillus velezensis* and *B. subtilis*. This finding offers an opportunity in the agricultural biotechnology field to study beneficial native species that could provide an alternative to the use of chemicals.

Keywords: Antagonism, agricultural biotechnology, biological effect, cucumber.

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INTRODUCTION

When the physicochemical characteristics of the soil and the exudates resulting from the physiological activity of plants are favorable, fungi and bacteria (part of the diverse microorganisms found in the rhizosphere) play a key role in the growth, nutrition, and health of plants [1]. Rhizobacteria found around root tissues have beneficial potential: they

can establish a symbiotic relationship with plants and work as antagonists of soil-borne phytopathogens [2]. The benefits of rhizobacteria include the improvement of nutrient availability and absorption; this antagonistic activity is also the result of hyperparasitism, antibiosis, and the competition with phytopathogenic organisms for space [3,4]. The rhizobacteria from genus *Bacillus* can form endospores as survival structures [5], which facilitates their use in stable commercial formulas [6].

Mexico is one of the most important producers of horticultural produce around the world. In particular, the state of Sinaloa produces a high volume of cucumber (*Cucumis sativus*) [7]. However, several phytosanitary problems have a severe impact on cucumber production, mainly as a consequence of the activity of phytopathogens. These pathogens include fungi from genus *Fusarium* [8], which cause withering, root rot, and plant death. Diseases caused by fungi from genus *Fusarium* are also considered soil-borne diseases with high pathogenic potential, whose resistance structures survive on the soil for several years [9,10].

Contemporary agricultural practices employed in the management of *Fusarium* in cucumber crops are highly-dependent on the use of synthetic fungicides. Additionally, the indiscriminate use of these compounds has created resistance among phytopathogenic organisms, as well as public health problems [11]. In this context, the use of rhizobacteria from genus *Bacillus* has become increasingly important for plant health management, particularly as a green and sustainable strategy for the protection of cucumber crops. Therefore, the aim of this research was to isolate rhizobacteria of genus *Bacillus* from the horticultural rhizosphere in Culiacan and use them as antagonists of the *Fusarium* spp. that impact cucumber plants.

MATERIALS AND METHODS

Sample collection

Soil samples were collected from January 12 to January 24, 2022, in the two agricultural plots of the Facultad de Agronomía of the Universidad Autónoma de Sinaloa (UAS). These plots house experimental tomato (*S. lycopersicum*) and cucumber (*C. sativus*) crops, both in the fruit production phenological stage. The tomato plot is located at 24° 37' 30.95" N and 107° 26' 35.75" W, while the cucumber plot is located at 24° 54' 50.71" N and 107° 37' 26.44" W. The cultivation of both crops has involved a low use of synthetic agro-inputs. The roots of 15 plants with soil-traces were collected from each plot, at a depth of 6 to 12 cm; the plants were randomly selected in a zigzag path. The roots and soil collected were placed in individual plastic bags; they were then labelled and sent to the Horticultural Diseases Laboratory of the Facultad de Agronomía, where they were kept at 4 °C, until they were processed.

Isolation, selection, and purification of rhizobacteria

The microorganisms were isolated according to the methodology proposed by Posada *et al.* (2016) [6], with minor modifications. The individual samples were processed as follows: 50 g of roots with soil traces were put inside a 1,000-mL beaker, into which 400 mL of sterile distilled water had been previously poured. An orbital shaker was used to shake the

mixture at 200 rpm for 60 minutes, keeping the temperature at 26±1 °C. Subsequently, a 1 mL aliquote was poured into a 12 mL test tube which contained 9 mL of sterile distilled water. Serial dilutions were carried out until a 10^{-8} dilution was achieved, 100 μL of which were distributed in a Petri dish with a nutrient agar medium. The dishes with the root and soil suspension were kept under lab conditions, at 26±1 °C for 48 h. Rhizobacteria colonies from the dishes with culture medium that formed an antibiosis halo were chosen; this halo limited the development of other nearby microorganisms and its macroscopic morphology (color, rim, texture, and elevation) was similar to bacteria from genus *Bacillus* [12,13].

The rhizobacteria colonies selected were isolated and purified in a nutrient agar medium and preserved at 4 °C in a phosphate buffer, until they were used.

Phytoparasitic organism

The phytoparasitic fungi was obtained from the strain repository of the Horticultural Diseases Laboratory of the UAS – Facultad de Agronomía. The strain was isolated from cucumber plants and identified as member of *Fusarium* spp. The phytopathogenic fungi was activated in the Potato Dextrose Agar (PDA) culture medium and incubated at 26±1 °C until it was used.

***In vitro* antagonisms of rhizobacteria against *Fusarium* spp.**

The dual culture technique was employed. Initially, a *Fusarium* spp. mycelium was allowed to grow in a 0.6-cm cylinder with a culture medium for 120 h. The cylinder was then placed in the middle of a Petri dish with PDA. Subsequently, 10 μL of a rhizobacteria suspension (3×10^8 UFC, according to the McFarland standards) were poured around the cylinder in which the mycelia had grown, 2.2 cm apart from each other, in the four cardinal points [14]. They were incubated at 26±1 °C. Sterile distilled water replaced the bacterial suspension in the cardinal points of the control treatment. The study consisted of a completely random design and eight repetitions per treatment (each Petri dish was considered to be a repetition). The growth of the mycelia of the fungi on the culture medium was measured in every dish when the mycelia in the control treatment reached 2.2 cm (7 days after the start of the treatment). The mycelia growth data determined the biological efficacy of the inhibition (Eterbarian *et al.*, 2005 [15]), according to the following formula:

$$n = a\frac{b}{a}\times 100$$

Where n=biological efficacy (%); a=radial growth of the control; and b=radial growth of the pathogen.

Antagonistic efficacy against *Fusarium* spp. in cucumber plants

Cucumber cv. "Poinsett 76" seeds were sown in polystyrene trays with 128 holes, using peat as substrate and vermiculite as cover. Once the seedlings had emerged and

they had two true leaves, the plants were inoculated, immersing their leaves for 3 min in a water suspension with a 3×103 mL^{-1} concentration of *Fusarium* spp. propagules [16]. Subsequently, the inoculated plants were transplanted to the center of a 3 kg plastic pot with 2.3 kg of a chromic vertisol [17] and peat moss mixture (7:3). Immediately afterwards, 10 mL of a water suspension rhizobacteria at a 1×108 mL^{-1} concentration were added around the neck of the plants. The seedlings were watered by hand daily (250 mL per pot). The damage severity caused by *Fusarium* spp. was determined using the symptom scale proposed by Marlat *et al.* (1996) [18] (Table 1). Additionally, the damage severity percentage was determined using the equation proposed by Ley *et al.* (2018) [19]:

$$\%SD = \sum\left[\frac{GD \times NP}{EM \times TP}\right]\times100$$

Where $\%SD$=damage severity (%); GD=damage degree; NP=number of damaged plants; EM=maximum damage degree in the severity scale; and TP=number of plants in the treatment.

The efficacy of the control was determined with the following equation (Ley *et al.*, 2018):

$$\%EB = \frac{100 - SD\,del\,tratamiento}{SD\,del\,control}\times100$$

Where $\%EB$=percentage of control efficacy; *SD del tratamiento*=damage severity mean per treatment; and *SD del control*=damage severity mean of the control. The study was established under greenhouse conditions, with a completely randomized design, consisting of eight treatments with seven repetitions per treatment. Each plot with a plant was considered as a repetition.

Identification of the bacteria

The bacteria were identified observing their morphology (cell shape, colony, and Gram stain) [13] and using molecular techniques. A rhizobacteria culture was grown for 48-72 hours before it was used. The DNA of the rhizobacteria was amplified through a Polymerase Chain Reaction (PCR) using the gene 16S

Table 1. Severity of the damage caused by *Fusarium* spp.

Scale value	Damage Description
0	Symptomless plant
1	slight chlorosis, wilting or stunting
2	Moderate chlorosis, wilting or dwarfing of the plant
3	Severe chlorosis, wilting or dwarfing of the plant
4	Dead plant

of the DNAr. The FD2 (5'-AGAGTTTGATCATGGCTCAG-3') and RP1 (5'-TACCTTGTTACGACTTCACC-3') universal initiators were used for this purpose, amplifying a 1,500 pb fragment with a T100™ Thermal Cycler (Singapore). According to the methodology proposed by Ley *et al*., (2018) [19], the following temperatures and times were used: enzyme activation at 95 °C for 5 min, followed by 30 cycles, including a denaturation at 94 °C for 1 min, an alignment step at 56 °C for 1 min, and an extension at 72 °C for 1 min. A final extension at 72 °C was carried out for 10 min, once the cycles were over.

The resulting fragments were visualized in a Powerpac™ chamber (Bio-Rad), through an electrophoresis process, using a 1% agarose gel. Likewise, the fragments were purified, sequenced, and compared with the nucleotide sequence available in the database of the National Center for Biotechnology Information (NCBI).

Statistical analysis

The resulting data were subjected to an analysis of variance (ANOVA), using the Minitab 19 statistical software. Likewise, the means were compared using Tukey's test ($p \leq 0.05$).

RESULTS AND DISCUSSION

Seven rhizobacteria were isolated from the experimental plots. They were then studied and codified as: FA11, FA12, FA13, FA14, FA15, FA16, and FA17.

In vitro antagonism of rhizobacteria isolates against *Fusarium* spp.

Table 2 shows the inhibitory effect of seven bacterial isolates on the *in vitro* growth of mycelia of *Fusarium* spp. The growth of the mycelia of the fungi under study was impacted by rhizobacteria, except for isolate FA11. Only isolates FA15 (1.35 cm) and FA16 (1.1 cm) recorded a significantly lower growth of the mycelia ($p \leq 0.05$) than in the control fungi.

Regarding the percentage of biological efficacy, only isolates FA15 and FA16 recorded a significant inhibition of mycelial growth ($p \leq 0.05$). Meanwhile, the percentage obtained with isolate FA15 was 11.36% higher than the percentage reported with isolate FA16 (Table 2).

Table 2. Inhibitory effect of rhizobacteria on the *in vitro* growth of the mycelium of *Fusarium* spp.

Treatments	Mycelial growth of *Fusarium* sp. *in vitro*	
	Mycelial growth (cm)	Biological effectiveness of inhibition (%)[A]
FA11	2.20±0.0 a	0±0 d
FA12	2.14±0.05* ab**	2.84±2.35 cd
FA13	2.11±0.06 ab	3.97±2.91 cd
FA14	2.08±0.04 ab	4.55±1.6 cd
FA15	0.85±0.09 d	61.36±4.21 a
FA16	1.10±0.08 c	50.00±3.43 b
FA17	2.14±0.05 ab	2.84±2.35 cd
Control	2.20±0.0 a	0±0 d

*Standard deviation. **Means not sharing a letter are significantly different according to Tukey ($P \leq 0.05$).

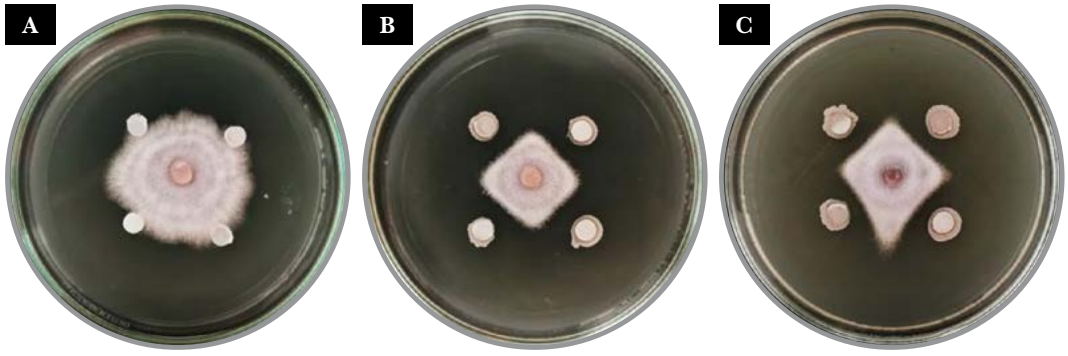

Figure 1. Inhibition of the mycelial growth of *Fusarium* spp., using a rhizobacteria cell suspension: A) control (*Fusarium* spp.), B) FA15 *vs. Fusarium* spp., and C) FA16 *vs. Fusarium* spp.

Efficacy of the antagonism of the FA15 and FA16 rhizobacteria against *Fusarium* spp. on cucumber plants

The efficacy of the antagonism against *Fusarium* spp. showed that plants treated with isolates FA15 and FA16 recorded a significantly lower severity of the disease ($p \leq 0.05$) than plants that were only inoculated with *Fusarium* spp. (Table 3). The severity of the damage on plants that were only inoculated with *Fusarium* spp. was 81.6% and 53.6% higher than plants treated with isolates FA15 and FA16, respectively. Consequently, the biological efficacy of FA15 was significantly higher ($p \leq 0.05$) than with isolate FA16: isolate FA15 had a 28% greater biological efficacy.

Identification of isolates FA15 and FA16

Colonies of FA15 and FA16 bacteria had similar shape and color; both colonies had a creamy consistency, a central elevation, and a mucus-like texture. FA16 colonies were smaller (1.5 mm) than FA15 colonies (2.0 mm). Both isolates recorded Gram-positive stains. After they were compared with the sequences previously reported in the Gen Bank database (NCBI), the sequences obtained from isolates FA15 and FA16 using the PCR molecular techniques showed a 100% identification with *Bacillus subtilis* and *Bacillus velezensis*, respectively. The sequences were deposited in the database, with accession numbers PP862827 (FA15) and PP862812 (FA16).

According to Phung and Dao (2024) [20], biodiversity conservation and the minimization of the negative environmental impact, among other factors, have placed

Table 3. Efficacy of the antagonism of isolates FA15 and FA16 against *Fusarium* spp. on cucumber plants.

Treatments	Antagonism on *Fusarium* sp. in cucumber plants	
	Severity of damage (%)	Biological efficacy (%)
FA15	14.82±13.36* c**	88.89±13.86 a
FA16	42.82±27.82 b	59.27±28.85 b
Fusarium sp.	96.42±9.45 a	---
Control (without fungus)	0±0 c	---

*Standard deviation. **Means not sharing a letter are significantly different according to Tukey ($P \leq 0.05$).

agricultural sustainability at the core of worldwide discussions. Therefore, further research should focus on beneficial microorganisms that can be used as part of an environmental, profitable, and sustainable agricultural strategy [21]. This interest has increased as a consequence of the efficacious promotion of plant growth of some bacteria and their role as biological control agents against various phytopathogens [2]. According to Tejera-Hernández *et al.* (2011) [22] efficacious beneficial bacteria must have >50% antagonistic efficacy against phytopathogen microorganisms. The FA15 and FA16 rhizobacteria chosen for this study had a >50% *in vitro* antagonistic activity against *Fusarium* spp. The morphological characteristics of these rhizobacteria belong to genus *Bacillus* [12,13], several species of which are known to have an antagonistic effect on phytopathogens [23]. The morphological characteristics of isolates FA15 and FA16 match the molecular identification, determining that isolates FA15 and FA16 belong to *Bacillus velezensis* and *B. subtilis*, respectively. The antagonistic effects of these isolates match the results obtained by Hasan *et al.* (2024) and Tian *et al.* (2023) [3,24]. This research proved that the *B. velenzesis* and *B. subtilis* rhizobacteria have biological efficacy against *Fusarium* spp. on cucumber plants. *B. velezensis* and *B. subtilis* can produce several highly-biodegradable cyclical lipopetides (including iturins, surfactins, and fengycins) with antimicrobial activity at a low concentration [5,24].

CONCLUSIONS

Two rhizobacteria with antagonistic efficacy against the *Fusarium* spp. pathogen that damages cucumber were isolated in Culiacán, Sinaloa. The rhizobacteria were identified as *Bacillus velezensis* and *B. subtilis*, both of which had a significant inhibition effect on the mycelia of *Fusarium* spp. Additionally, they proved to have potential as biological control agents on cucumber plants. This is an outstanding opportunity for the agricultural biotechnology industry to find native species that could be used as an alternative to chemical products.

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Colegio de Postgraduados

Glyphosate-, carbofuran-, and chlorpyrifos-tolerant *Priestia aryabhattai*, isolated from agricultural soils

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ABSTRACT
Objective: To isolate and carry out a molecular characterization of microorganisms potentially tolerant to high concentrations of different pesticides (glyphosate, carbofuran, and chlorpyrifos).
Design/Methodology/Approach: Based on the isolate project SIP20170193, only the strain GVE 5 was chosen for the experiment, as a result of its morphological and growth characteristics. The tolerance capacity (TC) of GVE 5 to three different pesticides (glyphosate, carbofuran, and chlorpyrifos) was evaluated in two different media (LB and M9). The only carbon source was 200 mg/L of each pesticide. GVE 5 was identified through the Polymerase Chain Reaction (PCR) molecular techniques and amplified by the 16S rRNA marker.
Results: Based on the TC analysis, the GVE 5 strain of *Priestia aryabhattai* recorded a growth with 200 mg L^{-1} of glyphosate, carbofuran, and chlorpyrifos in LB medium and M9 minimal medium.
Study Limitations/Implications: There were no limitations or implications for this study.
Findings/Conclusions: *Priestia aryabhattai* is tolerant to 200 mg L^{-1} of glyphosate, carbofuran, and chlorpyrifos. These results open new lines of research regarding the bioremediation of soils polluted by these agrochemicals. *Priestia aryabhattai* should be subjected to further evaluations as a plant growth promoter.

Keywords: pesticides, tolerance, *Priestia aryabhattai*.

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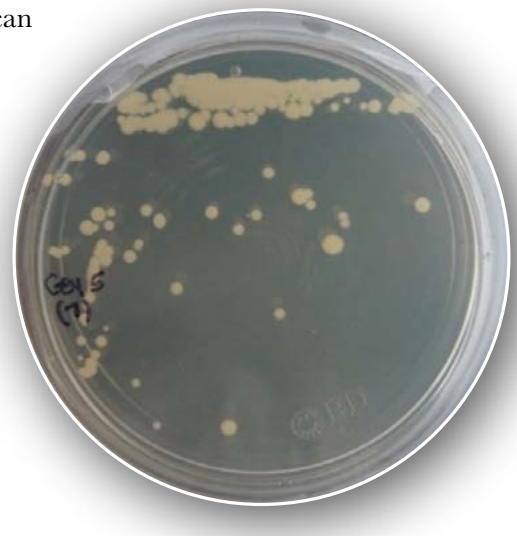
INTRODUCTION

The competition against weeds and pests can impact the quality and yield of food crops in agricultural systems. Throughout the world, agroecosystem sustainability is seriously threatened by the widespread use and release of several agrochemicals. In Mexico, pesticide production volume reached 59,157 t in 2017 (INEGI, 2018). For their part, Sinaloa producers use about 30% of the total pesticides applied in northeastern Mexico (Leyva *et al.*, 2014). Currently, the use of 183 active ingredients (AI) of highly



hazardous pesticides (HHP) has been authorized. Out of this total, 33% AI are included in the Official Pesticide Catalog (CICOPLAFEST 2016). If inhaled, these AI can be extremely dangerous and deadly. In addition, they are considered as probably carcinogen, carcinogen, mutagenic, bio-accumulative, persistent in water, soil, or sediments, and very toxic to aquatic organisms and bees.

The accumulation of these pesticides in the environment through various processes can reduce soil capacity, impacting biological production functions, environmental protection, and human health (Vallejo, 2013). Microorganisms can biodegrade pesticides and their metabolites from the environment, while microbial consortium can completely biodegrade them (Ortiz *et al.*, 2013). The capacity of microorganisms to tolerate and degrade pollutants is associated with their long-term adaptation to highly polluted environments. Commonly identified microbes used for bioremediation purposes against pesticides are: *Pseudomonas* spp., *Bacillus* spp., *Klebsiella* spp., *Pandoraea* spp., *Phanerochaete chrysosporium*, and *Mycobacterium* spp. (Odukkathil and Vasudevan, 2013). As a result of their biochemical capacity to adapt to the environment, bacteria can easily induce mutant strains. Consequently, further studies are required about this subject (Racke *et al.*, 1996; Oliveira *et al.*, 2015). During the last few years, efforts have been made to increase the sustainability of agriculture, seeking biological solutions to degrade pesticides. Therefore, the objective of this study was to isolate, identify, and characterize the molecules of bacteria that can tolerate a 200 mg L^{-1} concentration of glyphosate, carbofuran, and chlorpyrifos, under in vitro conditions. The aim was to determine a consortium and to develop a potential biotechnological catalog for the bioremediation of soils impacted by these agrochemicals. In the future, these microbial consortia could also have an additional purpose: to play a role in plant growth promotion.

MATERIALS AND METHODS

Chemical product

The commercial brands FAENA® (35.6% glyphosate), Furadan® (350L of carbofuran), and CHLORBAN® (480EC of ethyl chlorpyrifos) were used to evaluate tolerance in the M9 minimal medium.

Strain and pesticide selection and hemolytic activity assessment

Based on the results from the SIP20170193 project, a fast-growing strain (GVE 5) was chosen. GVE 5 came from Guasave, a region where potato crops dominate agricultural systems. The production or lack of hemolysis was determined in 5% blood agar from a bovine blood serum. Striatal-inoculation was carried out and, subsequently, the product was incubated at 28 °C for 18 h (Haubert *et al.*, 2017). A pesticide database was developed to determine the agrochemicals, taking into account family, degradation processes, and half-life (DT50), based on the following sources: TOXNET (https://toxnet.nlm.nih.gov), PubChem (https://pubchem.ncbi.nlm.nih.gov), and DISI (https://disi.gob.mx/agroquimicos/).

Evaluation of the tolerance capacity to 200 mg/L glyphosate, carbofuran, and chlorpyrifos

A M9 minimal medium was prepared: $Na_2HPO_4 \cdot 7H_2O$ 48 mM, KH_2PO_4 22 mM, NaCl 8.6 mM, NH_4Cl 18.7 mM, $MgSO_4$ 1 mM, and $CaCl_2$ 0.1 mM: pH 7-7.2 (adjusting HCL 1 M NaOH 5 M) (Graf and Altenbuchner, 2011; Shabbir *et al.*, 2018)II and III. Once the medium was sterilized, 200 mg L^{-1} of a commercial pesticide pattern (glyphosate, carbofuran, and chlorpyrifos) were added at 40 °C as the only source of carbon. A 2 g L^{-1} stock of each pesticide was dissolved in sterile deionized water. Subsequently, it was filtered in a 0.22 μm acrodisc and kept at 4 °C until it was applied with striatal Kolle handles. Afterwards, it was incubated at 28 °C for seven days, using a modified version of the methodology proposed by Shabbir *et al.* (2018). A blank and a control were used in the experiment to guarantee the reliability of the results and the control measures, as well as to obtain statistically significant data for the four repetitions.

