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Control of *Macrophomina pseudophaseolina* Crous, Sarr & Ndiaye with *Trichoderma* spp., and botanical and chemical pesticides

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ABSTRACT

Objective: The objective of the research was to evaluate in *in vitro*, greenhouse and field conditions, the effectiveness of biological, botanical and chemical pesticides for the control of *Macrophomina pseudophaseolina*.

Design/methodology/approach: *In vitro*, greenhouse and field experiments were carried out to evaluate the control effect of different pesticides for the control of *M. pseudophaseolina*.

Results: It was determined that all the evaluated strains of *Trichoderma* spp. they had a fungistatic effect against *M. pseudophaseolina*, and *T. reesei* showed the greatest antagonism and antibiosis against *M. pseudophaseolina*. High, medium and low doses of NeemAcar® and high and medium doses of Regalia® Maxx inhibited 100% the growth of *M. pseudophaseolina* mycelium. In the greenhouse, the lowest percentage of severity was obtained in the treatment with Regalia® Maxx + *T. reesei*. In the field, the lowest severity was determined with the application of NeemAcar® CE + Headline®.

Limitations on study/implications: Our results are essential for the management of this disease by producers.

Findings/conclusions: The implementation of the use of *Trichoderma* spp., botanical pesticides and chemical insecticides is recommended for the control of *M. pseudophaseolina*.

Keywords: Effectiveness, pesticide, chili crop, pathogen.

INTRODUCTION

The chili pepper crop (*Capsicum* spp.) is produced in extensive surfaces of the world and it is an essential condiment in Mexican culinary art (Olguín and Rojas, 2018). Production of the crop is affected by the attack of fungi in the soil, including *Macrophomina phaseolina* (Tassi) Goid. (Botryosphaeriaceae), which provoke charcoal rot of the root and neck and cause

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significant economic losses in this crop and in more than 500 species of plants (Verma *et al.*, 2007; Kaur *et al.*, 2012).

The control of *M. phaseolina* and other soil fungi is based on the use of chemical fungicides, although the intensive use of pesticides causes collateral damage including contamination of ecosystems and foods, harm to human health, and the appearance of strains that are resistant to the products (Gisi and Sierotzki, 2008); therefore, integrated strategies are required.

Biological control, including the use of *Trichoderma* spp. against soil fungi, has been documented (McLean *et al.*, 2004; Martínez *et al.*, 2013; Diánez *et al.*, 2016). In addition to biological control, another sustainable alternative to the application of chemical fungicides is the use of botanical pesticides with fungitoxic activity provoked by the action of terpenes, phenols, alkaloids, tannins, flavonoids, phytoalexins, essential oils, and other secondary metabolites produced by the plants (Martínez, 2012).

Under *in vitro* conditions, there are studies of *Trichoderma* against various pathogens; for example, Michel-Aceves *et al.* (2009) reported that the native a strain Thzn-2 of the species *Trichoderma harzianum* has the potential of biocontrol by inhibition and class 2 antagonism on *Fusarium subglutinans* and *Fusarium oxysporum*. Other authors such as Ruiz-Cisneros (2018) reported that the species of *Trichoderma* that they used presented a positive effect on tomato plants (*Solanum lycopersicum*) by improving the variables of height, biomass, chlorophyll, yield and fruit quality under greenhouse conditions. Likewise, Castro-del Ángel *et al.* (2021) mentioned that *Trichoderma* spp. reduced the impact of *Fusarium verticillioides* in corn genotypes (*Zea mays*) in the state of Veracruz; and Shawki *et al.* (2020) reported the use of potassium silicate, niacin, and antagonists *T. harzianum*, *T. hamatum* and *Bacillus subtili* as alternatives to chemical control of *F. verticillioides*. The hypothesis of this study was that the treatments with the combinations of fungicides + plant extracts and plant extracts + *Trichoderma* spp. presented greater effectiveness for the management of the pathogen in the chili pepper crop. Because of this, the effectiveness of biological, botanical and chemical pesticides for the control of *M. pseudophaseolina* was evaluated under *in vitro*, greenhouse and field conditions.

