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Original Article

Investigation of the Inhibitory Effect of Some Mites on Fungal Growth Against Leaf Spot Disease (*Alternaria alternata* (Fr) Keissler) Under *In Vitro* Conditions

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ABSTRACT: This study was conducted in vitro to determine the antifungal activity of acaricides containing the active ingredients Abamectin and Etoxazole against leaf spot disease (*Alternaria alternata* (Fr) Keissler) in vegetables. Acaricides used against *A. alternata* were added to the culture medium at three different concentrations, determined according to the recommended doses for the two-spotted spider mite (*Tetranychus urticae* Koch). The high dose was 50% higher than the recommended dose, the low dose was 50% lower than the recommended dose, and the recommended dose was 50% lower. The effect on the growth of the pathogen's colony and mycelium was as follows: At the high dose, Abamectin inhibited colony and mycelium growth by 40.18%, Etoxazole by 24.04%, and Abamectin-Etoxazole by 31.93%. At the dosage used, Abamectin inhibited colony and mycelial growth by 38.63%, Etoxazole by 16.81%, and Abamectin-Etoxazole by 15.56%. The effect of Abamectin on the spore count of the pathogen was as follows: At high doses, Abamectin inhibited colony and mycelial growth by 36.86%, Etoxazole by 22.15%, and Abamectin-Etoxazole by 39.06%. At the dosage used, Abamectin suppressed spore count and growth by 34.94%, Etoxazole by 17.50%, and Abamectin-Etoxazole by 35.21%. Based on these results, Abamectin, both alone and in combination with Abamectin-Etoxazole, was found to be a promising chemical control agent against *A. alternata*.

Keywords: Abamectin, *Alternaria alternata*, Etoxazole, Inhibition, *Tetranychus urticae*.
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1. INTRODUCTION

Acaricides, a type of pesticide used in agricultural production areas to combat mites, are commonly used to control red spider mites. The mechanisms of action of the acaricides are as follows: Abamectin is a locally and systemically effective acaricide. It stops the feeding of *T. urticae*, causing its death within one or a few days. Its effect is seen in both the adult and larval stages of *T. urticae*. Etoxazole is a contact acaricide. It disrupts exoskeleton formation by inhibiting chitin synthesis in *T. urticae*. This effect stops the development of *T. urticae*, especially in its early stages, causing the death of the pest. Etoxazole is a highly effective acaricide in both egg and larval stages.

Soil microorganisms provide ecological services that can aid crop growth by improving soil structure and nutrient cycling (Bahram et al., 2018; Qu et al., 2020; Ray et al., 2020; Zheng et al., 2022). Among soil microorganisms, phytopathogens have developed strategies and structural resilience to maintain continuity in their habitats and increase their chances of survival by multiplying in their environment. The influence of these factors creates an ideal environment for harmful microorganisms to act in the soil. As a result, the environment becomes favorable to pathogens. In particular, the fact that pesticides can be absorbed by plant roots and transported to other above-ground parts of the plant determines the rate of exposure between the plant and the phytopathogen (Xu et al., 2020; Qu et al., 2021). Phytopatho-

gens, which are soil microorganisms, have developed strategies and structural resistances to establish continuity in their habitats and increase their chances of survival by multiplying in their environment. As a result, an ideal environment is created for the activity of harmful microorganisms in the soil. Ultimately, the environment becomes favorable to the pathogen. In particular, the fact that pesticides can be absorbed by plant roots and transported to other above-ground parts of the plant determines the rate of exposure of the plant and the phytopathogen (Xu et al., 2020; Qu et al., 2021). In particular, the indiscriminate use of pesticides (acaricides, fungicides, insecticides, herbicides, etc.) activates resistance mechanisms in the vast majority of plant pathogens. Most importantly, this resistance is passed on to future generations. Pests that damage crops in agricultural areas cause varying degrees of yield loss. Many control methods, including cultural, physical, chemical, and biological, are used to prevent crop losses.

However, chemical control is preferred over other control methods due to the rapid results and ease of pesticide application (Mutlu, 2015). This situation also applies to *T. urticae* and *A. alternata*, which cause economic losses in greenhouse vegetable production areas. Environmental factors are very influential in the epidemic spread of a fungal disease. Furthermore, academic studies have determined that acaricides commonly used to control *T. urticae* and *A. alternata* in open and closed (greenhouse) vegetable production areas have different effects on and interact with plant pathogenic fungi, bacteria, and viruses.