Sanger sequencing molecular characterization: DNA extraction and PCR

Once the GVE 5 strain had been reactivated and purified, a repeated subculturing in stock was carried out with each strain, in order to obtain the biomass. Subsequently, the DNA was extracted according to the instructions of the DNeasy UltraClean Microbial Kit QIAGEN. Afterwards, the DNA was visualized in 1% agarose gel, stained with ethidium bromide. The 16S1 (AAGGAGGTGATCCAGCC) and 16S2 (GAGASTTTGATCHTGGTCAG) primers were used to amplify the areas preserved in 16S. A final volume of 25 μL was obtained, preparing the following amplification reactions: 5 μL DNA [20 ng], 0.5 μL of each oligonucleotide [10 μM], 5 μL buffer PCR [5X], 2 μL of $MgSO_4$ [25 mM], 0.5 μL of dNTP's [10 mM], 0.2 μL of Taq polymerase [5 U/μL], and ULTRAPURA water. The reactions were placed in a thermocycler, under the following conditions: initial denaturation at 95 °C for 4 min; denaturation at 95 °C for 1 min; alignment at 70 °C for 1 min; extension at 72 °C for 2 min, and final extension at 75 °C for 5 min. Afterwards, the PCR products were processed in an electrophoresis chamber, with a 1% agarose gel, at 90 V, during 35 min (Sambrook and Russell, 2001). The results were observed in a photodocumenter, under ultraviolet light. The PureLink™ PCR Purification Kit Invitrogen was used to purify the PCR products, following the instructions provided by the manufacturer.

Sequencing and phylogenetic analysis of 16S rRNA

A NanoDrop™ spectrophotometer was used to measure the purity and concentration of the PCR product. PCR products that complied with the specifications were sent to the Instituto de Biotecnología of the UNAM, where they were subjected to a Sanger sequencing, and to the MACROGEN company, where the FASTA archives and other quality archives were developed, using the CONSED packaging. The resulting sequences were analyzed with the BLAST program and the results were compared with those published by the National Center for Biotechnology Information (NCBI; http://www.ncbi.nlm.nih.gov). Subsequently, the MAFFT software (Katoh, 2002) was used to carry out a multiple alignment and the GBlocks software was used to filter them. GBlocks selects the

preserved blocks of multiple alignments to use in phylogenetic analysis. Once the analysis was completed, the Model Test software (Posada, 2006) identified the substitution model. The Silva database (https://www.arb-silva.de/aligner/) and the RAXML lab (Maximum likelihood) were used to build the phylogenetic trees.

RESULTS AND DISCUSSION

In order to purify the strain and to establish the morphological characterization, the assay, and the molecular identification, the GVE 5 strain was reactivated in the LB medium. These stick-shaped and aerobic bacterium is gram-positive, forms spore, has a negative hemolysis, and its optimal growth range is 28-37 °C. Based on these phenotypical characteristics, it can be classified as a mesophyll organism (Figure 1). This research also established its tolerance to 200 mg L^{-1} of glyphosate, carbofuran, and chlorpyrifos, in two different culture media (LB and M9). Figure 2 shows the assays that determined the GVE 5 growth for each pesticide. The results indicate that this strain could have a biochemical arsenal that could degrade these organic compounds, despite their capacity to persist in the environment. The molecular characterization continued at the 16S rRNA marker level. The PCR molecular technique was used for amplification purposes, obtaining a ≈1,500 pb area, which was purified and subsequently subjected to a sequencing process (Figure 3). Therefore, the genus *Priestia* predominated and the GVE 5 strain was identified as *Priestia aryabhattai*. The substitution analysis per area showed a 0.010 nucleotide distance (Figure 4). Narsing Rao *et al.* (2019) evaluated *Bacillus aryabhattai* and proposed its reclassification as an a posteriori heterotypic synonym of *Bacillus megaterium* de Bary 1884 (Approved lists 1980). Based on the conserved signature indels (CSI), Gupta *et al.* (2020) proved in October 2020 that many *Bacillus species* (including the *Subtilis* and *Cereus* clades) make up a total of new 17 individual clades. The authors proposed acknowledging these clades as new genus. In addition, they suggested naming the *Megaterium* clade as *Priestia* gen. nov., because it includes all the old *Bacillus* species: *B. megaterium*, *B. abyssalis*, *B. aryabhattai*, *B. endophyticus*, *B. filamentosus*, *B. flexus*, and *B. koreensis*. This situation is the result of the two CSI included in the oligoribonuclease (NrnB) that were shared by all the members of the clade (Gupta *et al.*, 2020). During the last years, the beneficial effect of *P. megaterium* on plant growth has

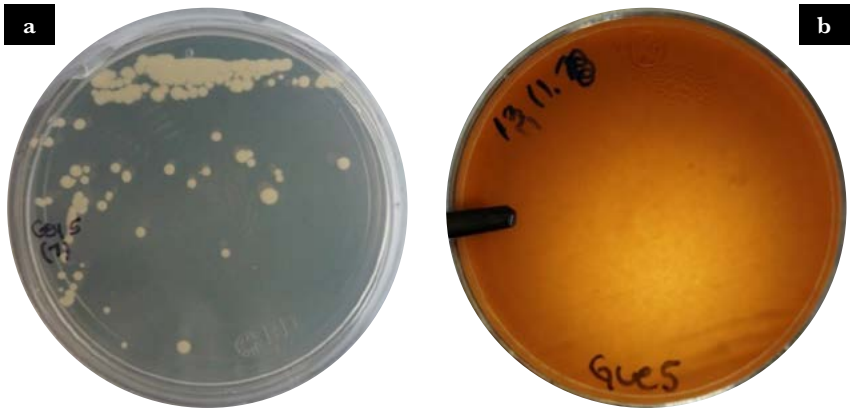


Figure 1. *Priestia aryabhattai* bacterium (GVE 5) (a) Morphology in LB agar (b) Blood agar hemolysis test.

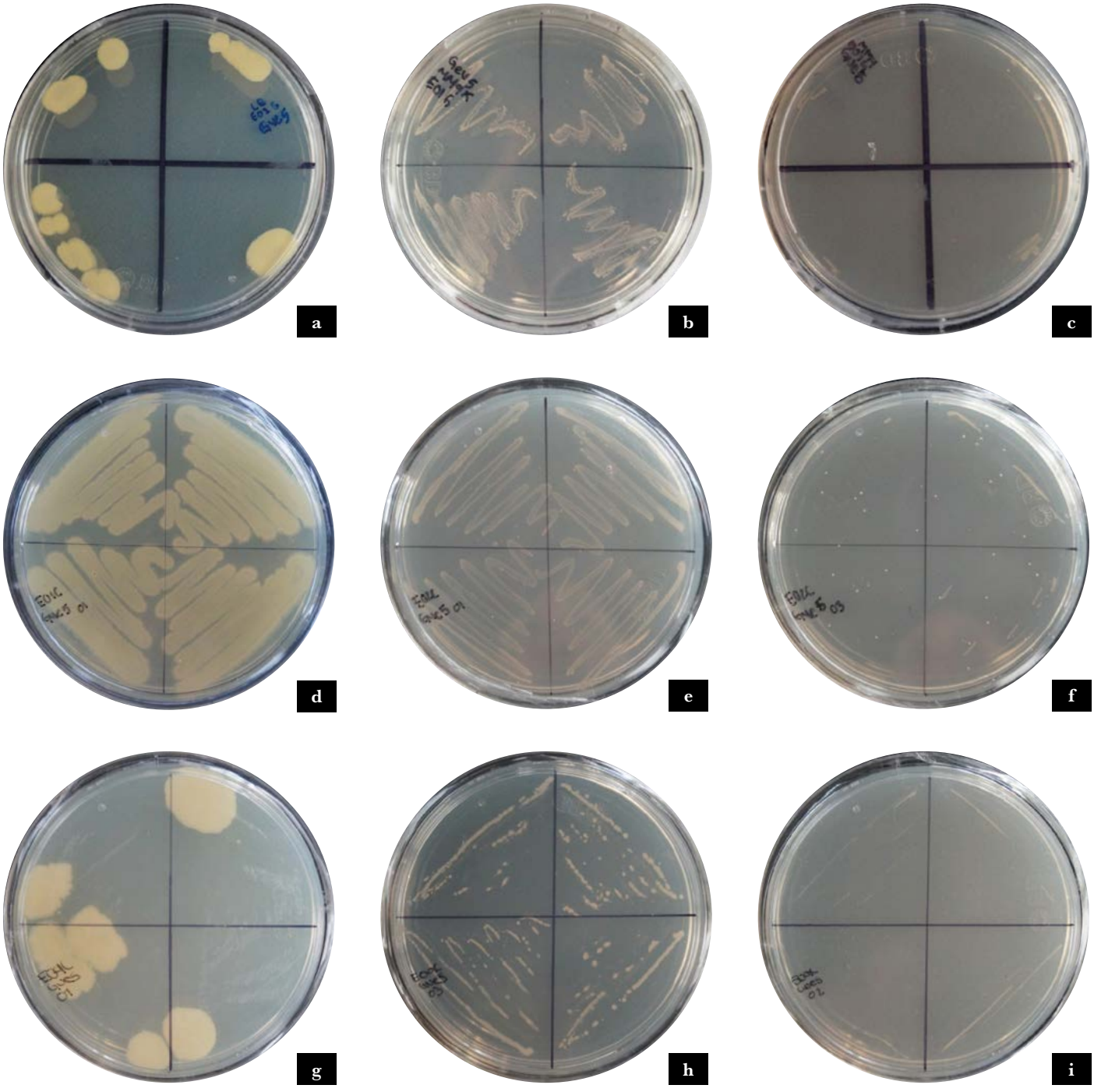


Figure 2. Glyphosate, carbofuran, and chlorpyrifos tolerance test. *Priestia aryabhattai* bacterium (GVE 5). LB medium 200 mg L^{-1} of the pesticide pattern of a commercial brand: glyphosate (a), carbofuran (d), and chlorpyrifos (g). M9 minimal medium 200 mg L^{-1} of the pesticide pattern of a commercial brand: glyphosate (a), carbofuran (d), and chlorpyrifos (g). M9 minimal medium without pesticide pattern (c), (f), and (i).

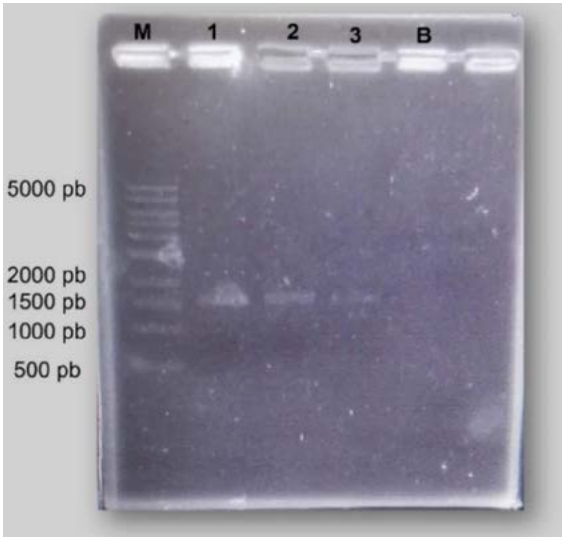


Figure 3. Electrophoresis results in 1% agarose gel (PCR product). Column (M): 500 pb DNA molecular weight marker, 200 lanes, columns (1), (2), and (3) GVE 5 strain, column (B) negative control.

Figure 4. Cladogram of the nucleotide sequencing alignment (GVE 5).

increasingly become a subject of interest (Biedendieck *et al*., 2021). In addition, Elarabi *et al*. (2020) isolated a strain that can tolerate 50, 100, 150, 200, and 250 mg mL^{-1} maximum concentrations of glyphosate. The evaluation was carried out using a mineral salt medium (MSM), quantifying the colony forming units (CFU) during seven days. The strain was identified as *Bacillus aryabhattai* FACU3 by 16S rRNA.

CONCLUSIONS

Priestia aryabhattai can tolerate 200 mg/L of glyphosate, carbofuran, and chlorpyrifos. Its TC was proved through the growth assay carried out in M9 minimal medium, taking into consideration the pesticide pattern as the only source of carbon. In addition, the 16S rRNA marker was used for molecular identification. In conclusion, *Priestia aryabhattai*

should be not just included in a biotechnological catalog for the bioremediation of soils polluted with these agrochemicals, but also should be subjected to further evaluations as a plant growth promoter.

ACKNOWLEDGEMENTS

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Colegio de Postgraduados

Infectivity and effectiveness of an arbuscular mycorrhizal fungi native inoculum on the growth and absorption of macroelements in maize (*Zea mays* L.) plants

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ABSTRACT

Objective: To evaluate the impact of an arbuscular mycorrhizal fungi (AMF) native inoculum on the growth and absorption of macroelements in maize (*Zea mays*) under seedbed conditions.

Design/Methodology/Approach: The experiment consisted of a completely randomized experimental design with four treatments (three inocula of arbuscular mycorrhizal fungi and an uninoculated control) and 30 repetitions, resulting in 120 experimental units. Two consortia of commercial AMF were used: AMF1, AMF2 and, one native AMF3 treatment. The experiment included a control (T) without inoculation. The variables evaluated were: total dry weight and mycorrhizal colonization in plants and nutritional content of nitrogen (N), phosphorus (P), and potassium (K) in plant tissue.

Results: The application of the native inoculum (AMF3) had a significantly greater impact on total dry weight, as well as on P and K content in plant tissue, than the rest of the treatments (particularly the control). AMF3 showed 18% more mycorrhizal colonization than the rest of the treatments.

Study Limitations/Implications: The experiment was carried out under seedbed conditions and did not include the production stage; therefore, the impact of the treatments on maize production is unknown.

Findings/Conclusions: Maize (*Zea mays*) plants had a positive response to inoculation with arbuscular mycorrhiza-forming fungi. The bio-technological potential of AMF3 (*Claroideoglomus claroideum*), a mycorrhizal consortium native to the rhizosphere, can be used to reinforce the development of maize plants, increasing the absorption of macroelements and inducing greater growth and root development.

Keywords: Root growth, mycorrhizal colonization, phosphorus, nitrogen, potassium.

INTRODUCTION

Maize (*Zea mays*) originated in the Americas, specifically central-eastern Mexico (Reyes *et al.*, 2022). The cultivation of maize has significant economic importance worldwide. Mexico ranks eighth in global production with 27,228,242 tons produced in 2019, behind

the United States, China, Brazil, Argentina, Ukraine, Indonesia, and India (FAOSTAT, 2022). Within Mexico, Sinaloa ranks first with 6,440,204 tons, surpassing states such as Jalisco, Estado de México, and Guanajuato (FAOSTAT, 2022). The plant development of this species is positively influenced by certain groups of naturally-occurring soil microorganisms, including arbuscular mycorrhizal fungi (AMF), which establish a symbiotic association with the roots of most vascular plants found in natural ecosystems and crops with economic and agricultural importance (Villavicencio and Garces, 2023).

However, several factors limit the production of maize (*Zea mays*), particularly the lack of nutrients and water, as well as pests and diseases caused by soilborne phytopathogens. These challenges have led to a dependence on the intensive use of synthetic products in maize cultivation, whose residues contribute to the pollution of soil-water-atmosphere ecosystems (Alvarado *et al.*, 2021).

Therefore, Palacios-Chávez (2023) and Torán-Figueroa (2023) suggest that the use of biofertilizers based on native arbuscular mycorrhizal fungi (AMF) and the application of vermicompost are viable alternatives that can reduce the use of chemical products, providing benefits to the plant and mitigating environmental problems. AMF are soil-borne fungi from the phylum *Glomeromycota* that establish symbiotic associations with more than 90% of terrestrial plants (Delgado and Gutiérrez, 2022; Schüßler *et al.*, 2001). Plants provide carbohydrates as a food source for the fungi, which in exchange offer various benefits to the plants. AMF form a mycelial network that allows greater soil exploration, enhancing the plants' capacity for water and nutrient absorption (Zhang *et al.*, 2020). They also provide resistance against biotic (pathogens, herbivores) and abiotic (drought, salinity, heavy metals) factors (Ravnskov *et al.*, 2020).

Meanwhile, consortia of native arbuscular mycorrhizal fungi are usually more effective than those composed of exotic species or a single species (Bashan *et al.*, 2000; Ortas and Ustuner, 2014), as a result of the adaptation of fungi to specific natural conditions. Their introduction to different environments, can lead to maladaptations to the new conditions (Rillig and Mummey, 2006).

Therefore, this study evaluated the impact of a native arbuscular mycorrhizal fungi inoculum on the growth and macronutrient absorption of maize (*Zea mays*) under seedling conditions.

MATERIALS AND METHODS

Study location

The research was conducted at seedbed-level under shade house conditions at the Faculty of Agronomy of the Universidad Autónoma de Sinaloa, in Culiacán, Sinaloa, Mexico, at 24° 37' 29" N, 107° 24' 30" W, and 38 m.a.s.l. From February 27 to May 8, 2020, the temperature ranged from 35 °C (maximum) to 18 °C (minimum).

Microbiological material

Two commercial consortia of arbuscular mycorrhizal fungi (AMF) were used, along with a native consortium and a control without AMF application. The commercial AMF consortia included AMF1 (*Glomus fasciculatum*, *G. constrictum*, *G. tortuosum*, *G. geosporum*,

Acaulospora scrobiculata, and *Gigaspora margarita*; from the MycorrazineVA brand, produced in Mexico) and AMF2 (formulated with spores from selected strains of vesicular-arbuscular mycorrhizal fungi, from the Glumix brand, distributed in Celaya, Guanajuato). Additionally, an AMF3 treatment with a native arbuscular mycorrhizal fungi inoculum (*Claroideoglomus claroideum*) was used. This inoculum was previously extracted from the rhizosphere of the huamuchil (*Pithecellobium dulce*) tree located in Ciudad de los Niños, Navolato, Sinaloa, with a radial biodiversity of watergrass (*Echinochloa crus-galli*) and Bermuda grass (*Cynodon dactylon*), as well as a control (T) without inoculation.

Seed sowing and inoculation

Seeds were sown on March 10, 2020, in 60-cell polystyrene trays, which were then cut into five sections of six cells each. One seed was planted per cell in a mixture of soil (characteristics specified in Table 1) and Kekkila® peat (1:1 v/v), which had been previously sterilized for two consecutive days (121 °C for 3 hours).

Inoculation with arbuscular mycorrhizal fungi was performed at the time of sowing, applying 0.5 g (AMF1), 0.5 g (AMF2), and 21.6 g (AMF3) of inoculum, to ensure that each plant received 20 spores. The plants were watered once every day with purified drinking water until the root ball was completely moistened. Additionally, the plants were fertilized every 30 days with a 25% Steiner solution without P (42 mg L^{-1} of N, 68 mg L−1 of K, 45 mg L^{-1} of Ca, and 12 mg L^{-1} of Mg), adjusting the pH to 6.5 using sulfuric acid.

Evaluated variables

The plants were evaluated and harvested 70 days post-sowing (dps) and total dry weight, mycorrhizal colonization, and macronutrient content were measured.

Dry biomass was obtained drying the samples in a Felisa™ FE-292 dehydration oven (70 °C for 72 hours) and separately weighing the shoots and roots using an Aczet CZ 30 analytical balance (Conquer Scientific).

The percentage of mycorrhizal colonization was assessed using the clearing and staining technique (Phillips and Hayman, 1970).

A sample of the shoot of the plants was taken from each treatment to determine the nutrient contents of N, P, and K in plant tissue. These samples were placed in a forced-air oven at 70 °C until a constant weight was achieved; subsequently, they were ground for laboratory analysis. The evaluation of N, P, and K content was performed using the methodologies described below. N was determined using the semi-micro Kjeldahl method (Etchevers, 1987). P was measured by colorimetry of molybdophosphate complexes reduced with ascorbic acid (AOAC, 1980). K was determined by the flame photometry methodology proposed by Rodríguez and Rodríguez (2015). Extracts obtained from dry

Table 1. Chemical and physical characteristics of the soil.

E.C. (mS cm^{-1})	pH	CEC ($Cmol_{(+)}kg^{-1}$)	Texture	N (mg kg^{-1})	P (mg kg^{-1})	K (mg kg^{-1})	Ca (mg kg^{-1})	Mg (mg kg^{-1})
0.85	7.11	44.46	Arcillosa	15.60	13.72	584.80	6687.50	973.30

E.C.: electrical conductivity; pH: hydrogen potential; CEC: cation exchange capacity.

digestion were used for K and P. To estimate the total contents, the concentrations of each element in the plant tissue were considered, along with the dry biomass weights of the shoot.

Experimental design

The experiment consisted of a completely randomized design with four treatments (three arbuscular mycorrhizal fungi inoculant and an uninoculated control) and 30 repetitions, resulting in 120 experimental units. An analysis of variance (ANOVA) and a means comparison test (Tukey, $\alpha=0.05$) were applied to analyze the data, using SAS for Windows (SAS Institute Inc., 2002).

RESULTS AND DISCUSSION

The inoculation of maize plants with AMF3 had greater impact on total dry weight, as well as the P and K content in plant tissue than the other treatments, particularly control. This phenomenon indicates the potential of this AMF consortium as a promoter of plant growth, particularly in maize.

In cases where crops are sown directly in the field and do not undergo a seedbed stage, the seed should be inoculated at the time of sowing to reduce costs and minimize competition with the inoculated microorganism. This measure also ensures a successful symbiosis during seed germination, allowing the plant root to recognize the presence of the inoculum, before native opportunistic microorganisms displace the inoculated microorganism (Zuluenta *et al*., 2021). The dry weight of the maize plant shoots was significantly higher ($P\leq0.05$, 10.91% difference) in AMF2 than in AMF1 (Figure 1). Meanwhile, the dry weight of the root, was significantly higher ($P\leq0.05$) with AMF3 (Figure 1). Compared to control, it had a 21.73% greater margin. Similarly, plants inoculated with AMF2 and AMF3 recorded 13.0% and 12.7% more total dry weight than the uninoculated control, respectively, indicating a significantly higher ($P\leq0.05$) difference than both the AMF1 and the uninoculated control (Figure 1).

For its part, N content of maize plants was statistically higher with AMF3 than AMF2, recording a 3.42% difference (Figure 3). However, both the control and AMF1 had lower values than AMF3, but no significant difference was observed.

P content was significantly ($P\leq0.05$) higher with AMF3 than with the other treatments, with a difference of 8.10% (*versus* AMF2) and 24.86% (*versus* AMF1 and the control (T)) (Figure 4). The AMF2 treatment was statistically higher (18.23%) than AMF1 and the control (T).

Meanwhile, K content variable was significantly higher ($P\leq0.05$) with the AMF3 treatment than the control (11.20% difference) and statistically similar to AMF1 and AMF2 (Figure 5).