MATERIALS AND METHODS

The strain of *M. pseudophaseolina* used was supplied by Colegio Superior Agropecuario from the state of Guerrero (CSAEGro-chADMF, accession KX757770.1), which was isolated from plant roots collected in *Capsicum annuum* L. (Solanaceae) chili pepper crop. On the other hand, the following native strains were used: 1) *Trichoderma asperellum* strain CSAEGro-Tas1-(KP639195.1), 2) *T. asperellum* strain CSAEGro-Tas2-(KP639196.1), and 3) *T. asperellum* strain CSAEGro-Tas3. In addition, the commercial strains: 4) *T. virens* strain G-41 (TvG-41) (PHC-Rootmate); 5) *Trichoderma* sp., strain TspF (Fithan®), and 6) *T. reesei* strain TrB (Bactiva®); which were obtained from the mycological collection of the Phytopathology Laboratory of the CEP-CSAEGro (Figure 1).

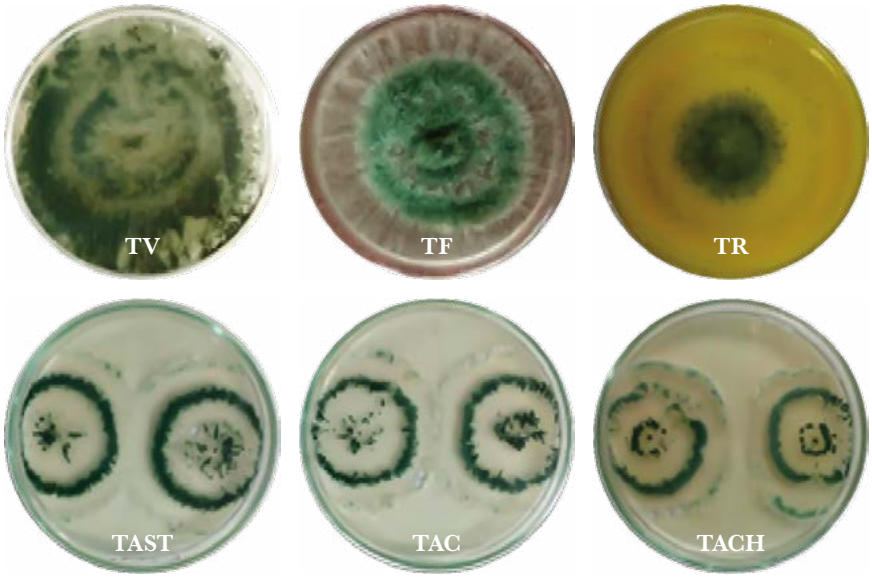

Figure 1. *Trichoderma* strains under study. TV = *T. virens* (PHC-ROOTMATE), TF = *Trichoderma* sp. (FITHAN), TR = *T. reesei* (BAC-TIVA), TAST = *T. asperellum* (native strain Santa Teresa, Guerrero), TAC = *T. asperellum* (native strain Cocula, Guerrero), TACH = *T. asperellum* (native strain from Chilapa, Guerrero).

In vitro biological control of *M. pseudophaseolina*

For this variable, two bioassays were conducted, the first was with the dual culture technique, where the treatments were: 1) Control, 2) Tas1, 3) Tas2, 4) Tas3, 5) G-41, 6) TspF and 7) TrB; first, 20 mL of PDA were emptied into the Petri dishes, then they were allowed to solidify, and a three day old 5 mm disc of *Trichoderma* spp. was placed on the corner of the dish, and in contrast a disc of *M. pseudophaseolina* was placed, giving a total of five replicas per treatment which were incubated at room temperature (±28 °C) in the laboratory; data of the variables were taken every 24 h (Larralde *et al.*, 2008).

