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Evaluation of the inhibitory activity of biofungicides, chemical inducers and plant extracts against conidial germination of *Erysiphe heraclei* DC, the causal organism of carrot powdery mildew

Abdel-Radi T. Bakeer [a], Hamada F. A. Ahmed [b, *], Hoda M. H. Ahmed [a], Mohamed R. A. Agamy [a]

[a] *Department of Plant Pathology, Faculty of Agriculture, Fayoum University, Fayoum P.O. Box 63514, Egypt*
[b] *Department of Ornamental, Medicinal and Aromatic Plant Diseases, Plant Pathology Research Institute, Agricultural Research Center, Giza P.O. Box 12619, Egypt*

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ABSTRACT

Powdery mildew of carrot, caused by *Erysiphe heraclei* DC, is one of the most important fungal diseases affecting the vegetative parts of the plant, leading severe damage to the resulting root crop. This study was designed to investigate the inhibitory activity of selected fungicide alternatives, i.e. biofungicides, chemical inducers, and plant extracts against *E. heraclei* conidial germination under *in vitro* conditions. The results showed that all biofungicides significantly inhibited conidial germination at all tested concentrations, especially at 2000 ppm compared to untreated spores. Bio-Mix (*Bacillus subtilis + Trichoderma harzianum*) was the most effective, followed by Bio-F (*B. subtilis*), while Trix-F (*T. harzianum*) was the least effective. The corresponding mean inhibition values were 74.1, 61.3, and 52.6%, respectively. Among the chemical inducers, salicylic acid at a concentration of 2000 ppm showed the highest inhibitory activity on spore germination (57.6%), followed by potassium silicate (44.8%), while citric acid showed the least activity (29.2%). As for plant extracts, the aqueous extract of germinated barley grains had the best effect (53.5%), followed by the germinated wheat grain extract (43.6%), lemongrass leaf extract (36.6%), and basil leaf extract (24.3%), while the germinated corn grain extract had the least effect (16.8%). The 40% concentration was more effective in inhibiting germination than the other concentrations. Complete inhibition of *E. heraclei* conidia germination was recorded using Micronite 80% at all tested rates, followed by Vinger 45% and Carbendazim 50%, recording 100, 88.9, and 85.5%, respectively.

1. Introduction

Powdery mildew of carrot (*Daucus carota* L.), caused by *E. heraclei* DC, is one of the most damaging fungal diseases of the crop and can be a major obstacle to carrot production due to severe yield and quality reduction [1]. The fungus appears as large areas of white mycelium on stems, leaves, and petioles [2]. Infected leaves become chlorotic and later necrotic, causing premature leaf senescence and reduced photosynthetic area, leading to yield loss [3]. *Erysiphe heraclei* is distributed worldwide and can infect many different hosts belonging to the Apiaceae family [4]. Synthetic fungicides have long been used to control powdery mildew diseases [5]. However, their extensive use has led to numerous environmental risks to humans, animals, fish, and even beneficial soil microorganisms, as well as the emergence of fungicide-resistant strains of the pathogen [6]. Consequently, it has become necessary to develop safe and effective alternatives for managing plant diseases [7]. Recently, there has been a trend toward the use of biocontrol agents, antioxidants, and botanicals/ or plant derivatives to suppress plant pathogens.

Biological control using microorganisms or their secretions is defined as suppressing pathogen populations to an acceptable level [8]. Numerous studies have documented various beneficial microorganisms possess antimicrobial properties against several plant pathogens [9]. More recently, several commercial biofungicide formulations based on beneficial bacteria, *i.e. Bacillus* spp., *Streptomyces* spp., and *Pseudomonas* spp., and beneficial fungi, *i.e. Trichoderma* spp., and *Coniothyrium* spp. [10]. Antioxidants are effective in managing plant diseases by enhancing plant resistance indirectly or by directly inhibiting plant pathogens [11]. Several reports have demonstrated the efficacy of potassium silicate against the growth of *Rhizoctonia solani*, *Fusarium solani*, *F. semitectum*, *F. oxysporum*, and *F. equiseti* [12], and against *Podosphaera aphanis* spore germination by 40–60% [13]. In a similar vein, treatment with salicylic acid and ascorbic acid significantly reduced the growth of *Macrophomina phaseolina*, *F. solani*, and *F. oxysporum* [14]. Similarly, the mixture of chitosan, humic acid, and salicylic acid significantly reduced spore germination and growth of *F. solani* and *R. solani* [15].