P plays a role in reactions that require energy within the cell, as an integral part of energy storage molecules (*e.g.*, adenosine triphosphate (ATP)). These molecules are formed as a result of photosynthesis and participate in plant respiration. Consequently, P is vital for the generation of new cells, for example, during the production of roots at the beginning of vegetative cycles. An increased P and K content was also observed in the plant tissue of

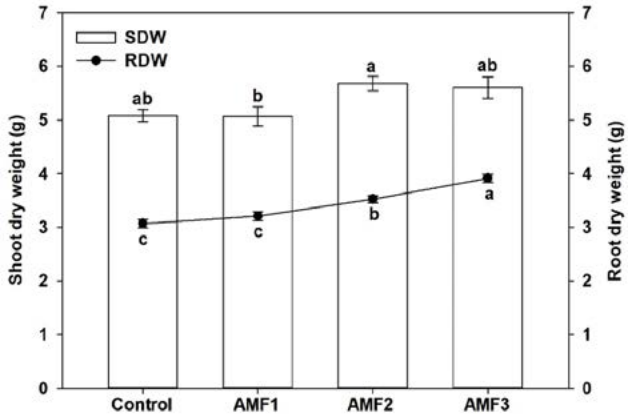

Figure 1. Root weight and shoot dry weight in maize plants after 70 days. Means standard error is shown; n=15. Identical letters above the bars indicate no significant difference (Tukey, 0.05). SDW=shoot dry weight, RDW=root dry weight. AMF1 *Glomus fasciculatum*, *G. constrictum*, *G. tortuosum*, *G. geosporum*, *Acaulospora scrobiculata*, and *Gigaspora margarita*. AMF2=consortium of arbuscular mycorrhizal fungi (Glumix®). AMF3=native arbuscular mycorrhizal fungi (*Claroideoglomus claroideum*). Control=uninoculated control.

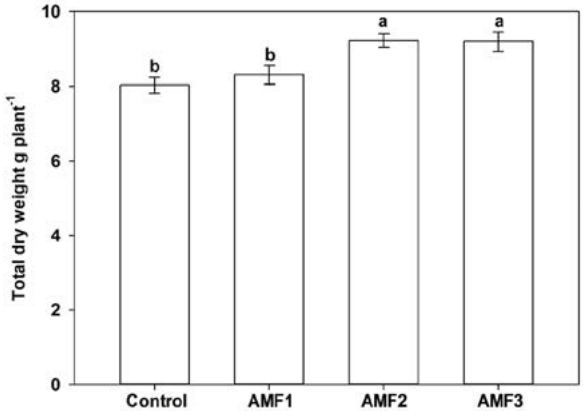

Figure 2. Total dry weight of maize plants after 70 days. Means standard error is shown; n=15. Identical letters above the bars indicate no significant difference (Tukey, 0.05). AMF1=*Glomus fasciculatum*, *G. constrictum*, *G. tortuosum*, *G. geosporum*, *Acaulospora scrobiculata*, and *Gigaspora margarita*. AMF2=consortium of arbuscular mycorrhizal fungi (Glumix®). AMF3=native arbuscular mycorrhizal fungi (*Claroideoglomus claroideum*). Control=uninoculated control.

maize inoculated with AMF, specifically with AMF3. This phenomenon can be attributed to the ability of AMF to absorb higher amounts of P. Faggioli *et al.* (2020) mention that AMF, through their hyphae and the secretion of extracellular phosphatases, are capable of capturing, transporting, and solubilizing the soil's scarce nutrient elements. Consequently, the plant's increased P uptake may have contributed to greater root development in maize plants. According to Paredes *et al.* (2021), phosphate strengthens the root system by promoting root extension and lateral branching. Additionally, the total dry biomass increased in plants inoculated with AMF, specifically with AMF3. A more developed root system allowed the plant to absorb more nutrients, which in turn resulted in higher dry biomass production, as noted by Ojeda *et al.* (2023).

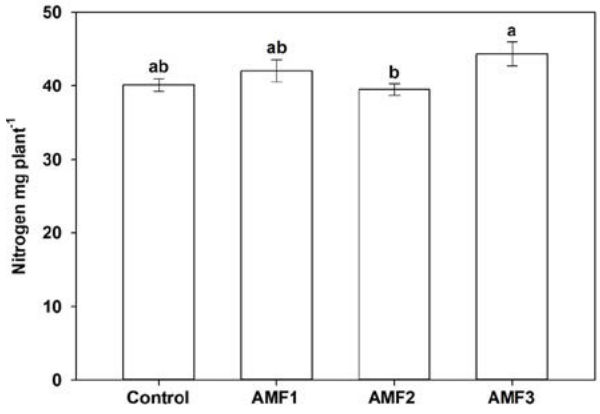

Figure 3. Nitrogen content of maize plants after 70 days. Means standard error is shown; n=15. Identical letters above the bars indicate no significant difference (Tukey, 0.05). AMF1=*Glomus fasciculatum*, *G. constrictum*, *G. tortuosum*, *G. geosporum*, *Acaulospora scrobiculata*, and *Gigaspora margarita*. AMF2=consortium of arbuscular mycorrhizal fungi (Glumix®). AMF3=native arbuscular mycorrhizal fungi (*Claroideoglomus claroideum*). Control=uninoculated control.

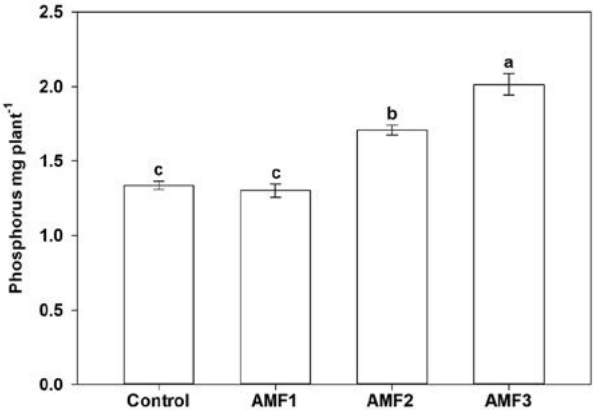

Figure 4. Phosphorus content of maize plants after 70 days. Means standard error is shown; n=15. Identical letters above the bars indicate no significant difference (Tukey, 0.05). AMF1=*Glomus fasciculatum*, *G. constrictum*, *G. tortuosum*, *G. geosporum*, *Acaulospora scrobiculata*, and *Gigaspora margarita*. AMF2=consortium of arbuscular mycorrhizal fungi (Glumix®). AMF3=native arbuscular mycorrhizal fungi (*Claroideoglomus claroideum*). Control=uninoculated control.

Total mycorrhizal colonization showed significant differences ($P \leq 0.05$) between AMF3 and AMF2, with a 78.53% difference. Specifically, AMF3 was significantly higher than AMF2 regarding vesicular colonization, with a 76.18% difference between these treatments. AMF3 had a statistically greater hyphal presence than AMF2 (80.31% difference). No mycorrhizal colonization was observed in AMF1 or control (T) (Table 2).

Mycorrhizal colonization was low in the commercial AMF treatments and absent in the uninoculated control; however, the response of the plants to the AMF2 and AMF3 consortium was favorable for most variables. Although the total mycorrhizal colonization percentage was low in AMF2, it did not limit the benefits of AMF for the plants. Therefore, the degree of mycorrhizal colonization is not always a clear indicator of its potential benefits for the host plant (Herrera, 2022).

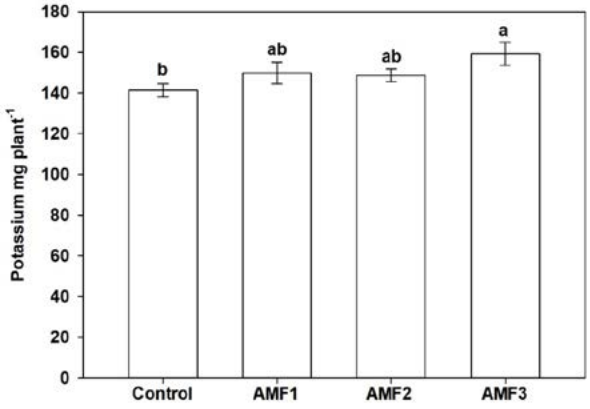

Figure 5. Potassium content of maize plants after 70 days. Means standard error is shown; n=15. Identical letters above the bars indicate no significant difference (Tukey, 0.05). AMF1=*Glomus fasciculatum*, *G. constrictum*, *G. tortuosum*, *G. geosporum*, *Acaulospora scrobiculata*, and *Gigaspora margarita*. AMF2=consortium of arbuscular mycorrhizal fungi (Glumix®). AMF3=native arbuscular mycorrhizal fungi (*Claroideoglomus claroideum*). Control=uninoculated control.

Table 2. Colonization of arbuscular mycorrhizal fungi in corn plants (*Zea mays*).

HMA	Total colonization (%)	Vesicles (%)	Hyphae (%)
Control	0.00 c	0	0
AMF1	0.00 c	0	0
AMF2	5.32 b	2.92	2.4
AMF3	24.79 a	12.26	12.19

AMF1=*Glomus fasciculatumi*, *G. constrictum*, *G. tortuosum*, *G. geosporum*, *Acaulospora scrobicurata*, and *Gigaspora margarita*. AMF2=arbuscular mycorrhizal fungi consortium (Glumix®). AMF3=native arbuscular mycorrhizal fungi (*Claroideoglomus claroideum*). Control=uninoculated control. The same letters after the means indicate no significant difference (Tukey, $\alpha=0.05$).

Therefore, the AMF-plant relationship is not considered specific, because any AMF species can colonize or form symbiosis with any plant (Delavaux *et al.*, 2019), as a result of their presence in virtually all types of soils (Guachanamá-Sánchez *et al.*, 2021). However, under certain edaphoclimatic conditions, some fungi may improve or provide a more significant benefit to a particular host (Zazueta *et al.*, 2021). In conclusion, the non-native commercial arbuscular mycorrhizal fungi (AMF1 and AMF2) exhibited very low colonization percentages in the root.

CONCLUSIONS

Maize plants responded positively to inoculation with arbuscular mycorrhizal fungi. The AMF3 consortium —sourced from the rhizosphere of the huamuchil (*Pithecellobium dulce*) tree in Sinaloa— had a significant effect on P and K absorption in maize, unlike commercial mycorrhizal consortia (AMF1 and AMF2) and an uninoculated control. Furthermore, the infectivity of the arbuscular mycorrhizal fungi consortium (AMF3) in maize plants was positive.

The biotechnological potential of AMF3 can be harnessed to enhance the initial development of maize plants, increasing the absorption of macronutrients, and promoting greater root growth and development.

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Colegio de Postgraduados

Frequency of fungi associated with strawberry dry wilt and *in vitro* antagonistic impact of *Trichoderma harzianum* and *Bacillus subtilis*

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ABSTRACT

Objective: To determine the frequency of fungi associated with strawberry dry wilt and to evaluate the antagonistic impact of *Trichoderma harzianum* and *Bacillus subtilis*.

Design/Methodology/Approach: Three sampling sessions were conducted in strawberry plantations in the Zamora Valley to isolate and identify fungi associated with strawberry dry wilt and to determine their frequency. *In vitro* antagonism tests were performed between *Trichoderma harzianum*, *Bacillus subtilis*, and fungi isolated from strawberry plants showing wilt symptoms. Additionally, the percentages of mycelial growth inhibition were determined.

Results: Six fungi were isolated from diseased plants showing wilt symptoms. The most frequent fungi were: *Neopestalotiopsis* sp. (54.7%), *Fusarium oxysporum* (50.6%), and *Rhizoctonia solani* (40.5%). *Trichoderma harzianum* inhibited >90% of the radial growth of *Rhizoctonia solani* mycelia, of *Cylindrocarpon* sp., >80% of *Fusarium solani*, and *F. oxysporum* mycelia, and 77.7% of *Neopestalotiopsis* sp. mycelia. *Bacillus subtilis* recorded the highest antagonism against *Rhizoctonia solani* (57%).

Study Limitations/Implications: This research faced no limitations.

Findings/Conclusions: *In vitro* tests determined that *Trichoderma harzianum* can inhibit the mycelial growth of fungi associated with strawberry dry wilt. *Bacillus subtilis* had a lower capacity to inhibit the mycelial growth of the confronted fungi than *Trichoderma harzianum*; however, it was the most effective bacterium against *Rhizoctonia solani*.

Keywords: strawberry, wilt, phytopathogenic fungi, biological control.

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INTRODUCTION

Mexico is the fourth largest strawberry producer worldwide, with an annual production of 578,142 tons and a *per capita* consumption of 1.9 kg. Michoacán is the most important producing state in the country, exceeding 354,000 tons, generating $8,113 million pesos, and accounting for 58.1% of the domestic production value [1]. However, strawberry

production is impacted by diseases such as wilt —caused by a consortium of fungi, with a variable incidence of the causal agents.

One of the most sustainable alternatives for the control of the fungal consortium responsible for strawberry wilt is the use of antagonistic fungi and bacteria. This biological control makes these microorganisms a valuable tool for agroecosystems, eliminating the need for chemical inputs [4]. *In vitro* research has shown an adequate antagonistic level of the T-H4 strain of *Trichoderma harzianum* against fungi associated with strawberry cultivation [5]. For their part, Pérez-Rodríguez *et al.* [6] reported that *Trichoderma harzianum* and *T. viridae* hindered the mycelial growth of fungi associated with strawberry crops. *Bacillus* strains have been proved to have a high potential as antagonists of *Fusarium oxysporum* [7]; additionally, *Bacillus subtilis* TS06 had an *in vitro* impact on the *Fusarium oxysporum* and *Verticillium dahliae* fungi that cause strawberry wilt [8] and inhibit the mycelial growth of *Fusarium equiseti* and *Fusarium solani* [9]. Consequently, the objective of this research was to determine the frequency of fungi associated with strawberry dry wilt and to evaluate the *in vitro* antagonistic impact of *Trichoderma harzianum* and *Bacillus subtilis* isolated from the rhizosphere of strawberry plants.

MATERIALS AND METHODS

Isolation of fungi associated with strawberry dry wilt and their frequency

The roots and crowns from wilted plants collected during three samplings from February to April 2024 in established plantations in the Zamora Valley, Michoacán, were washed to remove soil. Subsequently, they were sectioned into small pieces: 1 to 2 cm long roots and 1 cm thick crowns. The pieces were then subjected to a surface sterilization, immersing them in 3% sodium hypochlorite for 2 minutes and rinsed three times with sterile distilled water. The root and crown pieces were dried on sterile paper towels for 5 minutes and immediately placed onto a potato dextrose agar (PDA) medium. The inoculated dishes were incubated at 28 °C until colony growth was observed. Once the colonies had developed and after they had been identified, the isolation frequency of each fungus was determined.

Purification and identification of fungi

The mycelial growth of the various isolated fungi was transferred and purified using the hyphal tip technique on 2% water agar. Cultural identification was based on the color, appearance, and growth pattern of the colonies. Morphological identification was conducted using a compound microscope at 10x and 40x magnification, referencing the keys proposed by Nelson *et al.* [10], Sneh *et al.* [11], and Crous [12].

In vitro inhibition tests

Trichoderma harzianum was inoculated at one end of the Petri dish (a 5 mm diameter disc with antagonist growth) and the pathogenic fungus was inoculated at the opposite end. In the case of *Bacillus subtilis*, the bacteria were streaked at one end of the dish using the single-streak technique, while the pathogenic fungus was inoculated at the opposite end. As a control, a slice with the pathogen's mycelium was placed in the center of each Petri dish containing only PDA. All confrontation tests were performed in triplicate, while

the control fungi were tested in duplicate. The Petri dishes were incubated at 28 °C and the radial mycelial growth of the fungi (both confrontation and control) was measured in millimeters every 24 hours. The bioassay concluded once the mycelia of the control fungi had filled the Petri dish (8-12 days).

Determination of the inhibition percentage

The inhibition percentage of the mycelial growth of the pathogenic fungi was determined using the formula proposed by Patil *et al.* [13].

$$inhibition\,\% = \frac{D1 - D2}{D1} \times 100$$

Where $D1$ is the average radial growth of the fungus without antagonist and $D2$ the average radial growth of the fungus with antagonist.

Statistical analysis

The "percentage of mycelial growth inhibition" variable was subjected to an analysis of variance and Tukey's test ($p \leq 0.05\%$) using the Statistical Analysis System (SAS) software package. Prior to the analysis of variance, the percentage values were transformed using the arcsine transformation.

RESULTS AND DISCUSSION

Identification of fungi associated with strawberry dry wilt

Six fungi were isolated from strawberry plants with wilt disease. *Fusarium oxysporum* colonies are pink and may turn violet over time. *F. oxysporum* produced both microconidia and macroconidia. The latter were slightly curved and had 3 to 5 septa; intercalary chlamydospores were also observed. *Fusarium solani* is a fast grower that produces white mycelium, as well as microconidia and macroconidia; its chlamydospores usually appear as single entities.

Rhizoctonia solani has white, cottony colonies. As part of its specific characteristics, it forms hyphae at right angles and its septum is close to the point of origin of hyphal branching. Older colonies develop constricted mycelium. *Cylindrocarpon* sp. exhibited dark brown colonies with slow growth. Its conidiophores are short, while its macroconidia are generally cylindrical and develop septate. *Neopestalotiopsis* sp. developed a white, cottony colony with abundant acervuli on the surface. Under a compound microscope, light brown to dark brown macroconidia with septate hyphae were observed. *Alternaria* sp. displayed dark green mycelium; it produced light brown chains of oval conidia, with transverse and longitudinal septate.

Frequency of isolation of fungi associated with strawberry dry wilt

The most frequently isolated fungi associated with strawberry dry wilt were *Neoestalotiopsis* sp. (54.7%, second sampling), *Fusarium oxysporum* (50.6%, first sampling),

and *Rhizoctonia solani* (40.5%, third sampling). Less frequent fungi included *Fusarium solani*, *Cylindrocarpon* sp., and *Alternaria* sp. (Table 1). This variability in the frequency of isolated fungi may have been caused by the varied humidity and temperature conditions of the different times during which the samples were collected. Additionally, differences in fungal inoculum levels across plots and the susceptibility of various strawberry varieties grown in the Zamora Valley should be considered.

In vitro antasgonism tests with *Trichoderma harzianum*

Overall, the percentages of fungal growth inhibition increase over time. The inhibition of *Fusarium oxysporum* is high during the first 3 days of confrontation (54.5%); afterwards, progress is gradual, increasing by ≈10% every third day, until it reaches 83.7%. A similar percentage (85.8%) was recorded for *Fusarium solani* (Figure 1).

There were statistically significant differences ($p \leq 0.05$): the highest percentage of inhibition by *Trichoderma harzianum* against fungi associated with strawberry wilt was observed with *Rhizoctonia solani* (94%), with an outstanding increase (50%) in inhibition from the third to the sixth day. Other fungi that were satisfactoriy inhibited by this antagonist were *Cylindrocarpon* sp. (89.1%) and *Neopestalotiopsis* sp. (77.7%), with significant statistical differences between them. However, *Neopestalotiopsis* sp. was inhibited in only 6 days (Figure 1).

Trichoderma harzianum exhibited a strong antagonism against its competitors, demonstrating complete in vitro dominance, but with significant differences among the various fungi (Figure 2). These results are consistent with the findings of Guédez *et al.* [14] about pathogenic fungi that impact strawberries in the postharvest period. Additionally, the T-H4 strain of *Trichoderma* recorded an effective *in vitro* antagonism against strawberry fungi such as *Colletotrichum* sp., *Pestalotiopsis* sp., *Alternaria* sp., *Rhizoctonia* sp., and *Curvularia* sp. [5]. The biocontrol impact of *Trichoderma harzianum* and *T. viride* has also been demonstrated through a significant reduction of the mycelial growth of *Alternaria* sp., *Fusarium* sp., and *Rhizoctonia solani*, isolated from strawberry cultivation [6].

Table 1. Percentage of fungal isolations associated with strawberry dry wilt in three sampling conducted in the Zamora Valley, Michoacán.

Fungi	Frequency of isolations		
	Sampling (February)*	Sampling (March)	Sampling (April)
F. oxysporum	50.6%	20.0%	22.5%
F. solani	22.8%		
Rhizoctonia solani		10.5%	40.5%
Alternaria sp.	8.9%	7.4%	
Cylindrocarpon sp.	17.7%	7.4%	
Neopestalotiopsis sp.		54.7%	37.0%

*79 isolations in February, 95 in March, and 89 in April (2024).

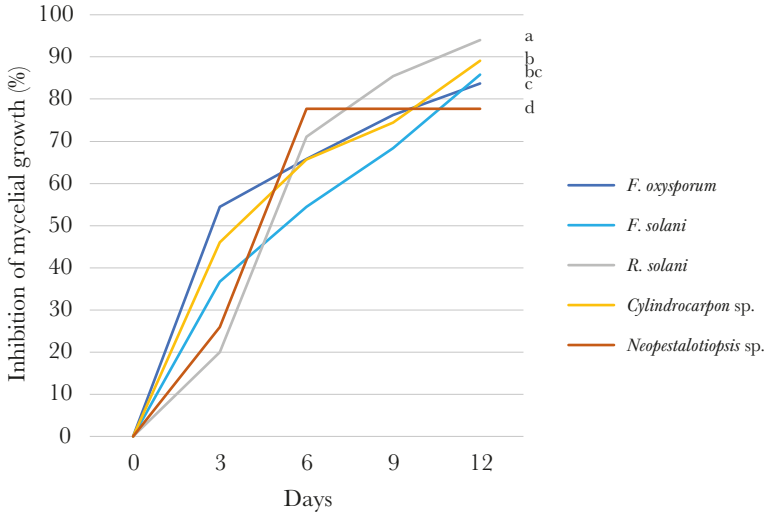

Figure 1. Percentage of mycelial growth inhibition caused by *Trichoderma harzianum* on fungi associated with strawberry dry wilt.