The second bioassay was carried out with the cellophane membrane technique (Patil *et al.*, 2014), cutting discs or circles of 8.5 cm of diameter of sweet cellophane paper (of the same diameter as the Petri dish), which were wrapped (without folding) in recycled paper and placed in a poly paper bag and sterilized in the autoclave at 15 lbf in^{2-1} for one hour. Later, a circle was placed on the PDA surface in the Petri dish and a 5 mm disc of PDA + *Trichoderma* (5 days of age) was sown in the center of cellophane paper, incubated for 48 h, and then the cellophane paper was removed together with the *Trichoderma* spp. colony, leaving the secondary metabolites produced by the antagonist fungus spread on the PDA; this, with the purpose of testing its effect on the pathogenic fungus, giving a total of five replicas per treatment.

In vitro effectiveness of botanical and chemical pesticides against *M. pseudophaseolina*

Botanical (Table 1) and chemical (Table 2) pesticides were used against *M. pseudophaseolina* with the infected culture medium technique (Kumar and Mane, 2017), in a completely random experimental design with five repetitions. The experimental

unit was the Petri dish with 20 mL of PDA + botanical or chemical pesticide, according to treatment (Tables 1 and 2). A 6-day-old disc (Ø=5 mm) with *M. pseudophaseolina* was sown in the center of the dish; it was incubated at 28±2 °C in light/dark and the diameter of the colony of *M. pseudophaseolina* was measured every 24 h for 72 h.

Variables evaluated

In bioassay one, the percentage of inhibition was measured every 24 h for 72 h with the Barari and Foroutan (2016) equation, where the inhibition percentage=[(a−b)/a]×100 (a=mycelium growth of the pathogen and b=mycelium growth of the pathogen in the presence of *Trichoderma* spp.).

In bioassay two, the diameter of the *M. pseudophaseolina* colony was measured every 24 h for 72 h and the percentage of inhibition of mycelium growth of the pathogen

Table 1. Doses of plant extracts evaluated *in vitro* against *M. pseudophaseolina*.

Product	Doses used in 20 mL of PDA (mg)	
[1]Neemix	High	14
	Mean	10
	Low	5
[2]Progranic® NeemAcar® CE	High	154 of *A. indica* + 42 of *C. zeylanicum*
	Mean	103 of *A. indica* + 28 of *C. zeylanicum*
	Low	51 of *A. indica* + 14 of *C. zeylanicum*
[3]Liquid Allium®	High	269
	Mean	179
	Low	90
[4]Capsi Oil	High	90 of *Cinnamomum* spp. + 60 of *P. nigrum*
	Mean	60 of *Cinnamomum* spp. + 40 of *P. nigrum*
	Low	30 of *Cinnamomum* spp. + 20 of *P. nigrum*
[5]Lipp Oil	High	120 of *L. graveolens* y *L. beriandieri* + 120 of *C. cassia* y *C. zeylanicum*
	Mean	80 of *L. graveolens* y *L. beriandieri* + 80 of *C. cassia* y *C. zeylanicum*
	Low	40 of *L. graveolens* y *L. beriandieri* + 40 of *C. cassia* y *C. zeylanicum*
[6]Cinn Oil	High	150 of *C. cassia* y *C. zeylanicum* + 45 of *L. graveolens* y *L. beriandieri* + 45 de *Allium* spp.
	Mean	100 of *C. cassia* y *C. zeylanicum* + 30 of *L. graveolens* y *L. beriandieri* + 20 de *Allium* spp.
	Low	50 of *C. cassia* y *C. zeylanicum* + 15 of *L. graveolens* y *L. beriandieri* + 10 of *Allium* spp.
[7]Alli Oil	High	270
	Mean	180
	Low	90
[8]Asphix®90	High	243.6
	Mean	162.4
	Low	81.2
[9]Regalia® (*Reynoutria sachalinensis*)	High	69
	Mean	46
	Low	23

Table 2. Doses of chemical fungicides evaluated *in vitro* against *M. pseudophaseolina*.