* Corresponding author.
E-mail address: dr.hamada.faa@gmail.com (H. F. A. Ahmed); Tel.: +201015628367

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Plant extracts are among the many fungicide alternatives that are receiving significant attention due to their non-toxicity, natural availability, and environmental friendliness [16]. Several plant extracts have potential as antimicrobial agents and can be used in the preservation of agricultural products, foods, and pharmaceuticals [17]. They contain numerous phytochemicals, *i.e.* alkaloids, flavonoids, phenols, polyphenols, glycosides, and tannins [18], which exhibit antimicrobial and cytotoxic effects on pathogens [19]. Several plant extracts have been tested for controlling powdery mildew in different plant hosts [20], and their effect on spore germination has also been evaluated [21]. Examples of these plant extracts include lemongrass (*Cymbopogon citratus*) [22] and basil (*Ocimum basilicum*) [23]. The present study aimed to investigate the inhibitory activity of certain biofungicides, chemical inducers, and plant extracts compared with three synthetic fungicides against conidial germination of *E. heraclei in vitro*. The hypothesis suggests that using safe and environmentally friendly materials can improve plant resistance to carrot powdery mildew.

2. Materials and methods

2.1. *In vitro* experiment and treatments description

The present study was conducted at the Plant Pathology Laboratory, Faculty of Agriculture, Fayoum University, to investigate the inhibitory activity of some fungicide alternatives against *E. heraclei* spore germination. The treatments included three biofungicides [Trix-F (*T. harzianum*), Bio-F (*B. subtilis*), and Bio-Mix (*B. subtilis+ T. harzianum*)], three chemical inducers (potassium silicate, salicylic acid, and citric acid), five plant extracts (germinated grains of wheat, barley, and corn; and basil and lemongrass leaves), and three synthetic fungicides (Carbendazim 50%, Micronite 80%, and Vinger 45%). Biofungicides were obtained from the Department of Plant Pathology, Faculty of Agriculture, Fayoum University, and chemical inducers, plant extracts and fungicides were obtained from the Agricultural Research Center, Egypt.

2.2. Preparation of cold watery plant extracts

Plant samples including leaves of lemongrass (*Cymbopogon citratus*) and basil (*Ocimum basilicum*), and grains of wheat (*Triticum aestivum*), maize (*Zea mays*), and barley (*Hordeum vulgare*). For the leaf samples, they were washed thoroughly with tap water, dried in the shade for 5 days, and then finely ground in a grinder. Approximately 100 g of leaf powder was soaked in a sufficient amount of distilled water for 24 h. For the grain samples, they were thoroughly washed with tap water and wrapped in a damp cloth for 36 h to induce germination. Approximately 100 g of germinated grains were added to a sufficient amount of distilled water and thoroughly blended in a blender. The filtrate from the leaves or germinated grains was filtered using filter paper (Whatman No. 1) and centrifuged at 300 rpm for 15 minutes to obtain a pure plant extract. The resulting extract was sterilized by a Millipore filter disc (0.45 µm). The final extract was diluted with sterile distilled water to obtain the concentrations of 10, 20, and 40%.

2.3. Evaluation of the inhibitory activity of selected treatments against conidial germination of *E. heraclei* under *in vitro* conditions

A spore germination test was performed to evaluate the antifungal or inhibitory activity of the treatments against *E. heraclei*. Spores were collected from small sporulation lesions on the infected parts and carefully placed on clean glass slides [24]. About 500, 1000, and 2000 ppm of biofungicides, chemical inducers, and fungicides, along with 10, 20, and 40% plant extracts, were prepared. About 2–3 drops of the prepared concentration were added to the spores, and the slides were then placed on glass rods in Petri dishes (9 cm in diameter) containing a small amount of water to provide the necessary humidity for germination. Spores treated with sterile distilled water served as a control. Three replicates were used for each treatment, and three dishes per replicate. The dishes were incubated for 48 h at 25 ± 1 °C. The germinated spores were counted using a hemocytometer, and the conidia germination percentage (CG%) and inhibition of conidia germination (ICG%) were calculated using the following equations:

$$CG\ \% = [(No.of\ germinated\ conidia\ /\ No.of\ conidia\ examined) \times 100] \quad (1)$$

$$ICG\ \% = [(CG\ in\ control - CG\ in\ treatment\ /\ CG\ in\ control) \times 100] \quad (2)$$

2.4. Statistical analyses

The obtained data were analyzed using analysis of variance (ANOVA) using Web Agriculture Stat Package (WASP 2.0, Central Coastal Agricultural Research Institute, Goa, India). The values shown represent the mean of the recorded measurements. To compare significant differences between treatments, Duncan's range test was applied at $p \leq 0.05$ [25].