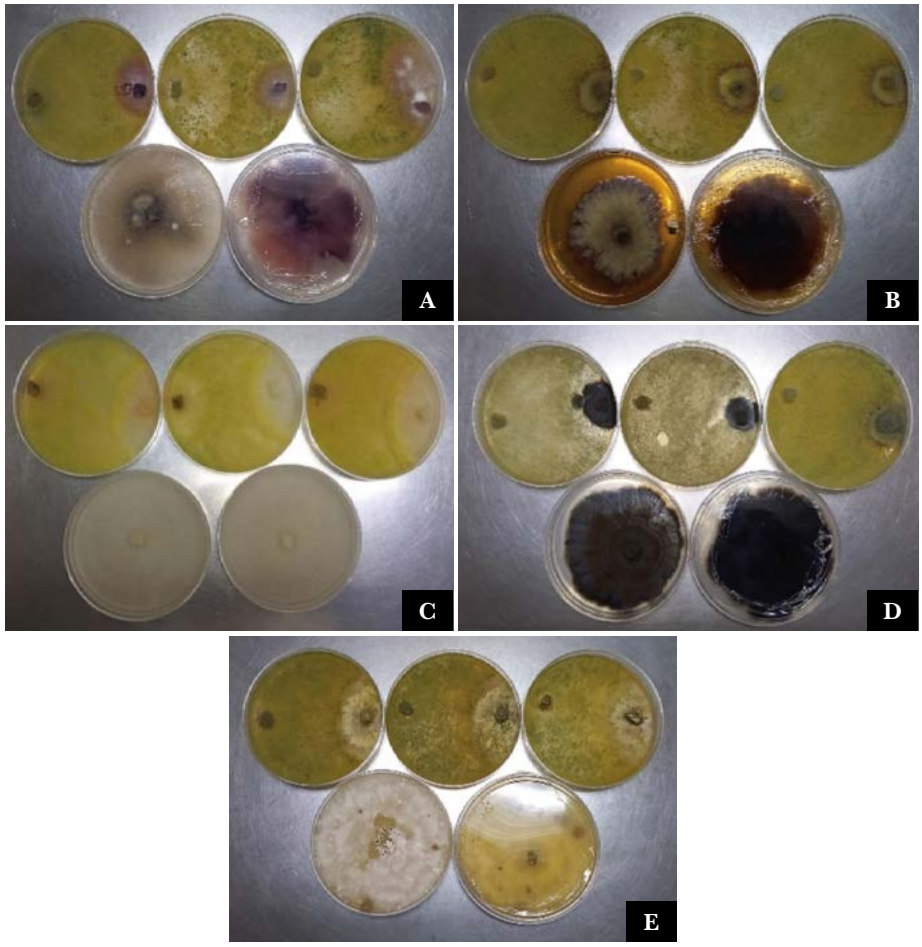

Figure 2. *Trichoderma harzianum* confrontation performed in triplicate *vs. Fusarium oxysporum* (A), *Fusarium solani* (B), *Rhizoctonia solani* (C), *Cylindrocarpon* sp. (D), and *Neopestalotiopsis* sp. (E). Growth of control fungi performed in duplicate.

Trichoderma exhibits several remarkable mechanisms that explain its microbial control of phytopathogens, including antibiosis, cell lysis, mycoparasitism, competition for space and nutrients, and environmental persistence [15-17]. Likewise, during the mycoparasitism process, *Trichoderma* species adheres to the hyphae of phytopathogenic fungi, frequently wrapping itself around them, and penetrating them; eventually, the degredation of their cells causes their complete weakening [18,19].

In vitro antagonism tests with *Bacillus subtilis*

Bacillus subtilis successfully inhibited the mycelial growth of some fungi associated with strawberry dry wilt. However, the inhibition percentages for three of the fungi were below 45%, while *Neopestalotiopsis* sp. recorded 53.2%, and the highest percentage was observed against *Rhizoctonia solani* (57%), with significant differences between them (Figures 3 and 4). These results are considerably lower than those reported by Jamali *et al.* [20], who achieved an 84% inhibition of *Rhizoctonia solani* in dual cultures with the *Bacillus subtilis* strain RH5 and concluded that the antagonistic activity of this bacterium is caused by the production of hydrolytic enzymes (chitinases, proteases, cellulases) and the synthesis of antimicrobial substances (bacillisin, surfactin, fengycin). In this regard, antibiosis is the most common mode of action of genus *Bacillus* [21].

Of the five fungi that the bacterium was tested against, only *Cylindrocarpon* sp. remained uninhibited until the second day. According to Michel-Aceves *et al.* [22], a shorter contact time (days) between the pathogen and antagonist causes greater aggressiveness in the antagonist and lower resistance in the phytopathogen. In this case, the bacterium only inhibited 45% of the mycelial growth of *Cylindrocarpon* sp.

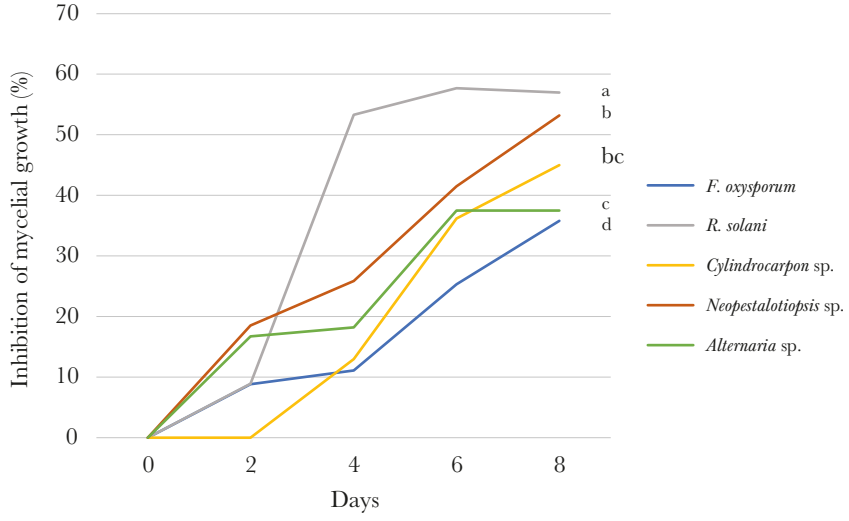

Figure 3. Inhibition percentages of the mycelial growth caused by *Bacillus subtilis* on fungi associated with strawberry dry wilt.

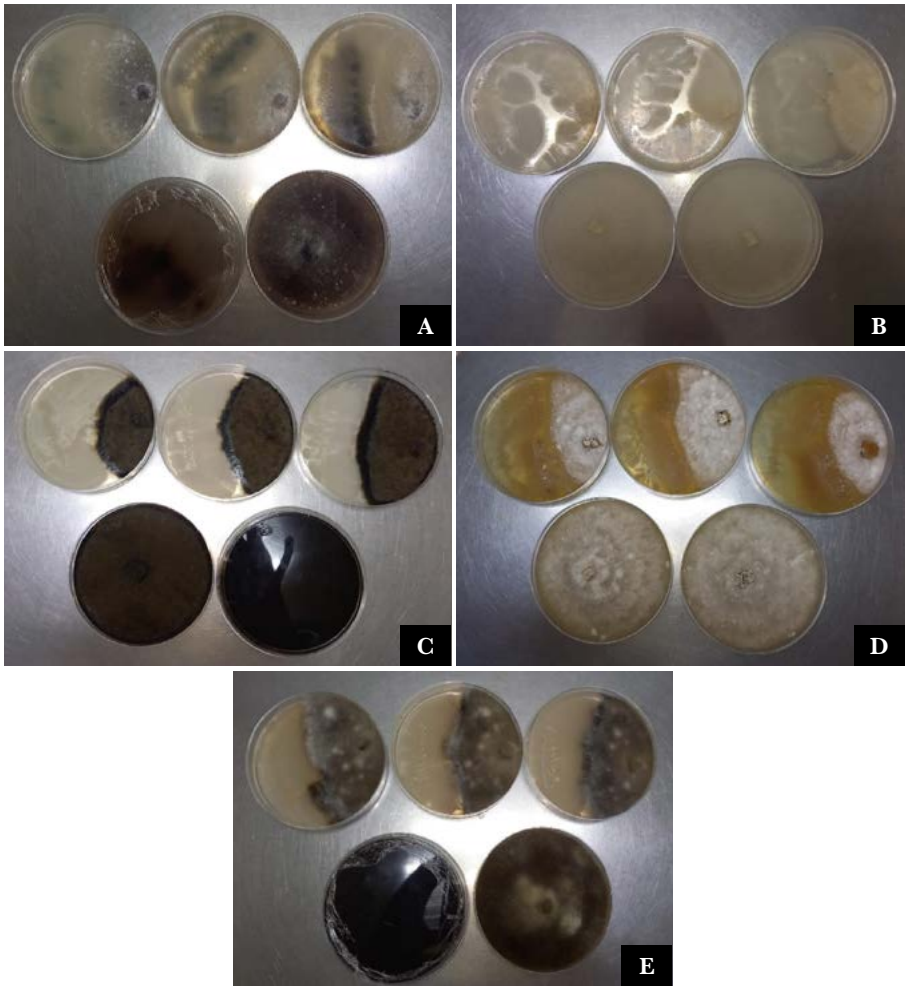

Figure 4. *Bacillus subtilis* confrontation performed in triplicate *vs Fusarium oxysporum* (A), *Rhizoctonia solani* (B), *Cylindrocarpon* sp. (C), *Neopestalotiopsis* sp. (D), and *Alternaria* sp. (E). Growth of control fungi performed in duplicate.

CONCLUSIONS

The most common fungi associated with strawberry dry wilt were *Neopestalotiopsis* sp. (54.7%), *Fusarium oxysporum* (50.6%), and *Rhizoctonia solani* (40.5%). *Trichoderma harzianum* can inhibit the mycelial growth of fungi associated with strawberry dry wilt. It mainly inhibited *Rhizoctonia solani* (over 90%), *Cylindrocarpon* sp., *Fusarium solani*, and *Fusarium oxysporum* (over 80%), and *Neopestalotiopsis* sp. (77.7%). *Bacillus subtilis* showed a lower capacity as an antagonist to inhibit the aforementioned fungi than *Trichoderma harzianum*. In this case, the fungus that was most effectively inhibited by the bacterium was *Rhizoctonia solani*.

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Physical, chemical, and microbiological parameters of soil under different tillage types

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ABSTRACT
Objective: To determine the effect on the chemical, physical and biological properties of soils under minimum tillage and conservation tillage in different locations in Sinaloa.
Design/methodology/approach: The treatments were evaluated using a completely randomized experimental design, with a total of eight treatments or sampling sites, where five samples per site were collected at a depth of 30 cm. A total of 40 samples were obtained, which were preserved in a thermal box and were transferred to the microbiology laboratory of the Faculty of Agronomy (UAS). The variables evaluated included the quantification of bacteria on plates, where the number of total bacterial colonies (TB), phosphate solubilizing bacteria (PSB), nitrogen-fixing bacteria (NF) and indole-promoting bacteria (IPB) were determined. In addition, organic matter, electrical conductivity and pH were determined.
Results: Soils under minimum tillage modality significantly promoted higher percentage of organic matter, a greater number of bacterial colonies and higher electrical conductivity compared to soils with conventional tillage modality.
Findings/conclusions: Agricultural tillage intervenes in the physical, chemical and microbiological properties of soils, as the sampling sites where minimum tillage is practiced show a higher concentration of organic matter and therefore leads to greater microbiology and electrical conductivity in those evaluated soils.

Keywords: Microbiological, Tillage, Organic matter.

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INTRODUCTION

In the state of Sinaloa, different types of tillage are used in agricultural practices. It is essential to understand how these affect the various properties of the soil, as it is a vital natural resource for the development of life and a key element in the natural cycle of matter and energy. To fulfill its functions in agriculture, the soil must have adequate physical, chemical, and biological conditions (Brito *et al.*, 2019). Soil is composed of minerals, organic matter, microorganisms, macroorganisms, air, and water. It is important to note that the plants and animals that grow and die in and on the soil are decomposed by the

action of microorganisms, transformed into organic matter, and mixed with the soil (FAO, 2019). However, by incorporating organic matter through soil tillage, the degradation of its arable layer increases, promoting the progressive degradation of the soil surface and facilitating erosion, one of the main methods of degradation resulting from agricultural practices (Gómez *et al.*, 2018).

Soil degradation not only decreases crop yields but also reduces carbon storage in agricultural ecosystems and biodiversity (Castro *et al.*, 2022). As a consequence of all the soil degradation problems, many developing countries face the challenge of implementing sustainable agricultural models that allow for food production to meet the growing demand of the population in a manner more compatible with the environment and natural resources (Thilagar *et al.*, 2016). Tillage systems directly influence soil properties, such as aggregate fractions (Tiemann *et al.*, 2015). The aggregation process is a dynamic one in which micro-aggregates bind together through roots, fungi, bacteria, organic polymers, and residues (Lehmann *et al.*, 2015).

Soil organic matter is composed of animal and plant remains, which serve as a substrate for plant growth and as a source of carbon and nitrogen for microorganisms, providing chemical and biological stability to the soil (Murillo *et al.*, 2020). A portion of this organic matter consists of living microorganisms, whose activity is crucial in the biogeochemical cycles of nutrients, as they are involved in the processes of nutrient mineralization and immobilization (Moreno, 2023). Babalola (2010) mentions that microbial biomass accelerates the availability and assimilation of soil nutrients through various mechanisms such as atmospheric nitrogen fixation and phosphorus solubilization. Additionally, it promotes plant growth and health by controlling damage caused by pathogenic microorganisms and synthesizing phytohormones like auxins, gibberellins, and cytokinins. Furthermore, symbiotic relationships are established between plants and microorganisms, strengthening their natural defense mechanisms (Beltrán & Bernal, 2022). For this reason, the objective of this research is to determine the effect of minimum tillage and conservation tillage on the chemical, physical, and biological properties of soils in different locations in Sinaloa.

MATERIALS AND METHODS

Rhizospheric soil was collected from four maize-producing areas under minimum tillage in the state of Sinaloa, along with four samples of rhizospheric soil from maize under conventional management, as shown in Table 1.

Table 1. Sampling locations and their physical properties (soil textures).

	Estación Bamoa/ MT	Palos Blancos/ MT	Las Tapias/ MT	La Unión/ MT	Costa Rica/ LC	Elota/ CT	Alhuey/ CT	San Pedro/ CT
Sand (%)	44	46	82	45	15	53	43	45
Clay (%)	34	38	8	39	64	21	39	36
Silt (%)	22	16	10	16	21	26	18	20
Texture	CL	CL	SCL	CL	C	SCL	CL	CL

MT=Minimum Tillage; CT=Conventional Tillage; CL=Clay Loam; SCL=Sandy Clay Loam; C=Clayey.

Laboratory and field analyses were conducted in the Microbiology Laboratory and in a greenhouse at the Faculty of Agronomy of the Autonomous University of Sinaloa (UAS), located at km 17.5 of the Culiacán-Eldorado highway, in the municipality of Culiacán, Sinaloa. According to the Köppen climate classification, modified by García (1973), the climate type is B1 S1, described as semi-arid, with summer rains, occasional winter rains, and an annual precipitation of 670 mm. The area has an average annual temperature of 24 °C, with a maximum of 41 °C in summer and a minimum of 5 °C in winter. The average annual relative humidity is 66.66%. Sampling was conducted in maize fields under minimum tillage and conventional tillage. The treatments were evaluated using a completely randomized experimental design, with a total of eight treatments or sampling sites. Five samples were collected from each site at a depth of 30 cm, resulting in a total of 40 samples. These samples were preserved in a thermal box and transported to the Microbiology Laboratory at the Faculty of Agronomy (UAS). The evaluated variables included the quantification of bacteria on plates, where the number of total bacterial colonies (TB), phosphate-solubilizing bacteria (PSB), nitrogen-fixing bacteria (NFB), and indole-promoting bacteria (IPB) was determined. Additionally, organic matter, electrical conductivity, and pH were measured. The quantification of bacterial colonies was performed according to the technique established by NOM-092-SSA1-1994 (method for counting bacteria on plates). The samples were processed in a laminar flow hood (61010-1, Labconco) under aseptic conditions. Ten grams of each sample were weighed and placed in glass containers with 90 ml of sterile distilled water (first dilution), and from this solution, serial decimal dilutions were made. Subsequently, 0.1 ml of each sample was added to Petri dishes and spread over the surface of the solid culture medium using an L-shaped cell spreader. Specific culture media were used: nutrient agar TB (DOF, 1994); NBRIP to detect phosphate-solubilizing bacteria (PSB) (Nautiyal, 1999); Rennie for nitrogen-fixing bacteria (NFB) (Rennie, 1981); and Luria-Bertani (LB) for indole-promoting bacteria (IPB) (Luria and Burrous, 1957). The colonies were quantified after three days in an incubator (9025E, Ecoshel) at 28 °C.

Specific culture media were used: nutrient agar TB (DOF, 1994); Pikovskaya agar to detect phosphate-solubilizing bacteria (PSB) (Nautiyal, 1999); Rennie for nitrogen-fixing bacteria (NFB) (Rennie, 1981); and Luria-Bertani (LB) for indole-promoting bacteria (IPB) (Luria and Burrous, 1957). The colonies were quantified after three days in an incubator (9025E, Ecoshel) at 28 °C. Additionally, the organic matter content of the soil for each treatment was evaluated. For this, 0.50 g of the sample was weighed, and 10 mL of 1 N potassium dichromate, 10 mL of sulfuric acid, 2 mL of phosphoric acid, and 200 mL of distilled water were added per sample. Subsequently, the sample was titrated with 0.5 N ferrous sulfate (Walkley and Black, 1934). The pH and EC were measured using the saturated paste technique, which involved bringing the sieved soil sample (2 mm sieve) to saturation with distilled or deionized water, and then extracting the filtered soil solution using a vacuum pump (Warncke, 1986). The pH and EC of this solution were determined using a Hanna HI98130 meter. The data obtained were analyzed using the SAS program for Windows (SAS Institute Inc., 2002). An analysis of variance was performed, along with a means comparison test ($p \leq 0.05$) and a Pearson correlation.

RESULTS AND DISCUSSION

The quantification of beneficial plant growth-promoting bacteria (PGPB) from four soils under minimum tillage and four soils under conventional tillage in the state of Sinaloa is shown in Table 2. In the analyzed soil samples, the presence of beneficial microbial populations (phosphate-solubilizing bacteria, nitrogen-fixing bacteria, and indole-promoting bacteria) was observed, along with a significant presence of total bacteria. It is worth noting that the sample from Unión, Angostura (37.6×10^6) promoted a significantly higher number of colony-forming units of bacteria compared to the other evaluated samples ($p\leq0.05$). On the other hand, the sample from Palos Blancos, Guasave (10.2×10^6) induced a significantly greater number ($p\leq0.05$) of colony-forming units of bacteria in Pikovskaya agar culture medium, achieving the best results compared to the other analyzed samples. Similarly, the bacterial colonies that developed in the Rennie culture medium from the soils of Palos Blancos, Guasave (11.6×10^6) and La Unión, Angostura (11.6×10^6) produced the best results, although it is worth noting that they are significantly similar ($p\leq0.05$) to the sample from Estación Bamoa, Guasave (11.3×10^6). Finally, the soil from Estación Bamoa, Guasave (2.4×10^6) showed the best results compared to the rest of the analyzed samples. However, it only showed a significant difference ($p\leq0.05$) with the soils from San Pedro (1.9×10^6), Costa Rica (1.8×10^6), Las Tapias (1.1×10^6), and Alhuey (1.0×10^6), respectively.

On the other hand, the physicochemical properties of four soils under conventional tillage and four soils under minimum tillage were analyzed, as shown in Table 3. The soil from Estación Bamoa, Guasave (2.1%) showed the best results in organic matter, although statistically ($p\leq0.05$) it was equal to the sample from Palos Blancos, Guasave (1.85%) and La Unión, Angostura (1.95%).

Regarding the EC variable, the soil from Estación Bamoa, Guasave (1.4 dS m^{-1}) obtained the best results, statistically ($p\leq0.05$) superior to the rest of the evaluated treatments. Finally, the sample from Alhuey, Angostura (7.4 pH) showed the best results in

Table 2. Microbiological properties of four soils with minimum tillage and four soils with conventional tillage in the state of Sinaloa.

Locality / Sample	Total bacteria	Pikovskaya (P)	Rennie (N)	Luria B. (indoles)
	CFU mL^{-1}			
Estación Bamoa, Guasave/ML	21.2×10^6 c	8.1×10^6 b	11.3×10^6 a	2.4×10^6 a
Las Tapias, Culiacán/ML	15.6×10^6 d	1.8×10^6 ef	8.2×10^6 b	1.1×10^6 c
Palos Blancos, Guasave/ML	34.8×10^6 b	10.2×10^6 a	11.6×10^6 a	2.0×10^6 ab
La Unión, Angostura/ML	37.6×10^6 a	5.8×10^6 c	11.6×10 a	2.1×10^6 ab
Alhuey, Angostura/LC	9.8×10^6 f	1.2×10^6 f	1.7×10^6 d	1.0×10^6 c
Costa Rica, Culiacán /LC	11.5×10^6 e	2.2×10^6 de	1.1×10^6 d	1.8×10^6 b
San Pedro, Navolato/LC	8.1×10^6 g	1.4×10^6 f	1.2×10^6 d	1.9×10^6 b
Elota, La Cruz/LC	15.2×10^6 d	2.4×10^6 d	3.9×10^6 c	2.2×10^6 ab

Means with different letters in a column are statistically different (Tukey, $p\leq0.05$). MT=Minimum Tillage, CT=Conventional Tillage, P=Phosphorus, N=Nitrogen.

Table 3. Physicochemical properties of four soils with minimum tillage and four soils with conventional tillage from the State of Sinaloa.

Sample / Locality	O.M. %	E.C. dS m^{-1}	pH 1-14
Estación Bamoa, Guasave/MT	2.1 a	1.4 a	7.1 c
Las Tapias, Culiacán/MT	0.9 b	0.9 bc	7.1 c
Palos Blancos, Guasave/MT	1.8 a	0.6 cd	7.2 bc
La Unión, Angostura/MT	1.9 a	1.1 ab	8.4 b
Alhuey, Angostura/CT	1.0 b	0.9 bc	7.4 a
Costa Rica, Culiacán /CT	1.1 b	0.5 cd	7.1 c
San Pedro, Navolato/CT	0.8 b	0.6 cd	6.7 d
Elota, La Cruz/CT	1.1 b	0.4 d	7.2 bc

Means with different letters in a column are statistically different (Tukey, $p \leq 0.05$). MT=Minimum Tillage, CT=Conventional Tillage, O.M.=Organic Matter, E.C.=Electrical Conductivity, pH=Hydrogen Potential.

terms of pH, being statistically ($p \leq 0.05$) better compared to the rest of the evaluated soil samples.

The correlation coefficients are shown in Table 4. This indicates that as the percentage of organic matter in the soil increased, the number of total bacteria rose by 85%. Additionally, the number of phosphate-solubilizing bacteria and nitrogen-fixing bacteria increased by 93% and 87%, respectively. On the other hand, the bacteria that promote indoles increased by 60%, and the electrical conductivity rose by 47% in response to the increase in organic matter. The pH showed no relationship with organic matter, presenting a null correlation.

Direct seeding is a management practice used to preserve the physical structure and increase the carbon stored in the soil. This way, it provides a habitat and higher quality substrates for the biota, improving the soil's biofertility (Holland, 2004). Each fraction of aggregates constitutes a microenvironment with unique physical, chemical, and structural characteristics that influence the microbial communities residing there (Mummey *et al.*, 2006).

Rojas and Camacho (2004) mention that bacterial populations will be higher in minimum tillage soils compared to conventional tillage soils. Additionally, Hernández and López (2002) agree that minimally disturbed soils are rich in carbon; therefore, they contain a greater microbial biomass compared to conventional tillage soils. Soil microbial communities play an integral role in nearly all ecosystem services, including nutrient cycling and the decomposition of organic matter (Trivedi *et al.*, 2017).

Table 4. Correlation analysis of organic matter with physicochemical and microbiological properties.