N°	Product*	Doses**	Doses per ha^{-1} (kg)	Doses (i.a.) used in 20 mL of PDA (mg)
1	Cercobin®-M (tiofanato de metilo)	High	0.75	10.43
2		Mean	0.62	8.61
3		Low	0.5	6.86
4	Rovral® 50 PH (iprodiona)	High	2.0	20
5		Mean	1.33	13.5
6		Low	1.0	10
7	Swich 62.5 WG (cyprodinil+fludioxonil)	High	1.2	9 of cyprodinil + 6 of fludioxonil
8		Mean	1.050	7.9 of cyprodinil + 5.3 of fludioxonil
9		Low	0.9 kg	6.8 of cyprodinil + 4.5 of fludioxonil
10	Pentaclor 600F (Quintozeno)	High	15 L	180
11		Mean	13.5 L	162
12		Low	12.0 L	144
13	Headline® (Piraclostrobin)	High	3.0 L	15
14		Mean	2.0 L	10
15		Low	1.0 L	5
16	Promyl® (Benomilo)	High	0.5 kg	5
17		Mean	0.45 kg	4.5
18		Low	0.4 kg	4
19	Sportak 45 CE (procloraz)	High	1.5 L	13.5
20		Mean	1.25 L	11.25
21		Low	1.0 L	9
22	Control	-	-	-

***** The dose was calculated considering a consumption of 1,000 L of water ha^{-1}.
****** The mean dose is that recommended by the product manufacturer (DEAQ , 2015).

colony was calculated with the equation by Patil *et al.* (2014), where the percentage of inhibition=[(D1−D2)/D1]×100 (D1=diameter of the fungus colony growing in dishes with PDA (control), D2=diameter of the fungus colony of the pathogen growing on PDA.

Control of *M. pseudophaseolina* in greenhouse

Only seven outstanding treatments were evaluated in the greenhouse and the field, which were distributed into divided plots, with five repetitions. The experimental unit was a black polyethylene pot of 13×18 cm with 1 kg of sterilized substrate (sand+mountain soil+vermicompost 1:1:0.25 v/v) and a Creole chili pepper plant (*Capsicum annuum*). The inoculum of *M. pseudophaseolina* was multiplied in sterilized seed of *Sorghum bicolor* L. (Poaceae). Likewise, inoculum from the strains of *Trichoderma* spp. was reproduced with ground corn. Ten days after the transplant, 13 g of infested sorghum seeds were inoculated with *M. pseudophaseolina* with 4.16×10^8 spores per mL in plants with 35 d of age and then the treatments were inoculated, where *Trichoderma* spp. at 2.3×10^8 spores per mL and the different botanical and chemical pesticides were used: a) Control, b) Regalia® Maxx (0.56 mL planta^{-1})+Swich® 62.5 (0.1 g), c) Regalia® Maxx (0.56 mL)+Headline® CE (0.11

mL), d) Regalia® Maxx (0.2 mL)+*T. reesei*, e) NeemAcar® CE (0.56 mL)+Swich® 62.5 (0.1 g), f) NeemAcar® CE (0.56 mL)+Headline® CE (0.11 mL), g) NeemAcar® CE (0.56 mL)+Cercobin®-M. (0.05 g).

Control of *M. pseudophaseolina* in the field

The treatments that were used in greenhouse conditions were evaluated in the field, which were distributed in divided plots. The experimental unit was three furrows, each with 5 m length and at 1.6 m of separation, with four plants of Creole chili pepper plants, at 0.7 m of distance between these (13.44 m^2). Plant transplanting was carried out at 24 d of age; 11 g of infected sorghum seeds were inoculated with *M. pseudophaseolina* at 4.5×10^5 spores per mL^{-1} in the plant neck of 79 days of age (55 days after transplanting); likewise, the application of the treatments was made with water expenditure of 112 mL per plant.