3. Results and discussion

3.1. *Inhibitory activity of fungicides against conidial germination of E. heraclei*

Data presented in Table (1) indicate that all fungicides significantly inhibited *E. heraclei* spore germination at all tested concentrations, especially at 2000 ppm compared to untreated spores. Micronite 80% completely inhibited spore germination, followed by Vinger 45% and Carbendazim 50%. The corresponding mean inhibition values were 100, 88.9, and 85.5%, respectively (Figure 1A). Current results are in agreement with those of Bakeer *et al.* [26], who found that complete inhibition of *E. heraclei* conidia germination was achieved using the fungicide score 25% (difenconazole), followed by thiovit-jet 80% (micronized sulfur), which recorded inhibition rates of 100% and 90%, respectively. Similarly, Ahmed *et al*. [27] reported that difenoconazole was the most effective in decreasing spore germination as well as germ tube length of the fungus *E. heraclei* by 97.1 and 92.4%, respectively. In the same vein, Ahmed *et al*. [28] reported that treatment with the fungicide propiconazole showed complete inhibition of *G. cichoracearum* spore germination. The inhibitory activity of chemical fungicides in reducing spore germination can be attributed to direct interference with the pathogen's cell wall synthesis, which increases wall permeability and destroys the plasma membrane, as well as preventing the biosynthesis of ergosterol, resulting in cell wall damage [29, 30].

Table 1: Effect of fungicides on conidial germination of *E. heraclei* after 48 hours of spore incubation at 25 ± 1°C.

Fungicides	Conidial germination %				ICG %
	Concentration applied (ppm)				
	500	1000	2000	Mean	
Micronite 80%	0.0 ± 0.00^{d}	0.0 ± 0.00^{c}	0.0 ± 0.00^{b}	0.0	100
Carbendazim 50%	6.0 ± 0.50^{b}	4.5 ± 0.43^{b}	0.0 ± 0.00^{b}	3.5	85.5
Vinger 45%	4.3 ± 0.43^{c}	3.8 ± 0.26^{b}	0.0 ± 0.00^{b}	2.7	88.9
Control	24.3 ± 2.08^{a}				–

ICG = Inhibition of conidial germination, which is calculated based on the control value. According to Duncan's multiple range test, values in the same column followed by different letters are significantly different (at $p \leq 0.05$). Mean ± standard error ($n = 3$).

3.2. *Inhibitory activity of biofungicides against conidial germination of E. heraclei*

Data presented in Table (2) indicate that all biofungicides significantly inhibited *E. heraclei* conidial germination at all tested concentrations, especially at 2000 ppm compared to untreated spores. Bio-Mix (*B. subtilis* + *T. harzianum*) was the most effective, followed by Bio-F (*B. subtilis*), while Trix-F (*T. harzianum*) was the least effective. The corresponding mean inhibition values were 74.1, 61.3, and 52.6%, respectively (Figure 1B). These results are in agreement with those of Bakeer *et al*. [31], who stated that among four commercial biofungicides tested against *E. heraclei* conidial germination, bio-zeid (*T. album*) was the most effective, followed by clean-root (*B. subtilis*) and bio-arc (*B. megaterium*), while blight-stop (*T. harzianum*) was the least effective. The corresponding inhibition values were 86.4, 81.6, 78.3, and 46.2%, respectively. Likewise, Bakeer *et al*. [26] reported that the biofungicide biobac 50% (*B. subtilis*) was the most effective in inhibiting *E. heraclei* conidial germination, followed by bio-arc 6% (*B. megaterium*), while plant-guard (*T. harzianum*) was the least effective, recording 61.9, 49.2, 32.9%, respectively. In addition, Ahmed *et al*. [27] reported that treatment with *B. subtilis*, *B. pumilus*, and *S. marcescens* recorded the highest reduction of *E. heraclei* spore germination, by 88.2, 84.1, and 81.1%, respectively, while *T. album* recorded the lowest reduction (59.6%). In general, the activity of biological control agents in inhibiting spore germination can be attributed to the ability of these bioagents to synthesize a wide range of antibiotics or other inhibitory substances, including cell wall-degrading enzymes, hydrogen cyanide, and siderophores, which degrade spore cell walls or inhibit enzymes necessary for germination [32]. For example, fungi belonging to the genus Trichoderma have great potential as bioagents due to their ability to synthesize several degrading enzymes, such as chitobiosidase, endochitinase, glucan 1,3-β-glucosidase, and N-acetyl-β-glucosaminidase, which strongly inhibit both germination and germ tube elongation of spores [33]. In the same context, it was found that various species of *Bacillus* have proven effective in suppressing many fungal and bacterial pathogens due to their ability to inhibit spore germination, as well as disrupting germ tube growth and mycelium development [34].