	BT	BSP	BFN	BPI	CE	pH
M.O.	0.85* p=.007	0.93* p=.001	0.87* p=.005	0.60 p=.115	0.47 p=.235	−0.02 p=.957

*Significant at $P \leq 0.05$. O.M.=Organic matter, TB=Total bacteria, PSB=Phosphate solubilizing bacteria, NFB=Nitrogen fixing bacteria, IPB=Indole promoting bacteria, EC=Electrical conductivity, pH=Hydrogen potential.

Soil organic matter contains about 5% total nitrogen, but it also includes other essential elements for plants, such as phosphorus, magnesium, calcium, sulfur, and micronutrients (Graetz, 1997). For this reason, organic matter in the soil increases cation exchange capacity, nutrient reserves, and mineralization processes (Julca *et al*., 2006). Although the decomposition of organic matter in the soil generates acids, such as carbonic acid, which can acidify the soil (Labrador, 2001), it was observed that organic matter and pH showed a null correlation. This may be due to the fact that the evaluated soils have high percentages of clay (except for the soil from Las Tapias). Sánchez *et al.* (2021) mention that clayey soils have a greater buffering capacity against changes in pH compared to sandy soils.

CONCLUSIONS

Agricultural tillage influences the physical, chemical, and microbiological properties of soils. In the sampling sites where minimum tillage was practiced, a greater concentration of organic matter was observed, leading to a higher number of bacterial microorganisms in the evaluated soils. Therefore, minimum tillage can be a viable strategy for agricultural production, contributing to soil conservation without negatively affecting properties that influence microbial activity.

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Colegio de Postgraduados

Potentially mineralizable nitrogen: Estimation of the labile and stabilized pools under woodland and cultivated soils in Mexico

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ABSTRACT

Objective: The aim of this work was to evaluate nitrogen pool and its variation due to land uses in different soil orders.

Design/methodology/approach: Alfisols, Entisols and Inceptisols under different uses (woodland and cultivated soils) were incubated for 20 weeks under controlled temperature and humidity. The mineralizable N (NO_3^- and NH_4^+) was obtained and the potentially mineralizable nitrogen (N_0) was estimated by the method of iterative adjustment. Nitrogen from 0 to 5 weeks was considered as labile nitrogen, and from 5 to 20 weeks was the stabilized nitrogen. The labile nitrogen was described by a potential model, while the stabilized nitrogen was described by a logistic model. The labile nitrogen and stabilized nitrogen pools were estimated after obtaining the first derivate and solving the integral of the potentially mineralizable nitrogen equation.

Results: The greatest estimated amounts of labile and stabilized nitrogen were detected in an Alfisol under woodland use, and the minimum values were obtained in an Entisol under agricultural use. Similar results were obtained for the estimated amount of labile and stabilized nitrogen pool.

Limitations on study/implications: It is important to measure the labile and stabilized fraction of nitrogen, which requires long-term incubations to obtain models of soil N pools, so other methods realiable and faster need to be considered.

Findings/conclusions: The potentially mineralizable nitrogen was positively related to the different fractions of labile and stabilized nitrogen under the different land uses.

Keywords: Nitrogen supply, soil fertility, woodland, agricultural soils

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INTRODUCTION

Extractive practices and low organic inputs reduce the content of the organic matter in the soil (MOS), which negatively impacts the sustainability of agricultural production systems. For increase the levels of MOS, organic inputs can be used to promote greater water storage capacity in the soil, enhance resistance to erosion, and nutritional contributions, among others. The C and N content in the soil results from a complex interaction between the C

and N additions from organic waste (plant and animal) and inorganic sources (fertilizers), with the C and N losses through decomposition and microbial mineralization and erosion (Gregorich *et al*., 1994; Cheng *et al*., 2016). The supply of nitrogen to crops consists of the nitrogen contained in the previous crop, which is incorporated into the soil, and eventually it is mineralized for unprotection of the aggregates of soil (Matus and Rodriguez, 1994). Galvis and Hernández (2004) mentioned that to increase the available nitrogen for crops, organic inputs of quick decomposition should be applied. Currently, research relating to the function of the edaphic component in the mineralization processes of organic materials are scarce; however, there has been an increased the interest in studying the influence of soil on mineralization processes and nitrogen protection that forms functional reserves, as well as the need to assess the rate of mineralization and accumulation of N. This goal can be achieved more efficiently with a better understanding of the factors that affect the mineralization processes and accumulation of nitrogen in the soil. This research evaluated nitrogen mineralization in relation to land use in different types of soil.

MATERIALS AND METHODS

Soil samples

Alfisols, Entisol, and Inceptisol were selected from the states of Nayarit and Campeche in Mexico. Soils were classified according to Soil Taxonomy (Soil Survey Staff, 2006). Soil samples were collected under different use, woodland and cultivated soils (Table 1). From each type of soil, 10 subsamples were taken from 0-20 cm, to form composite samples. These samples were air-dried in the shade, ground and sieved through a 2mm mesh. pH was measured using the potentiometric method, soil/water ratio 1:2, and electrical conductivity, with a conductimeter at a soil/water ratio of 1:5 (v/v).

Soil incubation

Residue of alfalfa (*Medicago sativa*, C/N13), previously dried at 65 °C, ground and sieved through a 40-mesh sieve was used. Amounts of residue applied to soil were equal to 10 t ha^{-1} of dry matter. Each treatment was repeated three times. Soil samples were incubated according to Stanford and Smith (1972) method, at 65% field capacity at a temperature of 30 °C for 20 weeks, each treatment was repeated three times. Mineralized N (NO_3^- and NH_4^+) from the soils was measured weekly until number 12 (1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12), after 15 and 20 weeks, an initial extraction was performed.

Table 1. Soil classification, site, vegetation and use.

Soil Classification	Land Use	Vegetation (Scientific name)	State
Entisol	Cultivated soil	(*Zea mays*)	Campeche
Inceptisol	Woodland	(*Haematoxylon campechianum*)	Campeche
Alfisol	Woodland	(*Cedrela odorata* L.)	Campeche
Entisol	Cultivated soil	(*Agave*)	Nayarit
Inceptisol	Cultivated soil	(*Saccharum officinarum*)	Nayarit
Alfisol	Cultivated soil	(*Solanum lycopersicum* L.)	Nayarit

Nitrogen Mineralization

Mineralized nitrogen (NO_3^- and NH_4^+) were measured using electrodes of selective ions (pH/mV, Coulter Beckman), and the potentially mineralizable nitrogen (N_0) was calculated (μg N g^{-1} suelo), N_0 was considered as an indicator of the soil's nitrogen supply for crop production.

Labile and Stabilized Nitrogen

The mineralized nitrogen (NO_3^- and NH_4^+) after incubation was divided in two phases, from 0 to 5 weeks was considered labile nitrogen (LN), while 5 to 20 weeks was considered stabilized nitrogen (SN) (Galvis and Hernández, 2004). The mineralized nitrogen was related to each type of soil use, and the tendency for labile nitrogen was described by a potential model $\left(y = ax^b\right)$, while the stabilized nitrogen was described by a logistic model $\left(y = C/1 + e^{(a-bx)}\right)$, where a is the amount of nitrogen mineralized (at first week of incubation or the change in tendency), b is the rate of nitrogen mineralization and y is potentially mineralizable nitrogen (N_0), for both labile and stabilized, respectively. Using the values of N_0 determined by the iterative adjustment method, Gauss-Newton non-linear procedure (NLIN) of the Statistical Analysis System program (SAS 8.01), a statistical means trial test (Tukey $\alpha = 0.05$) was carried out to determine if there were significant nitrogen mineralization effects related to soil use. The labile nitrogen and stabilized nitrogen pools were also estimated after obtaining the first derivative and solving the integral of the potentially mineralizable nitrogen equation by each type of use of soil.

RESULTS AND DISCUSSION

Soil pH values ranged from 5 to 8, and electrical conductivity was not higher than 1 dS m^{-1}, thus avoiding extreme values of acidity, alkalinity, or salinity, respectively that could affect nitrogen mineralization processes.

To analyze the soil nitrogen supply, mineralized nitrogen was obtained and potentially mineralizable nitrogen (N_0) was determined from 0 to 5 weeks (labile nitrogen), and from 5 to 20 weeks (stabilized nitrogen), the mean coefficient of determination was $r^2 = 0.97$ for the 18 trials.

The amount of N_0 and mineralization rate (coefficient b) of labile nitrogen (Table 2), showed a significant relation ($p < 0.05$) between the labile nitrogen and land use.

Values of labile nitrogen were 93 μg g^{-1} in the Alfisol under woodland use (*Haematoxylon campechianum*). In contrast, those obtained in the Entisol under agricultural use (*Zea mays*) were 15 μg g^{-1}, at the same mineralization rate (0.7 μg g^{-1} $week^{-1}$). The Entisol under agricultural use (*Zea mays*) exhibited lower nitrogen mineralization over time, resulting in a smaller amount of labile nitrogen compared to the Alfisol under woodland use (*Cedrela odorata* L.). This was due to soil order and land use; agricultural use (*Zea mays*) causes the extraction of nitrogen from crops with less nitrogen is replenished. Tillage also promotes the unprotection of the soil organic matter, thus diminishing supply nitrogen than under woodland use (*Cedrela odorata* L.). Stanford and Smith (1972) report that soils with N_0

Table 2. Labil nitrogen (L_N) and mineralization rate (R_{LN}) by soil order and land use.

Soil Order t	Land use	Vegetation	L_N ($N_L=a(x)^b$) $\mu g\ g^{-1}$	MSD	R_{LN} (value of b=rate) $\mu g\ g^{-1}$/week	MSD
Alfisol	Woodland	*Cedrela odorata* L.	93 a		0.7 c	
Inceptisol	Woodland	*Haematoxylon campechianum*	86 ab		0.8 c	
Inceptisol	Cultivated soil	*Saccharum officinarum*	33 ab		2.8 a	
Alfisol	Cultivated soil	*Solanum lycopersicum* L.	31 ab		2.1 ab	
Entisol	Cultivated soil	*Agave*	31 ab		1.3 bc	
Entisol	Cultivated soil	*Zea mays*	15 b	76	0.7 c	0.97

Mean values with different letters in the same column are statistically different, Tukey (α=0.05). MSD=Minimum Significant Difference.

between 11.5 to 13.5 percent of total N were associated with intensive cultivation with little or no application of N. Collins *et al.* (1992) explained that during the first few weeks, organic materials, such as simple sugars, organic acids and proteins, are consumed, which are more likely to be mineralized by the microbial biomass. Soil organic compounds that are mineralized in the first 5 weeks of incubation give place to a fraction of labile nitrogen, which is related to short-time crop nutrition.

In the Alfisol conditions there was a significant relation ($p<0.05$) between nitrogen mineralization rate (slope b) and land use (natural vegetation). While less is value of the slope (b), less is the nitrogen mineralization rate, which in turn corresponds to a slower loss of soil nitrogen after time (t). The Inceptisol, Alfisol and Entisol under agricultural use (*Saccharum officinarum*, *Solanum lycopersicum* L. and *Agave*), respectively showed a middle level of labile nitrogen (31 to 33 $\mu g\ g^{-1}$), at a rapid nitrogen mineralization rate over time (1.3 to 2.8 $\mu g\ g^{-1}\ week^{-1}$). This means that if the same soil and crop management practices continue over time, the nitrogen supply to crops will diminish more quickly, affecting the crop yield and soil fertility. Galvis (1998) mentioned that through soil tillage, the organic material becomes unprotected, which increases the activity of the microbial biomass and accelerates the mineralization process (Geisseler *et al.*, 2010); in other words, increases the mineralization rate.

Table 3 shows the amount (N_0) and mineralization rate (coefficient b) for stabilized nitrogen.

Similarly to labile nitrogen, there was a significant relation ($p<0.05$) between stabilized nitrogen and land use. Values of labile nitrogen were 128 $\mu g\ g^{-1}$ in the Alfisol under woodland use (*Cedrela odorata* L.), whereas those obtained in the Entisol under agricultural use (*Zea mays*) were 34 $\mu g\ g^{-1}$, at mineralization rates of 0.36 $\mu g\ g^{-1}\ week^{-1}$ and 0.56 $\mu g\ g^{-1}\ week^{-1}$, respectively.

This was also attributed to soil order and land use; agricultural use (*Zea mays*) that causes less accumulation of nitrogen (stabilized nitrogen) overtime compared to the Alfisol under woodland use (*Cedrela odorata* L.) where the process of nitrogen accumulation continues, leading to greater organic inputs. Vigil and Kissel (1991) found that after 17 weeks of incubation, there was an evident tendency of stabilization of accumulated mineralized N. They attributed this to the fact that the incorporation of

Table 3. Stabilized nitrogen (S_N) and mineralization rate (R_{SN}) by soil order and land use.

Soil order	Land use	Vegetation	S_N ($N_E=C/1+e^{(a-bx)}$) $\mu g\ g^{-1}$	MSD	R_{SN} (value of b=rate) mg g^{-1} $week^{-1}$	MSD
Alfisol	Woodland	*Cedrela odorata* L.	128 a		0.36 c	
Inceptisol	Woodland	*Haematoxylon campechianum*	122 ab		0.49 abc	
Alfisol	Cultivated soil	*Solanum lycopersicum* L.	98 ab		0.45 bc	
Inceptisol	Cultivated soil	*Saccharum officinarum*	68 ab		0.66 a	
Entisol	Cultivated soil	*Agave*	48 ab		0.59 ab	
Entisol	Cultivated soil	*Zea mays*	34 b	52	0.56 abc	0.19

Mean values with different letters in the same column are statistically different, Tukey (α=0.05). MSD=Minimum Significant Difference.

organic material is less than the material being lost from the soil across crop production systems. Galvis and Hernández (2004) mentioned that the mineralization rate after the fifth week of incubation declined compared to that observed in the first five weeks. This decline was attributed to the mineralization of organic compounds that are resistant to degradation by the microbial biomass. This stabilized nitrogen is associated with the long-term nitrogen supply, promoting microbial activity and enhancing the physical fertility of soil.

To evaluate the nitrogen pool, the labile and stabilized nitrogen pools were estimated by obtaining the first derivative and solving the integral of the potentially mineralizable nitrogen equation from each land use of soil order. The level and loss rate of nitrogen pool were compared using ANOVA (Table 4 and 5).

There was no significant difference ($p<0.05$) between labile nitrogen pool and land uses (Table 4). The results showed that in woodland soils, Inceptisol and Alfisol, the labile nitrogen pool were from 199 to 190 $\mu g\ g^{-1}$ respectively, and in cultivated soils (*Zea mays*), corresponding to Entisol, it was 30 $\mu g\ g^{-1}$. No significant differences were found in the nitrogen mineralization rate of the labile pool; these ranged from 12.2 $\mu g\ g^{-1}$ $week^{-1}$ in Inceptisol and Alfisol under woodland use to 2.2 $\mu g\ g^{-1}$ $week^{-1}$ in Entisols under agricultural use (*Zea mays* and *Agave*).

Table 4. Labile nitrogen pool (P_{LN}), and mineralization rate (R_{LNP}) by soil order and land use.

Soil Order	Land Use	Vegetation	P_{LN} $\mu g\ g^{-1}$	MSD	R_{LNP} (value of b=rate) $\mu g\ g^{-1}$ week	MSD
Alfisol	Woodland	*Cedrela odorata* L.	190 a		12.2 a	
Inceptisol	Woodland	*Haematoxylon campechianum*	199 a		16.8 a	
Inceptisol	Cultivated soil	*Saccharum officinarum*	123 a		19.2 a	
Alfisol	Cultivated soil	*Solanum lycopersicum* L.	103 a		12.9 a	
Entisol	Cultivated soil	*Agave*	89 a		8.0 a	
Entisol	Cultivated soil	*Zea mays*	30 a	177	2.2 a	17.5

Mean values with different letters in the same column are statistically different, Tukey (α=0.05). MSD=Minimum Significant Difference.

In contrast, there was a significant difference ($p<0.05$) between stabilized nitrogen pool and land uses (Table 5). The results showed that in woodland soils, Inceptisol and Alfisol, the level of stabilized nitrogen pool was from 922 to 891 $\mu g\ g^{-1}$, while in cultivated soils (*Zea mays*), corresponding to Entisol, it was 307 $\mu g\ g^{-1}$.

In this period, the microbial population is compelled to consume more resistant organic materials, which can lead to an increase in microbial activity as the system approaches a new equilibrium state (Collins *et al.*, 1992; Ajwa and Tabatabai, 1994, Wu *et al.*, 2024). The decrease in the value of the slope indicates a reduction in microbial biomass activity, leading to an increased accumulation of organic matter on the soil surface. The soil acts as a physical barrier, limiting the microbial biomass's access to the organic reserves, which in turn reduces the nitrogen mineralization.

Benbi and Richter (2002) suggest that, in order to obtain stable parameters in models describing N mineralization, incubation should continue until the mineralization rate declines to a minimum and reaches a constant level. This was achieved in most soils in the current study, in contrast to that reported by Wang *et al.* (2004), who observed a reduced and constant mineralization rate for some soils, but not for others. Even after 29 to 41 weeks of incubation, they did not identify stable nitrogen reserve sizes. Wang *et al.* suggested that extending the incubation time to 41 weeks would be practically unacceptable and of little relevance to understanding the N mineralization process. Galvis (1998) mentioned that when the soil is tilled, the organic material becomes unprotected, leading to an increase microbial biomass activity, which consequently accelerates the mineralization process, or in other words, increases the rate of mineralization. Other considerations must be considered in the evaluation of soils for agricultural use, which allow synchronizing nitrogen availability with plant demand (Grzebisz *et al.*, 2022, Hussain *et al.*, 2022; de Jesus *et al.*, 2024), or addressing salt-affected soils, which can modify nitrogen mineralization (Peangdin Chaiyapo, 2024). Further studies are needed to model and predict N dynamics in agricultural production systems based on soil types and climatic conditions, with the application of organic fertilizers and manures in different crops.

Table 5. Stabilized nitrogen pool (P_{SN}), and mineralization rate (R_{SNP}) by soil order and land use.

Soil Order	Land Use	Vegetation	P_{SN} $\mu g\ g^{-1}$	MSD	R_{SNP} (value of b=rate) $\mu g\ g^{-1}$ week	MSD
Alfisol	Woodland	*Cedrela odorata* L.	922 a		2.3 a	
Inceptisol	Woodland	*Haematoxylon campechianum*	891 a		1.2 b	
Alfisol	Cultivated soil	*Solanum lycopersicum* L.	598 ab		0.8 bcd	
Inceptisol	Cultivated soil	*Saccharum officinarum*	422 bc		0.3 cd	
Entisol	Cultivated soil	*Agave*	307 bc		0.2 d	
Entisol	Cultivated soil	*Zea mays*	233 c	331	0.2 d	0.73

Mean values with different letters in the same column are statistically different, Tukey ($\alpha=0.05$). MSD=Minimum Significant Difference.

CONCLUSIONS

The mineralized N from the labile fraction (LN) was adjusted to a potential model, while the stabilized fraction (SN) was fitted to a logistic model. Labile nitrogen (LN) and stabilized nitrogen (SN) were significantly higher ($p<0.05$) at 93 $\mu g\ g^{-1}$ and 128 $\mu g\ g^{-1}$ respectively, in the Alfisols under woodland (*Cedrela odorata*). In contrast, Entisol under agricultural use (*Zea mays*) was had the lowest LN (15 $\mu g\ g^{-1}$) and SN (34 $\mu g\ g^{-1}$). In these same soils, the labile nitrogen pool (LNP) for the Alfisols was higher than in the Entisol, with 199 and 30 $\mu g\ g^{-1}$ respectively, while the stabilized nitrogen pool (SNP) was 922 and 233 $\mu g\ g^{-1}$, respectively. In these soils, N_0 correlated positively with both the labile and stabilized nitrogen pool.

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Colegio de Postgraduados

Chemical Properties of a Water-Eroded Soil Amended with Mixtures of *Dunaliella salina* and Organic Fertilizers

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ABSTRACT

Objective: Evaluar el efecto de la incorporación de *Dunaliella salina* mezclada con fertilizantes orgánicos en un suelo erosionado hídricamente a través de algunas propiedades químicas, como una alternativa para mitigar los efectos causados por la erosión.

Design/methodology/approach: Se tomaron muestras de suelo erosionado, y este fue adicionando con dos dosis de fertilizante equino (C) y bovino (V) mezclado con *D. salina*, teniendo los tratamientos: C (control 0.2% de *D. salina*), Va (70,000 kg ha^{-1} y 0.2% de *D. salina*), Vb (35,000 kg ha^{-1} de abono y 0.2% de *D. salina*), Ca (70,000 kg ha^{-1} y 0.2% de *D. salina*), Cb (35,000 kg/ha^{-1} y 0.2% de *D. salina*). Después de la aplicación de cada tratamiento, se analizó materia orgánica, conductividad eléctrica, pH y mineralización de N a los 15, 30 y 45 días.

Results: Los tratamientos con mayor porcentaje de materia orgánica y conductividad eléctrica en todos los tiempos fueron Ca y Va. En los tratamientos Va y Ca, el pH se mantuvo neutro y con los valores más altos respecto a los demás tratamientos a los 15, 30 y 45 días después su incorporación.

Findings/conclusions: El tratamiento Va tuvo el mayor contenido de N desde los 15 días hasta los 45 días. Los resultados sugieren que la aplicación de abono orgánico con *D. salina* puede compensar los déficits en las propiedades químicas causados por la erosión hídrica.

Keywords: Erosion; organic matter; electrical conductivity; nitrogen mineralization.

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INTRODUCTION

Erosion is defined as a process of soil wear and degradation, through which the removal of soil particles occurs due to the individual and/or combined action of climatic agents (rain, wind, or ice), influenced by biota (vegetation, human activity), and topography (slope: length, shape, and degree of inclination), acting over time on soil resources (Toledo, 2013). In 2016, Bolaños and collaborators reported a 66% increase in water erosion at the national level, with 6% corresponding to severe or extreme erosion, 24% to moderate erosion, 36% to mild erosion, and finally, 34% classified as stable soil. The latter is characterized by no visible evidence of erosion either in satellite images or in-field evidence of surface runoff affecting the terrain. This typically occurs when vegetation cover is dense, vegetation is pristine, or in a very early stage of succession.

Specifically, the State of Mexico is among the most affected by water erosion, showing extreme erosion in the area of Santa María Zolotepec-Temoaya-Jiquipilco-San Lorenzo Malacota (western slope of the Sierra de Las Cruces) (Bolaños *et al.*, 2016).

The erosive phenomenon has a negative impact on the soil's physicochemical properties, particularly reducing profile depth, infiltration capacity, and water storage. It also leads to a decrease in organic matter and nutrient content, an increase in bulk density, changes in texture, and a reduction in carbon content (Arriaga & Lowery, 2003; Hincapié-Gómez & Salazar-Gutiérrez, 2011). This is reflected in the loss of soil fertility and decreased productivity in agricultural processes such as crop production (Brunel & Seguel, 2011).