Evaluation of variables in greenhouse and field conditions

The severity of the disease was measured by using the ordinal scale by Vakalounakis and Fragkiadakis (1999) (0=healthy plant, 1=chlorotic plant, 2=withered plant and 3=dead plant; the data were transformed into percentages of severity); and the data obtained were transformed with the Van der Plank (1965) formula, where $S=\left[\sum_i/N(VM)\right]\times100$ (S=percentage of severity, $\sum_i$= sum of values observed, N=number of sick plants sampled, VM=maximum value of the scale.

Statistical analysis

The data obtained *in vitro*, in the greenhouse and in the field were analyzed separately through ANOVA and Tukey's multiple range test (α=0.05) (Steel and Torrie, 1998) with the software SAS 9.4.

RESULTS AND DISCUSSION

Biological *in vitro* control of *M. pseudophaseolina*

On average, the strains Tas1, Tas2 and TrB took 2 d to make contact with the *M. pseudophaseolina* hyphae; and TvG-41, Tas3 and TspF, 2.2, 2.4 and 2.4 days, respectively. In the competition over space and nutrients, it was determined that the strains TrB and TspF inhibited the growth of the *M. pseudophaseolina* mycelium (Figure 2A) by 61.75 and 55.5%. These values are close to 54.6% produced from the effect of *T. harzianum* against *M. phaseolina* (Singh *et al.*, 2008), and different from the values of 75.55, 72.22, 68.88 and 48.88% of inhibition, caused by *T. harzianum*, *T. reesei*, *T. hamatum* and *T. pseudokoningii*, respectively (Karthikeyan *et al.*, 2015). Likewise, it was determined that the extra-cellular metabolites of all the strains of *Trichoderma* spp. had statistically similar fungistatic action against *M. pseudophaseolina* (Figure 2B).

It is considered that the less time that is required by the strains of *Trichoderma* spp. to make contact with the pathogen, the antagonist will have better chances to compete for space, nutrition primarily by microparasitism, and the production of secondary metabolites

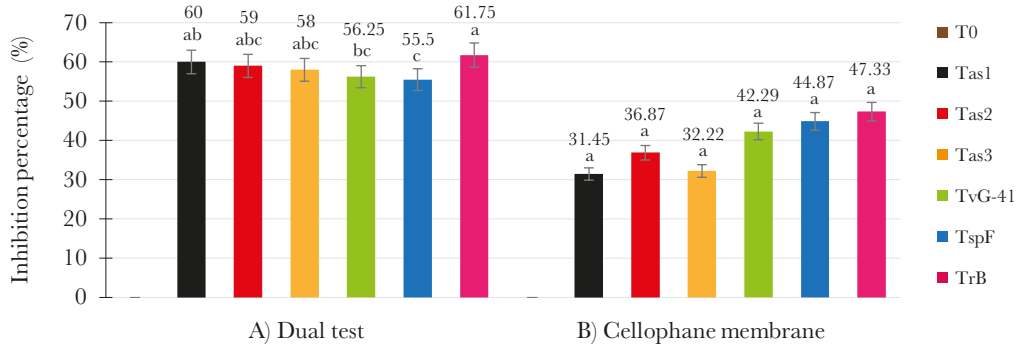

Figure 2. Percentage of inhibition of *M. pseudophaseolina* colonies at 72 h in the dual cultures and cellophane membrane bioassay. T0=Control, Tas1=*T. asperellum*, Tas2=*T. asperellum*, Tas3=*T. asperellum* native to Santa Teresa, Guerrero, Mexico, TvG-41=*T. virens* strain G-41 (PHC®-ROOTMATE®), TspF=*Trichoderma* sp. (FITHANMR), and TrB=*T. reesei* (BACTIVAMR). Letters that are equal are not statistically significant (Tukey α=0.05).

and high production of degrading enzymes such as chitinase, protease and lignase, which degrade the cell wall of the phytopathogenic fungus and use the cytoplasmic content for the nutrition of *Trichoderma* (De Marco *et al.*, 2004; Harman, 2006; Gonzalez *et al.*, 2012; Hernández-Melchor *et al.*, 2019).