Table 2: Effect of biofungicides on conidial germination of *E. heraclei* after 48 hours of spore incubation at 25 ± 1°C.

Biofungicides	Conidial germination %				ICG %
	Concentration applied (ppm)				
	500	1000	2000	Mean	
Bio-F (*B. subtilis*)	13.5 ± 0.43^{b}	7.4 ± 0.79^{c}	7.3 ± 0.26^{b}	9.4	61.3
Trix-F (*T. harzianum*)	15.2 ± 0.75^{b}	11.5 ± 0.86^{b}	7.8 ± 0.30^{b}	11.5	52.6
Bio-Mix (*B. subtilis + T. harzianum*)	10.3 ± 0.26^{c}	5.1 ± 0.45^{c}	3.5 ± 0.43^{c}	6.3	74.1
Control	24.3 ± 2.08^{a}				–

ICG = Inhibition of conidial germination, which is calculated based on the control value. According to Duncan's multiple range test, values in the same column followed by different letters are significantly different (at $p \leq 0.05$). Mean ± standard error ($n = 3$).

3.3. *Inhibitory activity of chemical inducers against conidial germination of E. heraclei*

Data shown in Table (3) indicate that among three chemical inducers tested for their inhibitory activity against *E. heraclei* conidial germination, salicylic acid recorded the highest inhibitory activity (57.6%), followed by potassium silicate (44.8%), while citric acid recorded the least activity (29.2%). The concentration of 2000 ppm showed significant superiority over the tested concentrations (Figure 1C).

Table 3: Effect of chemical inducers on conidial germination of *E. heraclei* after 48 hours of spore incubation at 25 ± 1°C.

Chemical inducers	Conidial germination %				ICG %
	Concentration applied (ppm)				
	500	1000	2000	Mean	
Salicylic acid	14.1 ± 0.87^{d}	9.8 ± 0.43^{c}	7.3 ± 0.26^{c}	10.3	57.6
Citric acid	20.4 ± 0.79^{b}	18.1 ± 2.26^{b}	13.1 ± 1.15^{b}	17.2	29.2
Potassium silicate	17.5 ± 0.43^{c}	12.3 ± 0.96^{c}	10.4 ± 0.60^{b}	13.4	44.8
Control	24.3 ± 2.08^{a}				–

ICG = Inhibition of conidial germination, which is calculated based on the control value. According to Duncan's multiple range test, values in the same column followed by different letters are significantly different (at $p \leq 0.05$). Mean ± standard error ($n = 3$).

Current results are in agreement with those of Kanto *et al*. [13], who stated that potassium silicate was effective against *P. aphanis* spore germination by 40 to 60%. Similarly, Abada and Ahmed [35] reported that treatment with salicylic acid recorded the lowest germination of *Leveillula taurica* conidia (41.6%), followed by chitosan (42.5%). In a similar vein, Bakeer *et al*. [31] reported that treatment with ascorbic acid, followed by potassium silicate recorded the maximum inhibition of *E. heraclei* conidial germination by 76 and 67.9%, respectively. Likewise, Bakeer *et al*. [26] tested the efficacy of three chemical

inducers against *E. heraclei* conidial germination and reported that ascorbic acid was the most effective, followed by sodium bicarbonate, recording 60.5 and 53.8%, respectively. In addition, Barakat *et al.* [36] reported that among three chemical inducers tested in inhibiting *E. cichoracearum* conidial germination, ascorbic acid was the most effective (65.3%), followed by potassium silicate (59.8%). Also, Ahmed *et al*. [37] reported that potassium silicate, followed by salicylic acid, each at 2000 ppm was the most effective in reducing the growth of *F. oxysporum*, *F. solani*, and *M. phaseolina*. The inhibitory activity of chemical inducers against spore germination may be due to the ability of these substances to damage or degrade the spore cell wall as a result of the complete contact between these substances and the spore surface [26].

