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.

Citation: Payán-Arzapalo, M. A., López-Avendaño, J. E., Cázarez-Flores, L. Ll., López-Inzunza, H. de J., Molina-Cárdenas, L., Vega-Gutiérrez, T. A., & Tirado-Ramírez, M. A. (2024). Effectiveness of Chemical Fungicides for the *in vitro* Control of *Fusarium* spp. Causing Basal Rot in Onion in Sinaloa, Mexico. *Agro Productividad*. https://doi.org/10.32854/agrop.v17i11.3115

Academic Editor: Jorge Cadena Iñiguez
Associate Editor: Dra. Lucero del Mar Ruiz Posadas
Guest Editor: Daniel Alejandro Cadena Zamudio

Received: May 14, 2024.
Accepted: September 18, 2024.
Published on-line: December XX, 2024.

Agro Productividad, 17(11) supplement. November. 2024. pp: 39-48.

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

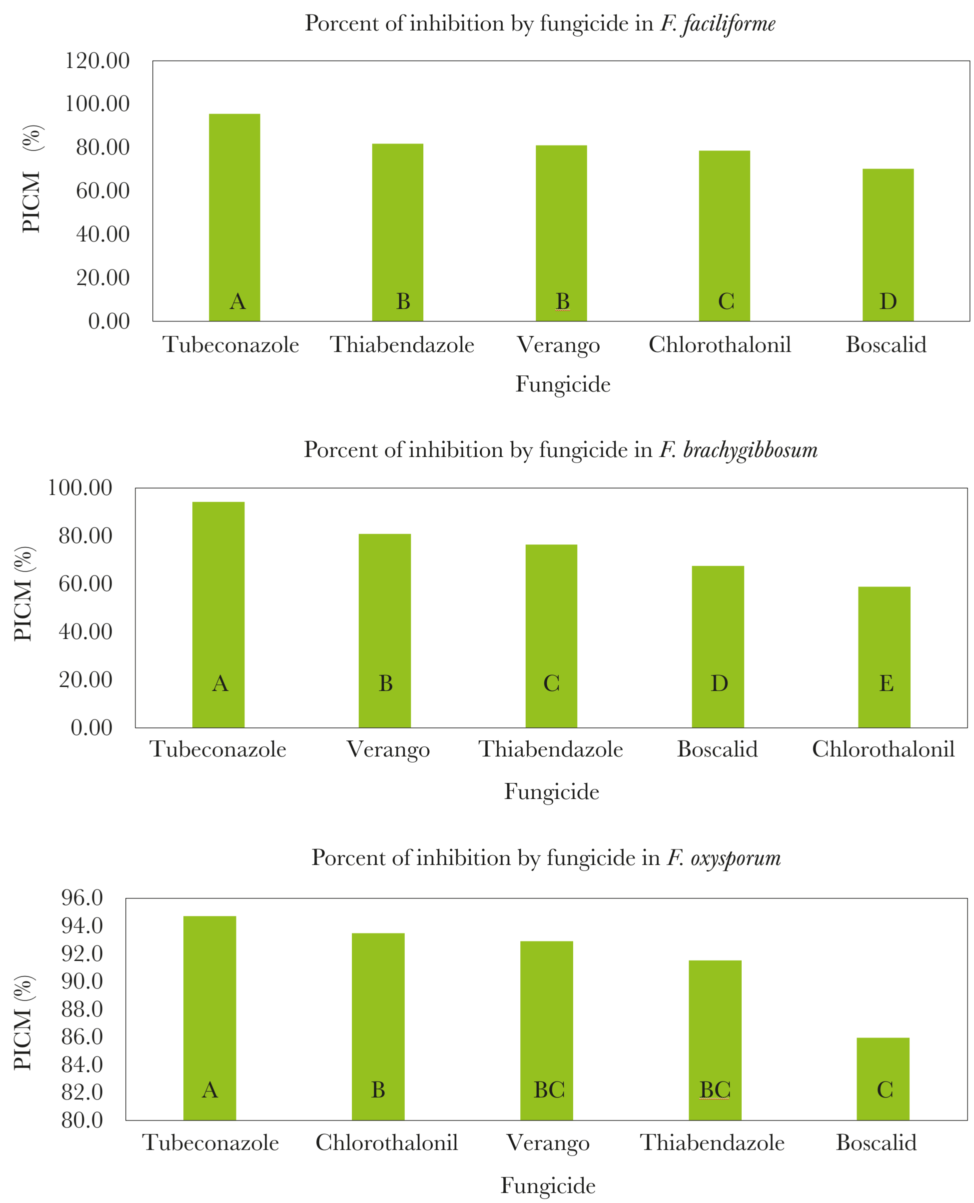

Figure 2. Percentage of Mycelial Growth Inhibition (MGIP) by fungicide in *F. falciforme*, *F. brachygibbosum*, and *F. oxysporum*. MGIP is expressed as a percentage, and the comparison of means was performed using Tukey's test with $p \leq 0.05$.

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).

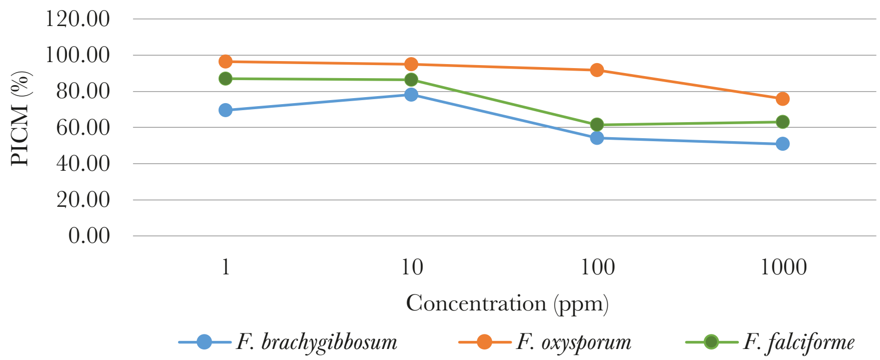

Figure 3. Percentage of mycelial growth inhibition (MGIP) by concentration in parts per million of *F. falciforme*, *F. brachygibbosum*, and *F. oxysporum*.

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**					
Fungicide - Concentration	***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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