The products Prograniс® NeemAcar CE (low, medium and high doses) and Regalia® Maxx (high and medium doses) obtained the highest effectiveness, because they exert fungicide action on the causal agent of charcoal rot, with 100% of pathogen inhibition (Table 3).

Effect of botanical and chemical pesticides on *M. pseudophaseolina*

It was found that the treatments with organic and chemical products caused highly significant differences in the percentage of inhibition of the *M. pseudophaseolina* colonies (P<0.0001) (Figure 3). A fungicide effect against *M. pseudophaseolina* was obtained with the applications of high and medium doses of Regalia® and with all the doses of NeemAcar® CE. All the other treatments presented fungistatic effect.

The low dose of *R. sachalinensis* decreased the growth of *M. pseudophaseolina* by 87.98%. The effectiveness of the Prograniс, Alli Oil, Capsi-Oil, Cinn Oil, and Asphix® extracts decreased when reducing the doses of the products; however, it was found that the low dose of Lipp-Oil was more effective than the higher dose, perhaps due to the interaction in high concentrations of all the components of this product (*L. graveolens* + *L. berlandieri* + *C. cassia* + *C. zeylanicum*), which could stimulate the mycelium growth of *M. pseudophaseolina*. When it comes to botanical pesticides, their inhibitory effect is because the secondary metabolites of the plants with which they are manufactured, such as phenols, terpenoids, alkaloids, carboxyl acids, and fatty acids, show insecticide properties (Avalos and Perez, 2009).

In this regard, Tandel *et al.* (2010) mentioned that with the extract of *A. cepa*, the effectiveness was 98.14%; Javaid and Asma (2011) also reported that the extracts of *Syzygium cumini* (L.) Skeels, *Eucalyptus citriodora* Roxb., *A. indica* L. and *Melia azederach* L., reduced

Table 3. Biological effectiveness of organic extracts on the growth of *M. pseudophaseolina* at 72 h.

Product	Dose	Mean (%)
Neemix 4.5% CE	High	12.35
	Mean	8.35
	Low	0
Prograniс® NeemAcar CE	High	100
	Mean	100
	Low	100
Liquid Allium®	High	30.68
	Mean	22.33
	Low	23.68
Capsioil	High	77.33
	Mean	66.68
	Low	61.35
Lippoil	High	60.33
	Mean	69.65
	Low	89.33
Cinnoil	High	51.33
	Mean	49.33
	Low	41.33
Allioil	High	18.98
	Mean	0
	Low	0
Asphix® 90	High	27
	Mean	4
	Low	0
Regalia Maxx®	High	100
	Mean	100
	Low	87.98
Control	--	--