An eroded soil has low fertility and is no longer considered agricultural soil. Due to this, the use of biostimulants, which contain substances that promote plant growth, such as auxins, cytokinins, and betaines, proves to be an alternative to mitigate the erosive effects on soil and improve crop yields (Barrios, 2007; Pérez-Madruga *et al.*, 2020). Various studies (Salim *et al.*, 2016; Wafaa *et al.*, 2017; El-Moursy *et al.*, 2019) have demonstrated that the combination of seaweed extracts with inorganic and organic fertilizers can achieve sustainable agricultural productivity (Pérez-Madruga *et al.*, 2020). *Dunaliella* is one of the most commonly used microalgae genera due to its commercial importance in obtaining bioactive compounds. These organisms contain between 50 and 60% protein in green cells based on dry weight and around 30% in red cells, with a high carotenoid content (Fimbres-Olivarría *et al.*, 2010).

The objective of this study was to evaluate the effect of incorporating *Dunaliella salina* mixed with organic fertilizers on the chemical properties of water-eroded soil as an alternative to mitigate the effects caused by the erosive phenomenon.

MATERIALS AND METHODS

Characteristics of the Sampling Area

A preferential sampling was carried out in the town of San Miguel Yuxtepec, located in the municipality of Jiquipilco, State of Mexico. The municipality of Jiquipilco is situated to the north of the Valley of Toluca and to the east of the Valley of Ixtlahuaca, occupying part of the Monte Alto mountain range. Its geographic coordinates are 19° 31' 58" latitude, 99° 41' 15" west longitude, and an altitude of 2723 m. It is characterized by a temperate sub-humid climate with summer rains, classified as C(w2) (Municipal Government Gazette, 2022). This site has soil with a high degree of water erosion (Bolaños *et al.*, 2016).

Sampling

With the help of a shovel, the first 0-20 cm of the eroded surface were taken, maintaining a cylindrical shape. The soil samples were then placed in containers with a capacity of 4 kg, each containing 3.6 kg of eroded soil. A total of 30 samples were collected.

Experiment Setup

The experiment was conducted under greenhouse conditions. A mixture of organic fertilizer (equine and bovine) with an extract of the *Dunaliella salina* algae was incorporated. The treatments were as follows: *D. salina* (C), bovine fertilizer at a high dose (70,000 kg ha^{-1})+*D. salina* (Va), bovine fertilizer at a low dose (35,000 kg ha^{-1})+*D. salina* (Vb), equine fertilizer at a high dose (70,000 kg ha^{-1})+*D. salina* (Ca), and equine fertilizer at a low dose

(35,000 kg ha^{-1})+*D. salina* (Cb). Each treatment had six repetitions under a completely randomized block design.

Sampling for Chemical Analyses

The sampling for chemical analyses was carried out at 15, 30, and 45 days after the application of each treatment. Approximately 250 grams of soil were collected from each experimental unit, at a depth of 0 to 15 cm, using a garden shovel. Subsequently, the samples were placed in plastic bags, labeled, and transported to the Soil and Environment Laboratory at the Autonomous University of the State of Mexico for further analysis.

Laboratory Analysis

Organic matter was determined using method (AS-07), electrical conductivity was measured by method (AS-18) using the CONDUCTRONIC PC18 conductivity meter, and nitrogen mineralization was assessed by method (AS-08) using the UDK139 Labolan distiller. These analyses were conducted following the guidelines outlined in NOM-021-2000-RECNAT.

Statistical Analysis

An ANOVA was performed for a completely randomized block design, along with Tukey's test to determine significant differences between the measured chemical properties. Both analyses were conducted with a 95% confidence level, using the Statgraphics Centurion 5.0 statistical package.

RESULTS AND DISCUSSION

According to the ANOVA test, the treatments that showed the highest and statistically significant values ($p<0.05$) of organic matter (OM) 15, 30, and 45 days after their incorporation were Ca (Horse manure at a dose of 70,000 kg ha^{-1}+*Dunaliella salina*) and Va (Cow manure at a dose of 70,000 kg ha^{-1}+*Dunaliella salina*). According to the classification of NOM-021-RECNAT-2000, the percentage for Ca (3.98%) was considered high at 15 and 30 days after application, while for Va (2.62%), the percentage was estimated as medium during the same period. At 45 days after the application of the treatments, the percentages for Ca (3.24%) and Va (3.16%) were classified as medium (NOM-021-RECNAT-2000) (Figure 1).

The results show that the treatments with 70,000 kg ha^{-1} of organic manure presented the highest values of organic matter (OM). This is consistent with the findings of Salazar-Sosa *et al.* (2010), who reported that the application of manure to the soil at doses of 40,000 to 160,000 kg ha^{-1} increases organic matter. Regarding the increase in OM in the treatment with horse manure, this can be attributed to the fact that this type of manure contains large amounts of carbon, organic matter, and micronutrients, which are released slowly, facilitating plant uptake (Huachi, 2008; Guzmán, 2021).

Regarding the treatment with cow manure, it should be noted that this manure harbors a rich microbial diversity that is important in the processes of organic matter mineralization (Behera and Ray, 2021).

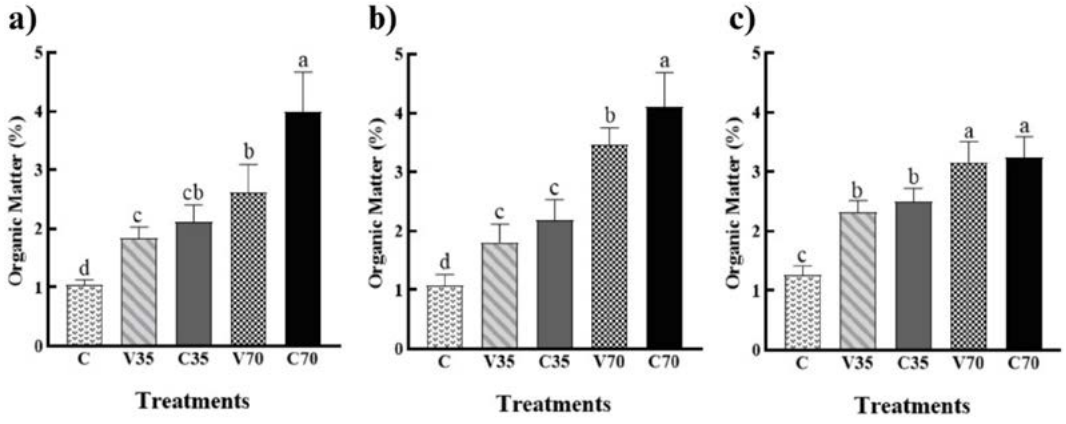

Figure 1. Organic Matter of a soil with water erosion treated with organic manure and *Dunaliella salina*. C (control with 0 kg ha^{-1} of manure and 0.2% of *D. salina*), Vb (35,000 kg ha^{-1} of cow manure and 0.2% of *D. salina*), Va (70,000 kg ha^{-1} of cow manure and 0.2% of *D. salina*), Cb (35,000 kg ha^{-1} of horse manure and 0.2% of *D. salina*), Ca (70,000 kg ha^{-1} of horse manure and 0.2% of *D. salina*) at a) 15, b) 30, and c) 45 days after application. Average ± standard deviation, bars with different letters denote significant differences ($p<0.05$).

As mentioned, the treatments consisted of a mixture of organic fertilizer and algal paste from the species *Dunaliella salina*. Regarding the latter, it has been found that algal-based biostimulants maintain the gradual release of nutrients, preventing their loss. Furthermore, it has been demonstrated that metabolites from microalgae improve soil fertility and nutrient absorption (Zhang *et al.*, 2024). According to the characteristics of *Dunaliella salina*, this is a microalga that can develop in extreme and deficient conditions. It has been primarily cultivated for the extraction of β-carotene and glycerol (Mendoza *et al.*, 2011). Glycerol serves as a suitable source of carbon for microorganisms and can increase microbial activity in the soil, thereby enhancing nutrient availability. This compound increases the soil's retention capacity and aids in nutrient absorption, enhancing the organic matter content of the soil (Betancourt-Aguilar *et al.*, 2016). Another property analyzed during this research was electrical conductivity (EC), where significant differences ($p<0.05$) were also observed among the treatments. The highest EC values were recorded in the treatments Ca and Va at 15, 30, and 45 days after their application (Figure 2).

At 15 days, the values for Ca (477.49 μS/cm) and Va (327.89 μS/cm) were classified as saline and moderately saline, respectively, according to the classification of NOM-021-RECNAT-2000. At 30 days after applying the treatments, the values for Ca (320.46 μS/cm) and Va (294.94 μS/cm) were considered moderately saline (NOM-021-RECNAT-2000). The Ca treatment recorded the highest electrical conductivity value at 45 days after application, measuring 324.56 μS/cm, which was classified as moderately saline according to NOM-021-RECNAT-2000.

The highest values found in the Ca and Va treatments can be attributed to the mineralization of manure, which releases high amounts of anions and cations, resulting in an increase in soil salinity. It has been reported that for every ton of manure applied to the soil, it receives between 15 to 50 kg of salts, depending on the quality of the manure (Trejo-Escareño *et al.*, 2013). Salazar-Sosa *et al.* (2010) and Cervantes-Vázquez *et al.* (2022) report

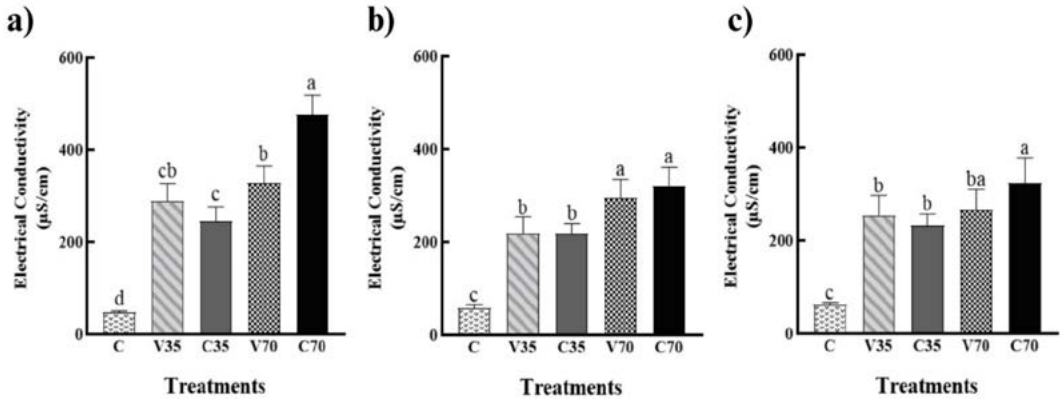

Figure 2. Electrical conductivity of a soil with water erosion treated with organic fertilizer and *Dunaliella salina*, C (control with 0 kg ha^{-1} of fertilizer and 0.2% of *D. salina*), Vb (35,000 kg ha^{-1} of cow manure and 0.2% of *D. salina*), Va (70,000 kg ha^{-1} of cow manure and 0.2% of *D. salina*), Cb (70,000 kg ha^{-1} of horse manure and 0.2% of *D. salina*), Ca (35,000 kg ha^{-1} of horse manure and 0.2% of *D. salina*) a) 15, b) 30, and c) 45 days after application. Mean ± standard deviation; bars with different letters indicate significant differences ($p<0.05$).

that the highest concentrations of salts are found in treatments with 80,000, 120,000, and 160,000 kg ha^{-1} of manure. Therefore, the treatments used in this study were less than 80,000 kg ha^{-1} to avoid increasing the amount of salts during the mineralization of organic matter, as the increase in Na^+ in the soil can lead to structural losses or inhibition of plant growth, affecting agricultural yield.

As with the content of organic matter and electrical conductivity, the highest pH values were observed in the Ca and Va treatments at 15, 30, and 45 days after application (Figure 3).

According to the classification of NOM-021-RECNAT-2000, the pH values were classified as neutral at all times (15, 30, and 45 days). At 15 days, the values were 6.96 and 6.71 for Ca and Va, respectively. At 30 days, the value for Ca was 6.87, and for Va, it was 6.70. At 45 days, the values for Ca and Va were 6.76 and 6.67, respectively. This

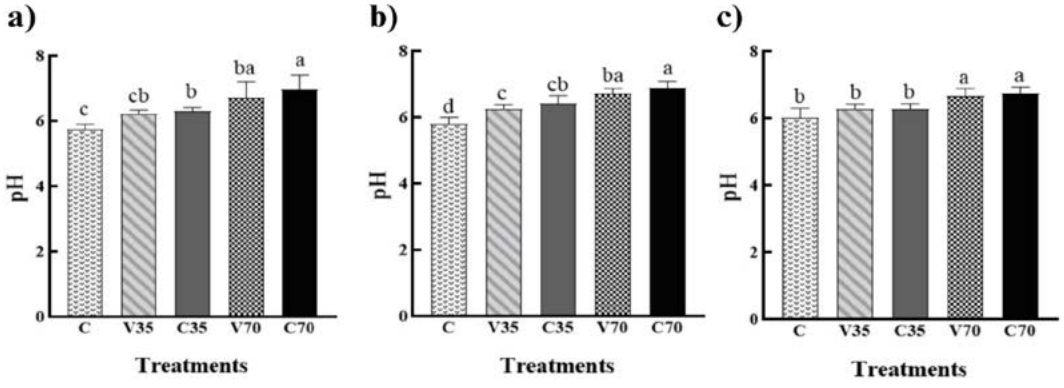

Figure 3. pH of an soil with water erosion treated with organic fertilizer and D. salina, C (control with 0 kg ha-1 of fertilizer and 0.2% D. salina), Vb (35,000 kg ha-1 of cow manure and 0.2% D. salina), Va (70,000 kg ha-1 of cow manure and 0.2% D. salina), Cb (70,000 kg ha-1 of horse manure and 0.2% D. salina), Ca (35,000 kg ha-1 of horse manure and 0.2% D. salina) at a) 15, b) 30, and c) 45 days after application. Average ± standard deviation, bars with different letters indicate significant differences ($p<0.05$).

can be explained by the findings of Trejo-Escareño *et al.* (2013), who reported that in soils treated with organic fertilizers, the pH increased due to the enrichment of cations and the ammonification of the organic matter present, as organic fertilizers promote proton attraction (Cervantes-Vázquez *et al.*, 2022). Finally, inorganic nitrogen was determined, and an increase in this element was observed in the treatments Va (23.27 mg kg^{-1}; 28.25 mg kg^{-1} at 15 and 30 days) and Ca (16.82 mg kg^{-1}; 15.32 mg kg^{-1} at 15 and 30 days after application) (Figure 4).

At 45 days, only the Va treatment presented the highest nitrogen value. This may be due to the concentrations of inorganic nitrogen depending on the activity of microorganisms under aerobic and anaerobic conditions. This process accelerates with an increase in temperature and is enhanced by adequate moisture and good oxygen availability. Cow manure has a more abundant and diverse microbiota compared to horse manure (Tian *et al.*, 2015; Pacheco-Torres *et al.*, 2021). However, the use of the algal paste can have a significant influence due to the glycerol it produces. Betancourt-Aguilar *et al.* (2016) report that glycerol serves as a suitable source of carbon for microorganisms and can increase microbial activity in the soil, leading to nitrogen being immobilized by microorganisms. Conversely, its wide range of pH values (ranging from acidic to alkaline) can affect the processes of denitrification and nitrogen immobilization differently and, in general, the soil biochemistry. This could explain the different concentrations of inorganic nitrogen in the soil.

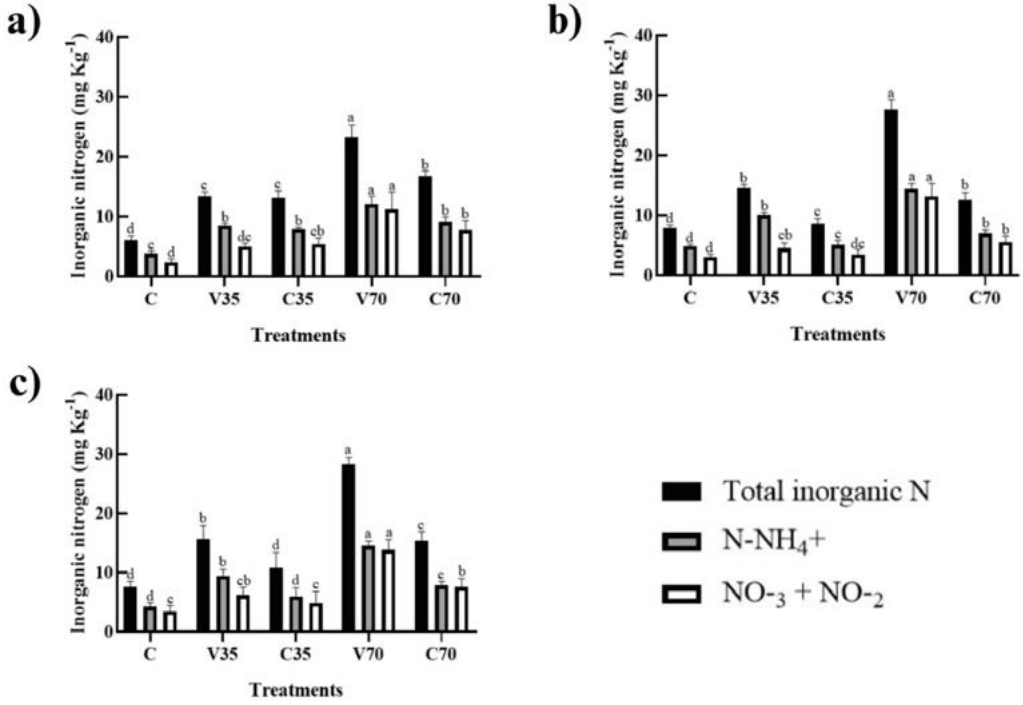

Figure 4. Nitrogen Mineralization of a soil with water erocion treated with organic fertilizer and *Dunaliella salina*: C (control with 0 kg ha^{-1} of fertilizer and 0.2% of *D. salina*), Vb (35,000 kg ha^{-1} of cow manure and 0.2% of *D. salina*), Va (70,000 kg ha^{-1} of cow manure and 0.2% of *D. salina*), Cb (70,000 kg ha^{-1} of horse manure and 0.2% of *D. salina*), Ca (35,000 kg ha^{-1} of horse manure and 0.2% of *D. salina*) at a) 15, b) 30, and c) 45 days after application. Average ± standard deviation; bars with different letters indicate significant differences ($p<0.05$).

In the case of the inorganic form NH_4^+, the highest values were recorded starting on day 30 in the treatments Va (7.80 mg kg^{-1}) and Vb (9.45 mg kg^{-1}). This increase may be due to the fact that cow manure contains 5.6% microorganisms, primarily ammonifying microorganisms that degrade nitrogen-containing compounds into smaller molecules that can be absorbed and utilized. Most of the ammonifying microorganisms found in this manure (fungi, bacteria, etc.) exhibit ammonification activities, particularly *Bacillus* spp., which shows high ammonifying activity and plays an important role in the nitrogen cycle. However, it has been found that temperature has a significant effect on ammonifying microorganisms, affecting the decrease in microbial activity at high temperatures. Many other studies have also demonstrated that inoculation with thermophilic microorganisms is effective for converting nitrogen in composting processes (Behera and Ray, 2021; Zhang, 2023).

For nitrates and nitrites, the highest values were recorded 30 days after the application of the treatments, with Ca (7.52 mg kg^{-1}) and Va (13.78 mg kg^{-1}) showing the highest values. This can be attributed to the findings of Salazar *et al.* (2007), who found that NO_3^- and NO_2^- increase with more than 60,000 kg ha^{-1} of manure applied to the soil. These results indicate that an increase in the dosage of manure application results in greater mineralization, increasing the amounts of NO_3^- and NO_2^- in the soil. However, they also reported a significant effect when applying manure at more than 40,000 kg ha^{-1}, resulting in an increase in all macronutrients necessary for plant development and production.

CONCLUSIONS

An increase was observed due to the application of the mixture of *Dunaliella salina* and organic fertilizers (cow and horse) on the chemical properties (organic matter, electrical conductivity, and nitrogen mineralization) of a water-eroded soil in the Ca and Va treatments.

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Colegio de Postgraduados

Effect of Laser Pre-Treatment on Peanut Seeds on Resveratrol Content and Fatty Acid Profile in Field-Harvested Seeds

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ABSTRACT

Objective: To evaluate the effects of laser radiation on peanut seeds as a pretreatment on the resveratrol content and fatty acid profile in the harvested seeds.

Design/methodology/approach: The experiment was established under field conditions located in the municipality of Nicolás Bravo, Chiapa de Corzo, Chiapas. The seeds were irradiated with a red laser with a wavelength of 636 nm and an intensity of 120 mW. The exposure time of the seeds to the laser was 15 min and non-irradiated seeds were used as a control.

Results: Laser radiation on seeds increased the resveratrol content in the harvested peanut seeds compared to control seeds, and changes were also observed in some fatty acids in peanut oil such as butyric acid, stearic acid and cis 11,14,17 eicosatrienoic acid.

Findings/conclusions: laser irradiation applied to seeds is a low-cost biotechnological alternative that allows generating positive changes in the quality of grains from seeds harvested under field conditions.

Keywords: physical biostimulation of seeds, resveratrol, fatty acids.