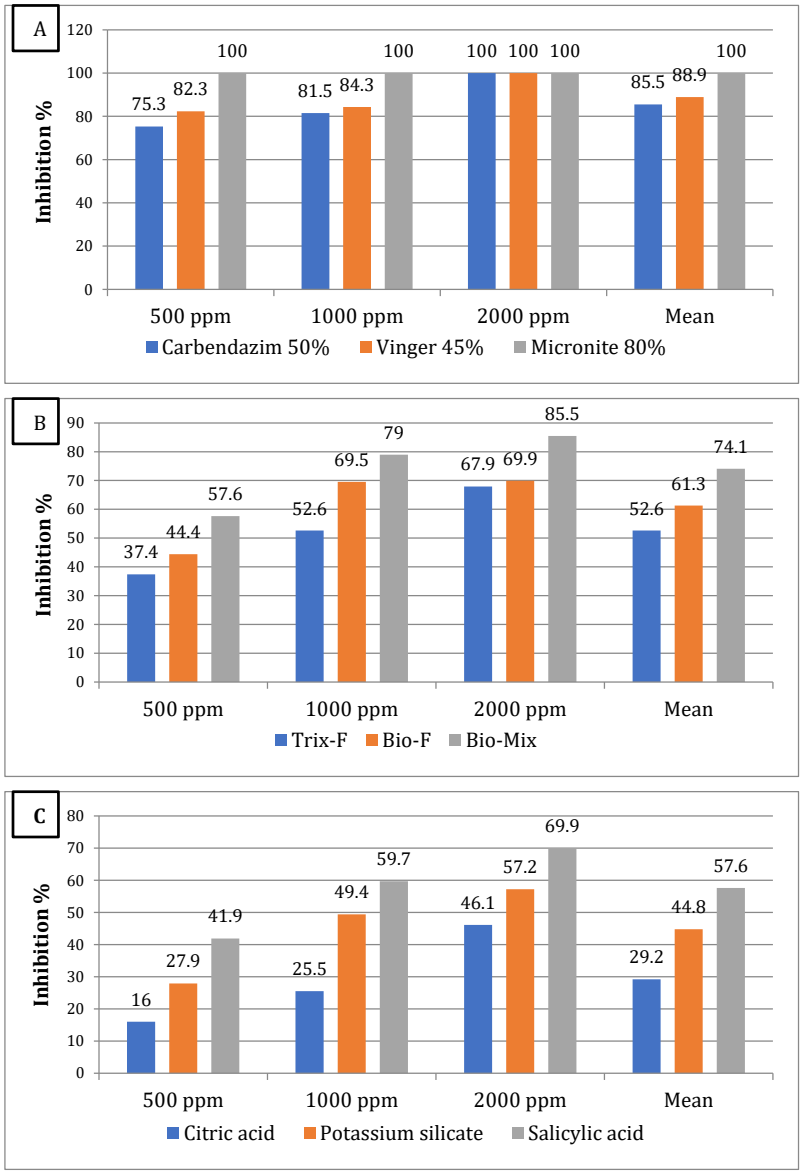

Fig. 1. Inhibitory activity of (A) fungicides, (B) biofungicides, and (C) chemical inducers, each at concentrations of 500, 1000, and 2000 ppm, against conidial germination of *E. heraclei in vitro* conditions.

3.4. *Inhibitory activity of plant extracts against conidial germination of E. heraclei*

Data offered in Table (4) show that among five plant extracts tested for their inhibitory activity against *E. heraclei* conidial germination, the aqueous extract of germinated barley grains had the best effect (53.5%), followed by the germinated wheat grain extract (43.6%), lemongrass leaf extract (36.6%), and basil leaf extract (24.3%), while the germinated corn grain extract had the least effect (16.8%). The 40% concentration was more effective in inhibiting germination than the other concentrations (Figure 2). These results are in agreement with those of Shalaby *et al.* [38], who tested the inhibitory activity of three plant extracts against *E. heraclei* spore germination and reported that garlic and blue gum extracts were the most effective in inhibiting germination, recording 75.8 and 72.6%, respectively. Similarly, Bakeer *et al.* [26] reported that garlic and moringa extracts recorded the highest reduction in *E. heraclei* spore germination, recording 47.9 and 36.8%, respectively. Additionally, Ahmed *et al.* [37] reported that the highest inhibition of the mycelium growth of *F. oxysporum*, *S. sclerotiorum*, *F. solani*, and *R. solani* was recorded by ginger, followed by lemongrass and lantana, each at 20%. From this perspective, plant extracts have great potential for various reasons, including their diverse origins and their superior abilities to inhibit mycelium growth and spore germination, and control spore viability [39].