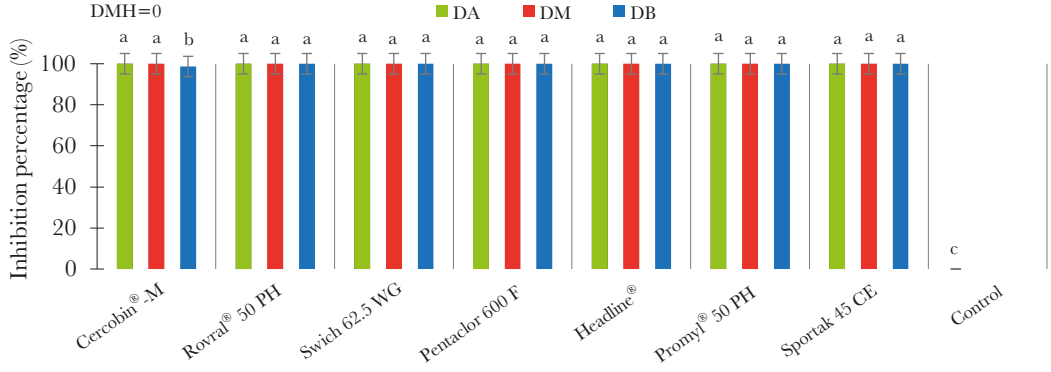

Figure 3. Percentage of inhibition of *M. pseudophaseolina* colonies due to the effect of chemical fungicides. Columns with the same literal are not statistically different. DMH=Tukey's minimum honest difference, α=0.05. DA=High dose, DM=Medium dose and DB=Low dose.

the biomass of *M. phaseolina*, while the extracts in acetate and chloroform of *A. indica* inhibited, the growth of *M. phaseolina* by 81 and 90%, respectively.

Muzammil *et al.* (2014) and Meena *et al.* (2014) reported that the extract of *A. sativum* reduced by 100% the growth of *M. phaseolina* in sunflower. Savaliya *et al.* (2015) also found that the extracts of *A. sativum*, *A. cepa* and *Zingiber officinale* Rosc., inhibited the growth of *M. phaseolina* by 77.65, 63.98 and 32.34%, respectively. In addition, they reported that the extracts of *A. sativum* and *A. cepa* suppressed the formation of microsclerotia of this phytopathogen. Baldiga *et al.* (2013) reported that carbendazim, iprodione and penflufen + fluoxastrobin applied at 40 mg L^{-1} had effectiveness of 5.6, 7.2 and 12.5%, respectively, against *M. phaseolina*. Reznikov *et al.* (2016) reported that pyraclostrobin + thiophanate-methyl increased the percentage of root development of soy seeds infected with *M. phaseolina*.

Control of *M. pseudophaseolina* in greenhouse conditions

Significant differences were found between treatments only in samples two and three. From the first to the last evaluation, it was found that the Regalia® Maxx + Swich 62.5 WG treatment stood out, because it presented the lowest averages of severity of charcoal rot. In evaluation three it was determined that in the plants treated with the mixture of these two products, there was 94.44% less severity than in the treatments with NeemAcar® + Cercobin®-M and Regalia® Maxx. Likewise, there was no difference in the application method, although the severity was slightly lower in the treatments applied preventively; that is, the time of application did not influence the severity of the disease in the chili pepper plants (Table 4).

Table 4. Comparison of the severity of *Macrophomina pseudophaseolina* on three evaluation dates under greenhouse conditions.

Treatments	Days after inoculation		
	7	14	21
T0	44.45a†	94.45a	100a
N1	5.56a	5.56b	5.56bc
N2	27.78a	33.33ab	27.78bc
N3	27.78a	5.56b	0c
N4	16.67a	50ab	50abc
N5	16.67a	66.67ab	66.67ab
N6	27.78a	72.22ab	100a
DMH	48.154	69.313	64.205
Valor of P	0.2758	0.0020	<0.0001

DT0=Control, N1=Regalia® Maxx + Swich 62.5 WG, N2=Regalia® Maxx + Headline, N3=Regalia® Maxx + *T. reesei*, N4=NeemAcar CE + Swich 62.5 WG, N5=Neemacar CE + Headline CE, N6=NeemAcar CE + Cercobin®-M. † Values with the same letters in the same column are not statistically different. DMH: Tukey's minimum honest difference α=0.05.