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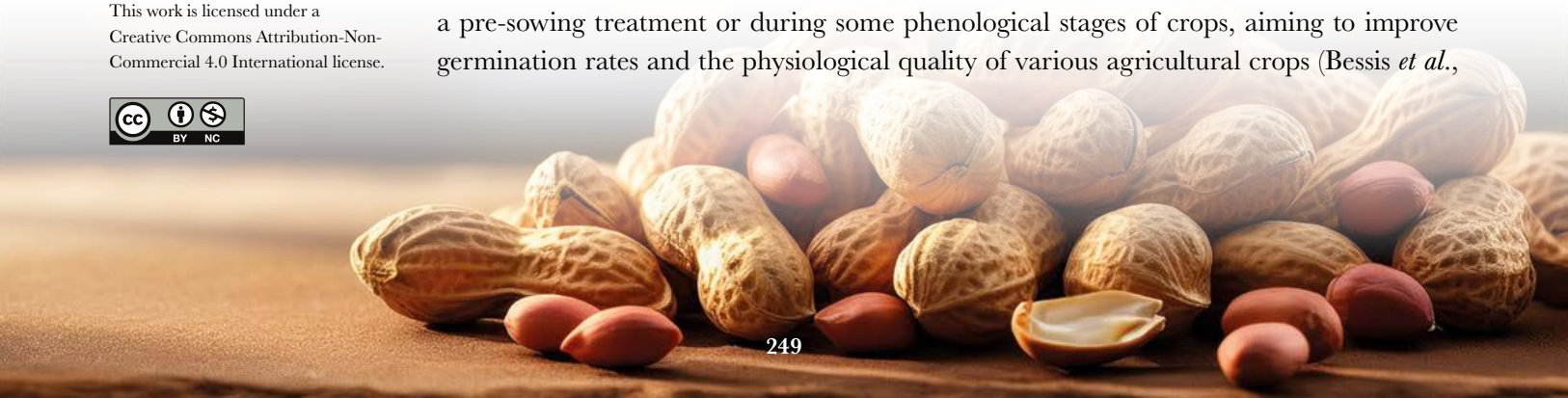
INTRODUCTION

The visible light spectrum consists of all light radiations of different colors: violet and blue, ranging from 290 to 500 nm; green, yellow, and orange from 530 to 610 nm; and red from 600 to 750 nm (Singh *et al.*, 2015). Plants in the vegetative and reproductive phases utilize chlorophyll and carotenoids as sensors to capture light; while in the germination phase, phytochromes present in the endosperm and embryo of the seed are the molecules responsible for capturing light radiation (Demotes-Mainarda *et al.*, 2016). Laser is a type of light amplified by stimulated emission of radiation, and due to its potential to concentrate large doses of energy, its application in various biological systems began as a pre-sowing treatment or during some phenological stages of crops, aiming to improve germination rates and the physiological quality of various agricultural crops (Bessis *et al.*,



1962). Bio-stimulation using He-Ne laser (632.8 nm) and laser diodes (650, 660 nm) has been applied in some cereals, such as *Zea mays* L. and *Triticum aestivum* (Toth *et al.*, 1993; Joshi *et al.*, 2012); in some vegetable seeds like *Raphanus sativus* L. and *Solanum lycopersicum* L. (Muszyñski & Gladyszewska, 2008; Álvarez *et al.*, 2011); in oilseeds such as *Carthamus tinctorius* L. and *Helianthus annuus* (Kumar & Srivastava, 2010; Perveen *et al.*, 2010); and in some leguminous seeds like *Medicago sativa* (Wilczek *et al.*, 2005). Currently, the emphasis of various studies is to increase the content of antioxidant compounds and improve the productivity and quality of crops to generate better ingredients and functional foods. Therefore, the objective of this research was to evaluate the effects of laser radiation on seeds as a pre-treatment before planting under field conditions, focusing on the resveratrol content and fatty acid profile of peanut seeds.

MATERIALS Y METHODS

The criollo variety peanut seeds used for this study were collected from the municipality of Jericó, Chiapas, Mexico. The selected seeds for this study had an average size of 1.5-2.0 cm in length and were kept in bags at a temperature and relative humidity of 20±2 °C and 45±2%, respectively, to minimize their metabolic activities and, consequently, loss of viability and energy until the start of the experiments.

The seeds were irradiated with a motorized red laser bar from the brand Steelpro® with a wavelength of 636 nm and an intensity of 120 mW. After conducting tests to select the appropriate exposure time, the conditions for the red laser treatment of the seeds were: an exposure time of 15 minutes at a distance of 15 cm from the seed. Non-irradiated seeds were used as a control. The control and treatment seeds were planted in the field under normal conditions, following the conventional planting method, with a spacing of 30 cm between plants and 60 cm between rows in the locality of Nicolás Bravo, Chiapa de Corzo, Chiapas. Each experimental unit consisted of 200 seeds and was conducted with 3 replications. The pods were harvested manually 115 days after planting; the pods were dried, and the hull was separated from the seeds. The extraction and quantification of resveratrol content (μg/g dry weight) in the hull and seed tissue was performed using the HPLC method described by Limmongkon *et al.* (2017) with some modifications. The separation was carried out on a C18 reversed-phase column. The mobile phase consisted of acetonitrile: water (40:60, v/v) and was run at a constant flow rate of 1 mL/min. The chromatograms were detected using a UV detector at 306 nm. Sigma Aldrich trans-resveratrol was used as a standard to create a calibration curve between the standard concentration and the average peak area. Oil extraction from seeds was conducted using the Soxhlet method, and the preparation of methyl esters of fatty acids followed the methodology described by Santos-Espinoza *et al.* (2020). Fatty acid compositions were analyzed using gas chromatography coupled with mass spectrometry (GC-MS). A completely randomized design was employed, using a simple ANOVA, with the Statgraphics statistical software.

RESULTS AND DISCUSSION

In Table 1, we can observe the resveratrol content in the hull and seeds. The results indicate that there were no statistically significant differences in resveratrol content in the

Table 1. Content of trans-resveratrol in the hull and peanut grains harvested from laser pre-treated seeds before field planting.

Treatments	Resveratrol (μg /g dry weight)	
	shell	Seeds
Control	14.87±0.570 a	7.50±4.27 b
Laser treatment	13.46±0.817 a	54.90±5.40 a
HDS	1.597	11.047

Average values with the same letter in the column are not statistically different (P≤0.05). HDS: Honest significant difference.

hull between the laser treatment and the control. On the other hand, in the seeds, it was observed that the resveratrol content increased sevenfold in the laser treatment compared to the control.

Few studies, such as that of Zhu *et al.* (2020), have investigated the increase in resveratrol content in peanuts in response to physical stimuli, finding that ultraviolet (UV-C) radiation promoted the accumulation of stilbenes by positively regulating the activity of the enzyme stilbene synthase, which is responsible for the synthesis of resveratrol via the phenylpropanoid pathway. They found this to be related to the increased transcription of genes in the stilbene biosynthesis pathway. The fatty acid profile of the oil from the peanut seeds subjected to laser treatment and the control is shown in Table 2. The results show that the most abundant fatty acids present in peanut oil were oleic acid (43-45%), linoleic acid (26%), palmitic acid (11%), stearic acid (4-5%), and cis 11,14,17 eicosatrienoic acid (1-2%), which is similar to what has been reported by various authors, such as Bravo *et al.*

Table 2. Fatty acid profile of the peanut grains harvested from laser pre-treated seeds before field planting.

Methyl esters of fatty acids	Treatments (relative abundance %)		
	Control	Laser treatment	HSD (95%)
Methyl butyrate	0.11±0.006 b	0.19±0.047 a	0.076
Methyl hexanoate	0.18±0.045 a	0.25±0.071 a	0.135
Methyl decanoate	0.04±0.015 a	0.06±0.002 a	0.024
Methyl myristate	0.17±0.081 a	0.09±0.039 a	0.144
Methyl palmitate	11.45±0.264 a	11.70±0.546 a	0.972
Methyl palmitoleate	0.09±0.006 a	0.20±0.117 a	0.189
Methyl heptadecanoate	0.18±0.018 a	0.14±0.035 a	0.063
Methyl stearate	4.96±0.047 a	4.06±0.540 b	0.870
Methyl cis 9-oleate	45.63±0.725 a	43.68±2.199 a	3.711
Methyl linoleate	26.32±0.336 a	26.33±0.533 a	1.010
Methyl arachidate	2.29±0.048 a	1.92±0.365 a	0.591
Methyl cis 11-eicosenoate	1.93±0.004 a	1.64±0.313 a	0.502
Methyl cis 11,14,17 eicosatrienoate	4.27±0.071 b	5.04±0.355 a	0.581
Methyl lignocerate	2.48±0.229 a	1.98±0.631 a	1.076

Average values with the same letter in the row are not statistically different (P≤0.05). HDS: Honest significant difference.

(2018) and Santos-Espinoza *et al.* (2020). The results demonstrate that the laser treatment significantly modified the fatty acid profile of peanut oil, resulting in increases in the relative abundance percentage of butyric acid and cis 11,14,17 eicosatrienoic acid, along with a significant decrease in stearic acid.

In the same way, it was demonstrated that the laser treatment did not affect the most abundant fatty acids in peanut oil, which correspond to unsaturated fatty acids such as oleic and linoleic acids, nor the ratio of oleic to linoleic acid, which mediate the quality of the oil. This is the first report analyzing the effect of laser radiation on seeds as a pre-treatment before planting on the fatty acid profile of peanuts cultivated in the field.

CONCLUSIONS

The laser radiation technique on peanut seeds before planting is a low-cost biotechnological alternative that helps increase the resveratrol content in the seeds. Additionally, it generated changes in the fatty acid profile, which did not affect the most abundant fatty acids in the oil, such as oleic and linoleic acids, thus ruling out the possibility that this technique may have negative effects on the quality of the seed oil.

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Colegio de Postgraduados

Morphological, Molecular, and Pathogenic Characterization of *Rhizoctonia solani* Isolates Associated with Bean Drying in Northern Sinaloa, Mexico

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ABSTRACT

Objective: The objective of this study was to characterize, using morphology, DNA sequence analysis, and pathogenicity, *R. solani* isolates associated with bean plants with root rot symptoms in commercial plots in northern Sinaloa.

Design/methodology/approach: During the 2020-2021 cycle, diseased plants infected by *Rhizoctonia* were collected in the municipalities of Ahome, El Fuerte, and Guasave. Pure fungal isolates were obtained in specific media; which were morphologically characterized in PDA medium and preserved. Subsequently, the pathogenicity of the isolates was evaluated and they were molecularly identified. Genomic DNA was extracted from the isolates, part of the RPB2 gene was amplified by PCR, and the amplified products were sequenced.

Results: Phylogenetic analysis with RPB2 sequence data confirmed the identification of 63 isolates as *R. solani* and allowed them to be assigned to the anastomosis group (AG): AG-4. Of the total isolates analyzed, 86% correspond to the AG-4 HGI anastomosis subgroup and 14% to the AG-4 HGIII subgroup. In pathogenicity, the percentage of germination and severity of the isolates were evaluated, showing different levels of pathogenicity.

Limitations of the study/implications: None.

Findings/conclusions: *Rhizoctonia solani* AG-4 anastomosis subgroups HGI and HGIII are associated with bean drying in northern Sinaloa. Therefore, this study will serve as a basis for other studies that generate control strategies for this pathogen.

Keywords: pathogenicity, sclerotia, RPB2, phylogenetic analysis, anastomosis.

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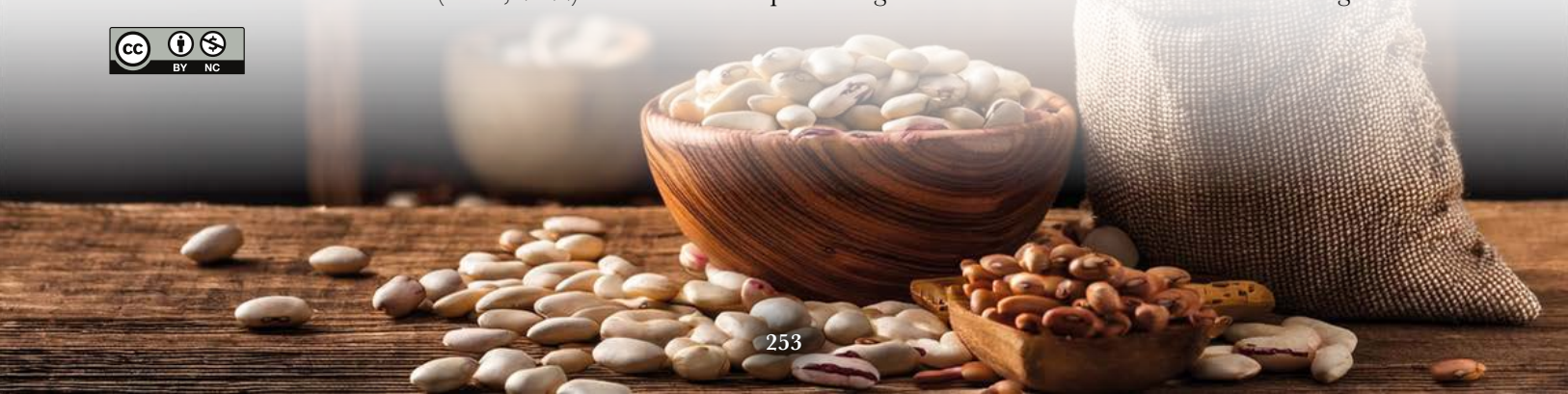
INTRODUCTION

The production of beans (*Phaseolus vulgaris* L.) is one of the most significant agricultural activities in Mexico's economy. The annual income generated by bean cultivation in the country ranges around 17 million pesos, with a production exceeding 965 thousand tons (SIAP, 2022). The main bean-producing states are Zacatecas and Sinaloa. Bean agricultural



production in Sinaloa takes place during the autumn-winter cycle and covers a planted area of 63,262 hectares, of which 70% is established in northern Sinaloa, in the municipalities of Ahome, El Fuerte, and Guasave (SIAP, 2024). Local bean production is characterized by poor organic matter soils, highly fragmented without crop rotation schemes, and very variable environmental conditions, which favor the incidence of pests and diseases that jeopardize and limit this activity. A recurring problem in northern Sinaloa is bean drying, and associated with this, phytopathogenic fungi have been found, among which the species *Rhizoctonia solani* occurs most frequently.

The basidiomycete *Rhizoctonia solani* Kühn (teleomorph *Thanatephorus cucumeris*) is considered a heterogeneous genus of fungi that inhabit the soil, with a global distribution. It is a phytopathogen that infects a wide variety of agriculturally important crops (Chavarro *et al.*, 2015; Gonzalez, 2006; Kanetis *et al.*, 2016). The genetic, morphological, and pathogenic variability of *R. solani* strains has classified it into 14 anastomosis groups (AG) that are reproductively incompatible with each other (Carling *et al.*, 2008). The anastomosis groups are numbered from AG1 to AG13 and AG BI (Cubeta and Vilgalys, 1997; Hane *et al.*, 2014). The complexity of the species within the anastomosis groups has led to the subdivision of many AG into anastomosis subgroups that differ in one or more biochemical, genetic, or pathogenic characteristics (Cubeta and Vilgalys, 1997).

Traditionally, AGs were assigned by observing the fusion of hyphae with known tester isolates. However, molecular analysis is now the most efficient method for the classification of *Rhizoctonia* (Sharon *et al.*, 2006). This allows for the determination of the species, the anastomosis group, and the corresponding anastomosis subgroup. In beans, the reported anastomosis groups are AG 1, AG 2, and AG 4. Individually, they have been associated with different types of diseases. For example, AG 1 and its various registered anastomosis subgroups in beans include AG-IA, AG 1-IB, AG 1-IE, and AG 1-IF, which cause damage to foliage, resulting in blighting, necrotic and watery lesions on the leaves (Gonzalez, 2008; Valentin *et al.*, 2016). In contrast, AG 2 and AG 4 cause root lesions, such as dark cankers, corky lesions, and plant drying (Woodhall *et al.*, 2008). Host specificity among anastomosis groups is variable (Carling *et al.*, 2002; Fiers *et al.*, 2011). AG 4 was originally thought to infect only potatoes; however, it has been reported that isolates from this group also infect soybean, corn, tobacco, tomato, sugar beet, beans, among others.

In bean plants, the AG-4 anastomosis group of *R. solani* is recognized for causing seed rot, post-emergence wilting, tip rot, and root rot in important crops such as beans.

In bean plants, the AG-4 anastomosis group of *R. solani* is recognized for causing seed rot, post-emergence wilting, tip rot, and root rot in important crops such as beans. Currently, Mexico lacks research on this species; previous studies have only focused on *Rhizoctonia solani* in vegetables like potato, as it has had the most significant problems with this pathogen. Regarding bean cultivation in Sinaloa, there are no studies related to *Rhizoctonia solani*, despite the ongoing and aggressive presentation of Rhizoctoniasis. In this regard, the objective of the research is to morphologically, molecularly, and pathologically identify the anastomosis groups and subgroups of *Rhizoctonia* present in bean cultivation, which will help determine control strategies that should focus on seeking sustainable alternatives.

MATERIALS AND METHODS

During the period from 2020 to 2021, 50 bean lots were inspected in the municipalities of El Fuerte, Ahome, and Guasave in northern Sinaloa. Each lot was sampled using the "five of gold" technique (Martínez, 2012), with 2 samples taken per sampling site, resulting in 10 samples of infected tissue from each lot. These samples were placed in plastic bags labeled with the location and date, geographical coordinates, variety, and symptoms, and then stored in a cooler at approximately 8-10 °C (Agrios, 2005) for transportation to the laboratory for processing. For the isolation of *Rhizoctonia solani*, 0.5×0.5 cm cuts were made from the margins of the lesions and were planted in 8.5 cm diameter Petri dishes containing acidified PDA (potato dextrose agar) with lactic acid (Yang *et al.*, 2014; Muzhinji *et al.*, 2015).

The cultures were incubated at 25 °C for 48 hours and observed under a biological microscope to purify the colonies of *Rhizoctonia solani*. The isolates were transferred to 1.6% water agar (WA) and incubated at 25 °C. After 24 hours, the plates were examined under the microscope to select a hyphal tip, which was then transferred to PDA medium (Sneh, 1996) and incubated at 25 °C for 7 days. Subsequently, the isolates were preserved in 30 ml Falcon tubes with sterile substrate and sterile distilled water.

From the pure strains obtained from *Rhizoctonia solani*, morphological characterization was performed by taking macro and microscopic variables such as colony color, sclerotium formation, hyphal diameter, and number of nuclei per hypha (Sneh *et al.*, 1991). The isolates were grown on PDA and incubated at 25 °C for 30 days, with daily inspections to measure the time of sclerotium formation and colony color. To measure the diameter of hyphae and nuclei per hyphal cell, 7-day-old colonies were used. The mycelium from each isolate of *Rhizoctonia solani* was transferred to a glass slide and stained with Safranin-O (Bandoni, 1979). Twenty hyphal cells were examined under a biological microscope with an Olympus Lx micrometer.

To evaluate the anastomosis reaction among the isolates, Petri dishes containing 10 ml of agar water were used, with a sterile slide placed inside. On one end of the slide, a 0.5 cm^2 piece of mycelium agar from one isolate was placed, and on the other end, a different isolate was added for confrontation. The setup was incubated at room temperature for 24 hours, and then observed under a microscope to check if the hyphae from the two isolates made contact. If contact occurred, the contact zone was stained with 0.05% trypan blue, and the type of anastomosis reaction was determined according to Carling (1998).

For molecular identification, genomic DNA was extracted from each of the two isolates. The DNA extraction was performed using the CTAB method (Murray and Thompson, 1980; Doyle and Doyle, 1990; Porebski *et al.*, 1997) from 50 to 100 mg of mycelium. Partial fragments of the second largest subunit of the RNA polymerase II gene (*rpb2*) were amplified and sequenced using the primer pairs RBP2-980F (5'-TGYCCIGCIGARACICCHGARGG-3') and RPB2-7Cr (5'-CCCATRGCTTGYTTRCCCAT-3') (Liu *et al.*, 1999; Reeb *et al.*, 2004), respectively.

Subsequently, the sample was sequenced. The sequence data obtained were compared using a BLAST search in the National Center for Biotechnology Information (NCBI) database to determine the anastomosis group (AG) of the individual isolates. The sequences

were aligned using the Clustal W algorithm integrated into the MEGA 6.0 software package (Tamura *et al.* 2013), and the phylogenetic relationship among isolates was calculated using the Neighbor-Joining (NJ) method (Saitou and Nei 1987) under the Kimura two-parameter model (Kimura, 1980) as the substitution model, omitting all sites with gaps. For comparison purposes, ITS rDNA sequences from other known AG isolates were obtained from GenBank and used for phylogeny. The Bootstrap analysis was performed using 1000 pseudoreplicates of the dataset. The sequence of *Botryobasidium simile* (GenBank accession number GEL2348) was used as the outgroup for rooting.

Pathogenicity tests for each isolate were conducted by inoculating 5 seeds of common bean, variety Azufrado Higuera, planted in 2 kg pots with agricultural soil. A total of 50 ml of a mycelial suspension adjusted to a concentration of 1×10^5 mycelial fragments/ml was placed directly onto the seeds.

As a control, five seeds of common bean, variety Azufrado Higuera, were used without inoculation. All plants were maintained in a greenhouse for 15 days at temperatures ranging from 22 to 32 °C. Four repetitions were made for each treatment. Germination percentages were evaluated 10 days after planting, and symptoms of root rot, stem canker in the seedlings, and wilting were assessed 30 days after planting. The pathogenicity test was conducted twice with similar results. The fungi were re-isolated from the infected roots and found to be morphologically identical to the isolates used for inoculation, thereby fulfilling Koch's postulates. To assess the severity in the roots and hypocotyls of each plant, an ordinal scale from 0 to 5 was used, developed by Cardoso and Echandi (1997). ANOVA was performed with a $p > 0.05$.

RESULTS AND DISCUSSION

During field surveys, the symptoms observed in common bean crops in the municipalities of Fuerte, Ahome, and Guasave included plant wilting, corky lesions at the base of the stem, dark cankers, and root rot (Figure 1), as described by Woodhall and colleagues (2008) for *Rhizoctonia* spp.

Figure 1. Symptoms in field bean plants. A. Bean plant wilt. B. Dark cankers at the base of the stem and root. C. Corky lesions.

A total of 71 isolates were obtained, named from FAVF400 to FAVF470. The colonies of isolates from bean plants exhibiting wilt symptoms showed a cottony mycelial growth ranging from white to brown, turning the medium amber. The mycelium of the isolates is septate, hyaline, with constriction at the basal cell, forming branches at right angles, with hyphae measuring 5-8 μm in width and sclerotia 1-3 mm in diameter, with no presence of spores (Figure 2), typical characteristics of *R. solani* (Sneh *et al.*, 1991; Wantanabe, 2002; Zitter *et al.*, 2004). Staining of nuclei revealed the presence of only multinucleate isolates, and in the anastomosis test, all isolates anastomosed with each other, classifying into categories C3 and C2 (Figure 3), according to Carling *et al.*, 1988 and MacNish *et al.*, 1993.

The molecular identification of 63 isolates, based on partial fragments of the second largest subunit of RNA polymerase II gene (*rpb2*), using the primer pairs RBP2-980F and RPB2-7Cr, revealed that the 63 sequences had 99-100% similarity to *Rhizoctonia solani* with homologous sequences from NCBI. Phylogenetic analysis was performed with the sequences of the isolates of Rhizoctonia solani, Rhizoctonia oryzae-sativa, and Botryobasidium simile. A matrix was processed comprising the RPB2 regions of 84 isolates, including those from the present study and 21 reference strains. This analysis established the identity of a single anastomosis group for Rhizoctonia solani AG-4, and two subgroups of this, where 54 isolates were found to belong to AG-4 HGI and 9 isolates to the AG-4 HGIII subgroup (Figure 4).