This activity can be attributed to the presence of various phytochemicals, *i.e.* carbohydrates, flavonoids, alkaloids, triterpenes, and saponins [40]. These phytochemicals exhibit various antimicrobial and cytotoxic effects on plant pathogens [19]. For example, the inhibitory activity of lemongrass extract may be due to plasma membrane disruption, mitochondrial disorganization, and leakage of calcium, potassium, and magnesium ions, the loss of ions could further impact signal transduction and fungal germination [41].

Table 4: Effect of plant extracts on conidial germination of *E. heraclei* after 48 hours of spore incubation at 25 ± 1°C.

Plant extracts	Conidial germination %				ICG %
	Concentration applied (%)				
	10	20	40	Mean	
Germinated barley grains	14.5 ± 0.55^{e}	11.9 ± 0.96^{e}	7.5 ± 0.45^{c}	11.3	53.5
Germinated corn grains	22.2 ± 1.47^{b}	21.1 ± 2.40^{b}	17.2 ± 0.52^{b}	20.2	16.8
Germinated wheat grains	18.3 ± 1.27^{d}	13.5 ± 0.55^{d}	9.3 ± 0.34^{d}	13.7	43.6
Lemongrass leaves	20.1 ± 0.85^{c}	17.3 ± 2.33^{c}	8.8 ± 0.30^{dc}	15.4	36.6
Basil leaves	22.0 ± 1.64^{b}	20.5 ±1.40^{b}	12.7 ± 0.34^{c}	18.4	24.3
Control	24.3 ± 2.08^{a}				–

ICG = Inhibition of conidial germination, which is calculated based on the control value. According to Duncan's multiple range test, values in the same column followed by different letters are significantly different (at $p \leq 0.05$). Mean ± standard error ($n = 3$).

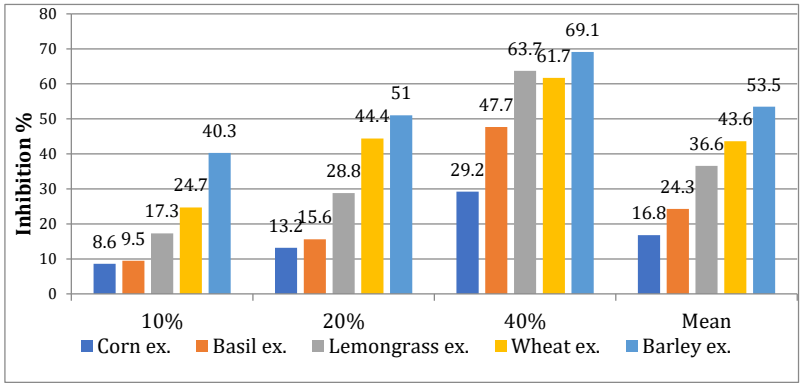

Fig. 2. Inhibitory activity of five plant extracts, each at concentrations of 10, 20, and 40%, against conidial germination of *E. heraclei in vitro* conditions.

4. Conclusions

In the current study, we tested the inhibitory activity of selected fungicide alternatives against *E. heraclei* conidial germination in vitro. The results showed the efficacy of biofungicides and chemical inducers as good inhibitors, especially when used at a concentration of 2000 ppm, as well as the efficacy of plant extracts, especially when used at a concentration of 40%. The results concluded the superiority of Bio-Mix (*B. subtilis* + *T. harzianum*), Bio-F (*B. subtilis*), salicylic acid, and germinated barley grain extract on all tested treatments. The corresponding inhibition values were 74.1, 61.3, 57.6, and 53.5%, respectively. Treatment with Micronite 80% has achieved a complete inhibition of spore germination at all tested concentrations.

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Author Contributions

All authors contributed to this work as follows: Conceptualization: A.T. Bakeer and H.F.A. Ahmed; data curation: H.F.A. Ahmed and H.M.H. Ahmed; formal analysis: H.M.H. Ahmed and M.R.A. Agamy; investigation: A.T. Bakeer and H.F.A. Ahmed; methodology: H.M.H. Ahmed and M.R.A. Agamy; resources: H.M.H. Ahmed and M.R.A. Agamy; software: H.F.A. Ahmed and H.M.H. Ahmed; writing—original draft: A.T. Bakeer and H.F.A. Ahmed; writing—review and editing: A.T. Bakeer, H.F.A. Ahmed, H.M.H. Ahmed and M.R.A. Agamy. All authors have read and agreed to the published version of the manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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