The effect of the application of Regalia® Maxx may be because the components of this extract can stimulate the synthesis of phytoalexins and C-glycosdsil (cucumarin) which accumulate in the penetration site of the fungus, in addition to promoting the lignification of the cell wall and an increase in the activity of the enzymes chitinases, peroxydases and β-1,3 glucanasas, which affect the colonization and survival of the pathogen (Fofana *et al*., 2005) Similarly, Margaritopoulou *et al.* (2020) reported that the extract of *Reynoutria sachalinensis* causes defenses in the plants, especially as consequence of the induction of the salicylic acid path. Su *et al.* (2012) mention that the extract of *R. sachalinensis* can induce resistance in the plants through induction of phytoalexins and production of phenolic compounds, an increase in the production of proteins related to the defense, and accumulation of species that are reactive to oxygen, lignification, and formation of papillas on the cell walls.

The favorable effect obtained with the *Trichoderma* strains is because this bio controlling fungus presents fast growth, broad ecological plasticity (Harman, 2006), and mechanisms of direct action such as competition over the substrate, antibiosis, and microparasitism against *M. phaseolina* and other phytopathogens that live in the soil (Khaledi and Taheri, 2016). In addition to these effects, *Trichoderma* spp. colonize the root zone limiting their growth to the external layers of the root and do not penetrate the vascular bundle, which improves the development of the plants (Poveda, 2020).

Integrated control of *M. pseudophaseolina* in the field

In the statistical analysis, significant differences were detected only in samples two and three. It was found that the treatments with NeemAcar® CE + Headline CE and NeemAcar® + Cercobin®-M presented the lowest averages (32.9 and 33.3%) of severity, respectively, making them the most effective treatments to counteract charcoal rot (Table 5).

NeemAcar® CE is formulated from *A. indica* and *C. zeylanicum* and presently it is known that the first has several bioactive compounds (Atawodi and Atawodi, 2009); meanwhile, Lu *et al.* (2011) mention that cinnamon oil (*C. zeylanicum*) shows synergistic effects when

Table 5. Comparison of the percentage of severity of *Macrophomina pseudophaseolina* on the three evaluation dates.

Treatments	Days after inoculation		
	7	14	21
Control	42.1 a*	63.4 a	84.7 a
Regalia® Maxx + Swich 62.5 WG	40.7 a	41.2 ab	37.5 b
Regalia® Maxx + Headline CE	29.0 a	44.0 ab	40.7 b
Regalia® Maxx + *T. reesei*	31.5 a	25.9 b	39.8 b
NeemAcar® CE + Swich 62.5 WG	43.5 a	39.8 ab	49.5 b
NeemAcar® CE + Headline CE	33.8 a	33.3 ab	32.9 b
NeemAcar® + Cercobin®-M	21.2 a	25.9 b	33.3 b

*Values with the same letters in the same column are not statistically different (Tukey, α=0.05).

it is combined with other oils of plant origin to control both "gram positive" and "gram negative" bacteria, which explains why the use of NeemAcar® was superior.

The indiscriminate use of pesticides has reduced and damaged the agricultural activity of the country (Zepeda-Jazo, 2018), not just in chili pepper cultivation, but in every crop. Ortiz *et al.* (2014) suggest that despite the regulations and restrictions of pesticide use, they can represent a serious problem not only in the soils and waters in Mexico, but also for the health of workers and populations exposed. The appearance of resistance or multiple resistances from pests, diseases and weeds to different active ingredients must be mentioned. This study's results will be very important for the control of this disease by producers of chili pepper, because it includes strategies of integrated management, not only in the use of chemical control; however, some of the implications for the future are to take into account the cost/benefit and to compare these management strategies with conventional management, as well as the elaboration of these types of extracts to try to reduce the contamination from chemical pesticides and the production costs of this crop for producers.

CONCLUSIONS

The research determined that all the strains evaluated of *Trichoderma* spp. had a fungistatic effect against *M. pseudophaseolina*. The high, medium and low dose of NeemAcar® and high and medium dose of Regalia® Maxx inhibited the growth of the mycelium of *M. pseudophaseolina* by 100%. The lowest percentage of severity was obtained under greenhouse conditions, in the treatment with Regalia® Maxx + *T. reesei*. The lowest severity was determined in the field with the application of NeemAcar® CE + Headline®.

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