In Mexico, these anastomosis subgroups have been reported in cultivated plants such as hibiscus, potato, chili, and tomato (Moreno *et al.*, 2013; Ortega *et al.*, 2022), but not in

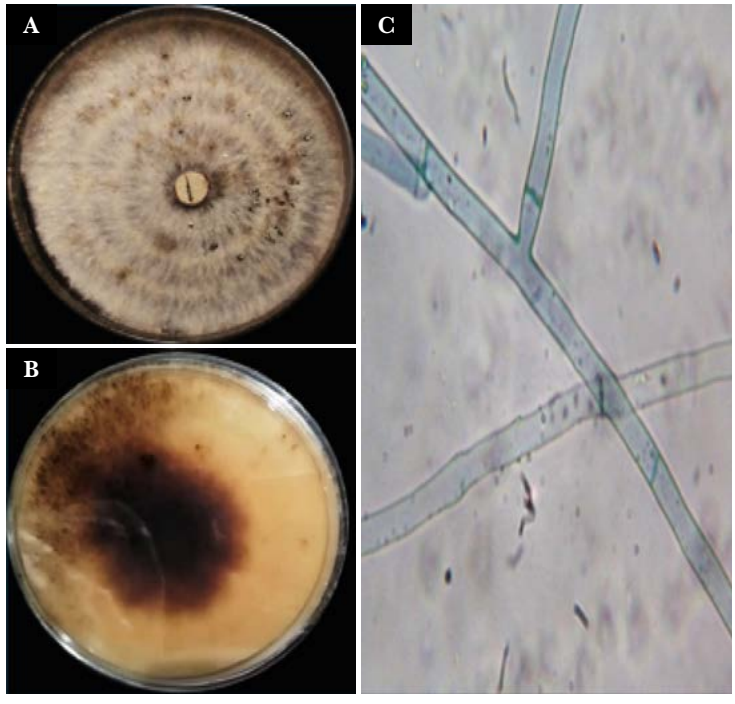


Figure 2. Morphology of the colonies and coloration of the medium in PDA of *Rhizoctonia* spp. A. Cottony mycelium and microsclerotia on the surface in PDA. B. Color change of the culture medium. C. Septate mycelium, constriction at the basal cell, and formation of right angles.

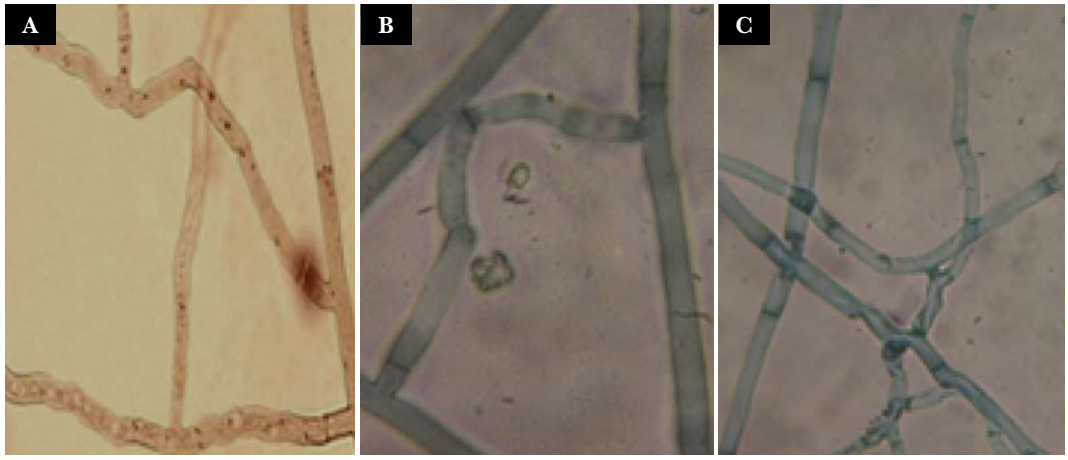


Figure 3. Staining of nuclei and anastomosis reaction. A. Multinucleated hyphae. B. Wall fusion, fused membranes; the anastomosis point is not very frequent. The diameter of the anastomosis is almost always equal to that of the hypha. Anastomosis reaction C3, positive, indicates that the confronted isolates belong to the same AG. C. Obvious wall connection; it is unknown whether there is membrane exchange. Adjacent cells die, and the fusion diameter is very narrow. Anastomosis reaction C2, positive, indicates that the confronted isolates belong to the same AG but a different subgroup.

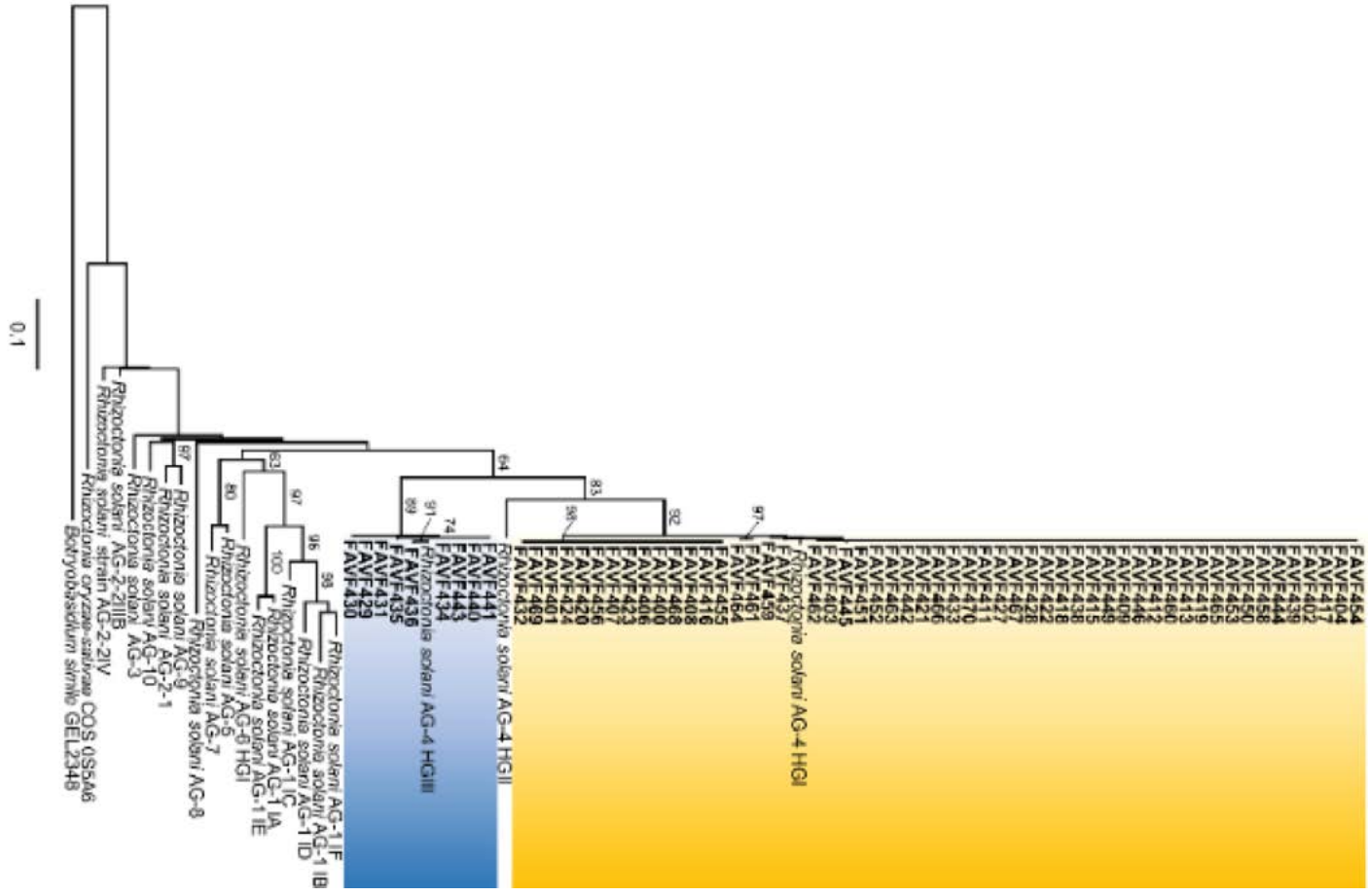


Figure 4. Phylogeny by ML of the concatenated RPB2 gene sequences of 63 *Rhizoctonia* spp. isolates representative of 70 obtained from bean plants exhibiting wilting and root rot symptoms. Bootstrap of 1000 replicates; outgroup: *Botryobasidium simile*. AG=Anastomosis group.

common bean. However, studies in other bean-producing countries report the presence of anastomosis groups AG-1, AG-2, AG-3, AG-4, AG-5, AG-6, AG-9, AG-10, and AG-11. In the northern region of Sinaloa, Mexico, the results showed that all the *R. solani* isolates obtained from common bean crops in the municipalities of El Fuerte, Ahome, and Guasave belong to the anastomosis group AG-4. This aligns with previous reports indicating that *R. solani* AG-4 is the predominant AG in bean production areas, causing root rot and plant wilting worldwide, followed by AG-1 and AG-2 (Gülsüm *et al.*, 2024). The HGI and HGIII subgroups were the most prevalent.

In the pathogenicity test, all isolates were found to be pathogenic, directly affecting the germination process of common bean seeds by up to 100% compared to the controls (Figure 5). Symptoms associated with rhizoctoniasis appeared 10 days after inoculation, with infected plants showing root rot, dark cankers on the root and hypocotyl, corky lesions, and wilting. The treatments applied showed significant differences compared to the control (Figure 6), with the HGI subgroup being the most severe.

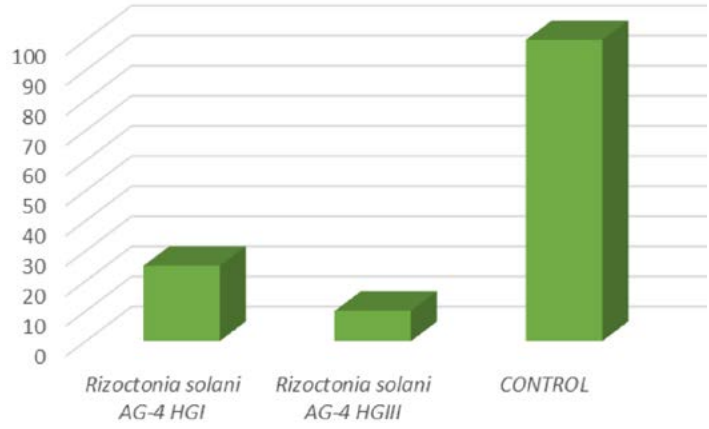


Figure 5. Percentage of bean seed germination.

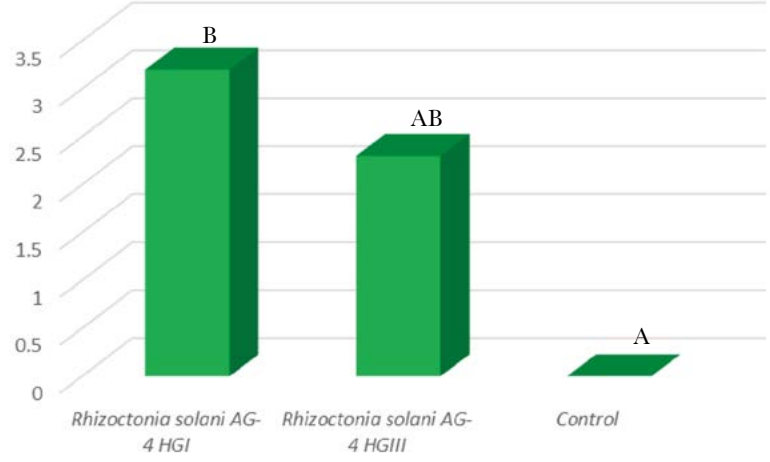


Figure 6. Severity of anastomosis subgroups. Means with a common letter are not significantly different ($p>0.05$).

This demonstrates that the fungi isolated from *Rhizoctonia solani* AG-4 HGI and HGIII are causal agents of root rot and wilting in beans in northern Sinaloa. The results of the seed germination percentage and the severity of the isolates showed that these pathogens are capable of limiting crop development, leading to decreased yield and production of this grain, resulting in economic losses due to root diseases in the studied regions.

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CONCLUSIONS

Rhizoctonia solani was found naturally causing corky lesions, black cankers, root rot, and plant wilt in common bean crops in northern Sinaloa, particularly in the municipalities of Ahome, El Fuerte, and Guasave. The anastomosis group present in the bean crop in northern Sinaloa is AG-4, along with its subgroups HGI and HGIII. Pathogenicity tests indicated that both anastomosis groups cause disease in beans, being extremely severe, preventing seed germination, and leading to the manifestation of symptoms such as plant wilt.

Future studies should focus on the search for common bean varieties resistant to rhizoctoniasis, as well as on disease control and pathogenicity in other crops, to develop a management strategy against the different groups and subgroups of anastomosis of *Rhizoctonia solani*. The pathogenicity tests indicated that both anastomosis groups cause disease in beans, being extremely severe, preventing seed germination, and leading to the manifestation of symptoms such as plant wilt. Future studies should focus on the search for common bean varieties resistant to rhizoctoniasis, as well as on disease control and pathogenicity in other crops, to develop a management strategy against the different groups and subgroups of anastomosis of *Rhizoctonia solani*.

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Extraction of nitrogen and distribution of dry matter in forage corn (*Zea mays* L.) in a clayey soil

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ABSTRACT

Objective: To analyze nitrogen extraction in forage maize (*Zea mays* L.) and its distribution in the plant's stems, leaves, ears, and husks under different fertilization rates, and to determine the total dry matter (DM) yield in the plant and in each of its organs.

Design/methodology/approach: Plots of forage maize were established to evaluate treatments with different nitrogen fertilization rates in a randomized complete block design with four replications in clay-textured soil.

Results: The behavior of nitrogen extraction in forage maize production on clay soil was observed. No significant differences were found among the treatments. The DM yield was very low, ranging from 13.72 to 16.52 t ha^{-1}. The percentage of DM distribution was higher in the ear and lower in the husks, and the same pattern was observed for N extraction.

Findings/conclusions: The dry matter yield in forage maize was not significantly affected by the applied nitrogen rates, and the yield was very low. The percentage of dry matter in the ear did not reach the 45% that the crop should have.

Keywords: N rates, yield, plant organs.

INTRODUCTION

Maize (*Zea mays* L.) is one of the most widely used forages in the world, as it grows quickly, produces a large amount of biomass, has a good nutritional level, and adapts to a wide variety of climates and regions. In Mexico, maize silages have a low net energy value for lactation (<1.5 Mcal kg^{-1} of dry matter) compared to maize silages in the United States of America and Europe (Chalupa, 1995). This can be attributed to the fact that

in the past, the emphasis was primarily on yield per hectare in maize silage production, without considering its nutritional value, as there was no available information regarding the importance of this aspect (Núñez *et al.*, 2006). In the Comarca Lagunera, dairy farming is intensive or technified, with an intensive use of inputs and a high degree of mechanization. In milk production in the Lagunera Region, it is very important to analyze forage production as it is the nutritional support for regional livestock (Rector Plan 2001-2006). The production of forage maize is one of the most important agricultural activities in the Comarca Lagunera, as it is the second source of forages that meet the growing demand of Mexico's main dairy basin (Salazar *et al.*, 2003).

Absorption studies aim to quantify, in some way, the requirements, extraction, or consumption of nutrients by a crop to complete its production cycle. These studies quantitatively contribute to strengthening the recommended fertilization programs, as they specifically allow us to know the amount of nutrients absorbed by the crop (Bertsch F., 2003). Fertilization is an important component of forage maize production technology. Soil and plant analyses are also important tools for making appropriate decisions on what and how much to fertilize. Nitrogen is the nutrient that forage maize requires the most and is the one that most commonly limits yield. The nitrogen taken up by plants from the soil can come from fertilizers, manure, or residual nitrogen in the soil. However, when nitrogen is applied in excess, what the plant does not absorb can be lost from the soil through various processes, such as ammonium $\left(NH_4^+\right)$ volatilization and nitrate $\left(NO_3^-\right)$ leaching, which can contaminate the aquifer. The extracted nitrogen is the nitrogen removed by the crop in its above-ground parts; research has estimated that forage maize extracts an average of 14 kg of nitrogen per hectare per ton of dry matter produced (Núñez *et al.*, 2006).

The yield of a crop is determined by its ability to accumulate biomass (fresh and dry matter) in the organs designated for harvest, and a proportional increase in the biomass allocated to these organs ensures an increase in yield. Thus, the distribution of dry matter among the different organs of the plant plays a fundamental role in crop production (Peil *et al.*, 2005). Dry matter yield per hectare and digestibility are important because they largely determine the potential for milk production (Núñez *et al.*, 2003). Other studies also highlight the contribution of the nutritional characteristics of leaves and stems to the digestibility of maize hybrids. The objective of this study was to determine the total dry matter (DM) yield and its distribution in stems, leaves, ears, and husks in forage maize according to different nitrogen fertilization rates; additionally, to evaluate the total nitrogen (N) extraction in each of the plant's organs.

MATERIALS AND METHODS

The study was conducted at the La Laguna Experimental Field (CELALA, by its acronym in Spanish) of the National Institute of Forestry, Agricultural and Livestock Research (INIFAP) in Matamoros, Coahuila, located in the Comarca Lagunera, which has an average annual rainfall of 243.5 mm, an elevation of 1,355 meters above sea level, and an average annual temperature of 24 °C. In the spring-summer cycle, plots were established with forage maize to evaluate treatments with different nitrogen fertilization rates. The treatments were distributed in a completely randomized block design with four

replications. The soil where the crop was established is clayey, with the properties noted in Table 1.

For the data collection of the variables, plant sampling was conducted, and the samples were divided by organs (stems, leaves, ears, and husks). The samples were dried and ground to analyze total N in the laboratory using the Kjeldahl method (Jones, 2001). Statistical analyses were performed using SAS version 9.1 (SAS Institute, 2003) with Duncan and Tukey methods. These analyses allow determining if the explanatory variables are associated with the applied N treatments, and in what way this association occurs, *i.e.*, whether the values of each dependent variable tend to increase or decrease as the N dose levels of the treatments increase. The fertilization doses were applied in fractions as described in Table 2. The sowing date was May 19, 2008, and the variety used was SB-302. The N source was ammonium sulfate (20.5% N), which was diluted in water before each application.

A pre-sowing irrigation or flood irrigation was applied to moisten the soil, ensuring good seed germination; subsequently, four supplemental irrigations were given at 23, 44, 60, and 78 days. The harvest took place on September 1 of the same year, 105 days after sowing.

RESULTS AND DISCUSSION

Researchers from the forage program at the La Laguna Experimental Field of INIFAP mention that the selection criteria for forage maize hybrids are based on the expected yields and the nutritional quality of the forage. For this reason, a practical way to select maize hybrids is that they should have a potential yield of more than 18 tons per hectare of dry matter, more than 45% ear, less than 55% neutral detergent fiber, or less than 28% acid

Table 1. Soil properties where the crop was established.

Parameter	Unit	Value
pH		8.14
Electrical conductivity	dS m^{-1}	0.42
Texture		Clayey
Sand	%	20.4
Clay	%	48.6
Nitrate	mg kg^{-1}	13.4
Ammonium	mg kg^{-1}	12.4

Table 2. N dose and percentage applied in sowing and relief irrigation.

N dose (kg/ha^{-1})	Soeing	1er Irrigation	2o Irrigation	3er Irrigation
	Applied % of N rate			
70	15	85	--	--
190	15	45	40	--
310	15	40	35	10
430	15	40	35	10

detergent fiber (INIFAP, 2004). In the results obtained from the variables analyzed in this study, there were statistically no significant differences in any of the applied treatments; however, there are differences among the means of each treatment, which are explained below. The total dry matter yield varied from 13.72 to 16.52 t ha^{-1}, with the highest DM yield occurring in treatment four, showing very little difference compared to the other treatments depending on the fertilizer dose applied (Table 3).

In terms of dry matter yield by organs, we observed that the highest yield occurred in the ears, while the lowest was in the husks across all treatments. Overall, the DM yield is low, as other research conducted in the same region has reported yields ranging from 19.145 to 22.368 t ha^{-1} (Reta *et al.*, 2000). Our low yield may be attributed to the fact that planting was not done within the recommended spring date range for the Lagunera Region, which is from March 20 to April 30, as most hybrids decrease their forage production when planted late (Núñez *et al.*, 2006). Our planting date was 19 days late outside the mentioned range. In treatment three, the lowest DM yield (13.72 t ha^{-1}) was obtained, despite the nitrogen application dose being higher than that of treatments one and two. The percentage distribution of DM by organs was highest in the ears across all fertilization treatments applied (Table 4).

Regarding the total nitrogen extraction, there were no statistically significant differences in any of the treatments, nor in the amount of nitrogen extracted from each of the plant organs (Table 5).

N extractions of 7.2, 9.07, 8.25, and 9.4 kg N/ha per ton of DM were obtained in treatments 1, 2, 3, and 4, respectively. The highest N extraction occurred with the application of 430 kg N ha^{-1}, but it is still very low compared to the average N extraction that the plant should have. In treatment 3, lower N extraction was observed than in treatment 2, despite the fact that the N application rate was much higher, which suggests

Table 3. Dry matter yield obtained with the different treatments.

Dose (kg ha^{-1}) N	Dry matter yield (t ha^{-1})				
	Stem	Leaf	Cob	Bract	Total
70	4.36	2.67	5.79	1.04	13.86
190	3.88	3.07	6.14	1.37	14.46
310	4.81	2.41	5.23	1.27	13.72
430	5.12	2.79	7.26	1.35	16.52

All differences are non-significant according to the analysis of variance, Duncan and Tukey test, 0.05.

Table 4. Dry matter distribution percentage.

Dose (kg ha^{-1}) N	dry matter distribution (%)			
	Stem	Leaf	Cob	Bract
70	31	19	42	8
190	27	21	42	10
310	35	18	38	9
430	31	17	44	8

Table 5. Nitrogen extraction.

Dose (kg ha^{-1}) (N)	Nitrogen extraction (kg ha^{-1})				
	Stem	**Leaf**	**Cob**	**Bract**	**Total**
70	15.03	17.67	63.08	4.01	99.79
190	12.33	34.41	78.43	6.04	131.21
310	17.06	26.75	64.41	5.00	113.22
430	20.89	36.75	92.26	5.42	155.32

All differences are non-significant according to the analysis of variance, Duncan and Tukey test, 0.05.

a significant loss of N. These low N extraction may be due to the residual N content in the soil; in the 0-30 cm extract alone, there are 25.8 mg kg^{-1} of inorganic N, which is approximately equivalent to 100 kg ha^{-1}. The amount of N taken up by the plant organs was higher in the ear in all treatments, and lower in the husks.

CONCLUSIONS

The dry matter yield in forage maize was not significantly affected by the applied N doses, and the yield was very low, as in the Lagunera region, a good potential yield should exceed 18 tons per hectare of dry matter. The dry matter percentage in the ear did not reach the 45% that the crop should have. The fertilizer doses had no statistically significant influence on the distribution of dry matter in the plant organs. Similarly, there were no statistically significant differences in nitrogen extraction across any of the treatments analyzed. Average extractions of 7.2, 9.07, 8.25, and 9.4 kg N/ha per ton of dry matter were obtained in treatments 1, 2, 3, and 4, respectively. These values are very low compared to the average nitrogen extraction that forage maize should achieve.

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