Recent studies have shown that pesticide application in agricultural areas has varying effects on the density of certain phytopathogens. These effects include increases, decreases, and unchanged density. Furthermore, while one pesticide may increase the growth of one pathogen, the same pesticide may decrease the growth of another. The pesticide(s) used must always have a suppressive effect on phytopathogens. Pesticides should decompose rapidly in the soil and have minimal negative impact on beneficial microorganisms in the soil. Studies were conducted to determine the effects of acaricides on phytopathogens. Sun et al. (2005) found that AVM B1a inhibited the growth of existing fungi in the soil to a certain extent. In a study evaluating the effects of Abamectin, a commonly used agricultural agent, on soil microorganisms, researchers found that after a 7-day exposure period following application, Abamectin altered the composition of soil microbial communities, disrupted microbial interactions, and reduced community complexity and stability. They also found that treatment with Abamectin at a concentration of 1.0 mg/kg increased the density of soilborne pathogens such as *Ralstonia spp*. in the soil (Qiu et al., 2022). In studies conducted to determine the effects of herbicides on phytopathogens, it was generally determined that hormone-based herbicides increased plant infection with fungal diseases in their environment. It has been reported that the incidence of maize smut disease in plots treated with 2,4-D herbicide increased by 25% compared to untreated plots (Dığrak & Özçelik 2002; Şevik et al., 2004; Ören et al., 2009).

In his study, Sato (1987) identified the negative effects of many pesticides on beneficial microorganisms, and additionally stated that they also inhibited ammonification and denitrification. Özörgücü et al. (1992) stated that anthracol, used as a fungicide against blue mold disease (*Peronospora tabacina*) in tobacco, reduced the number of soil microfungi, in a study investigating mite-fungal interactions. Dönel et al. (2014) isolated microfungi from the body surfaces and interiors of rafignatoyid mites, reviewed the relationships between the isolated fungi and the mites, and identified a total of 25 fungal species belonging to the genera *Alternaria, Acremonium, Aspergillus, Beauveria, Chrysosporium, Cladosporium, Mucor, Penicillium, Rhizoctonia, Trichoderma, Trichothecium,* and *Ulocladium* on the body surfaces of rafignatoyid mites. Pesticides applied indirectly or directly to the soil leave residues in the soil, causing the destruction or temporary inhibition of soil microorganisms (Dığrak et al., 1996; Greaves et al., 1981). Studies have emphasized that the negative effects of pesticide application on microbial populations in soils are due to differences in chemical composition and environmental factors (temperature, soil pH, soil structure, soil and air humidity, etc.). This study aimed to investigate the *in vitro* antifungal effects of acaricides containing Abamectin and etoxazole against *A. alternata*, a pest causing economic losses in greenhouse production areas in Elazig province.

2. MATERIALS AND METHODS

2.1. Fungal Microorganism *A. alternata*

In this study, the *A. alternata* ALT128 isolate, purified from tomato plants by the single-spore method at the Mycology Laboratory of Firat University Keban Vocational School and grown in stock cultures (paper cultures), which exhibited high virulence (78%) in pathogenicity tests, was used.

2.2. Culture Medium

Potato Dextrose Agar (PDA 39 g, 150 mg Streptomycin sulfate, 1000 ml pure water), a standard culture medium for many fungal plant pathogens, was used for the multiplication of microorganisms and the determination of anti-fungal activity (Merck KGaA, Darmstadt, Germany).

2.3. Acaricides

In our country, acaricides containing the active ingredients Etoxazole and Abamectin, which are licensed against *Tetranychus urticae*, were used in the study, as in Table 1.

Table 1. Active ingredients and dosages of acaricides used in the study (*in vitro*)

Active Ingredient	Recommended by the Company			Doses and A.U. (1 L su)		
	Plant	R. D (100 L su)	A.U. (1L su)	Hd	Rd	Ld
Abamectin 18 g/L EC	Tomato (*T. urticae*)	25 ml/da	0.63 ml/da	0.94	0.63	0.32
Etoxazole 110 g/L SC	Eggplant (*T. urticae*)	35 ml/100L	0.35 ml/L	0.53	0.35	0.18

Legend: Hd: 50% high dose; Rd: recommended dose; Ld: 50% low dose; A.U.: amount used.

2.4. Studies conducted *in vitro*

In vitro studies investigated the effects of acaricides on pathogen colony growth and spore number.

2.4.1. Impact on Colony Development

In adjusting the dosage of acaricides, 50% more than the usage dose against *T. urticae* was added to the culture media as the high dose (Hd), 50% less as the low dose (Ld), and the usage dose (Rd). The pathogen culture, developed in PDA medium for 7 days, was cultured by taking 5 mm diameter fungal discs from the end portions where growth continued using a fungal drill. One disc at a time was transferred to the center of the acaricide-added PDA medium and incubated at 25±1 °C for 7 days. As a control, the pathogen was transferred to PDA medium without acaricide. The developing fungal colonies were measured daily using a digital caliper. The study trials were designed according to a randomized complete inhibition design with 4 characters (3 different doses + control) and 10 replications. The measurement of the pathogen colony diameter in the Petri dish was performed by measuring the fungal colony diameter in separate directions perpendicular to each other (Benjilali et al., 1984). The percentage (%) inhibition rates of acaricides compared to the control were calculated using the following formula (Deans and Svoboda, 1990).

$$E(\%) = \left(\frac{K-M}{K}\right) X100 \quad (1)$$

Where E = inhibition rate (%); K = colony diameter during control (mm); and M = colony diameter in practice (mm).

2.4.2. Impact on the number of sports

In this study, culture media additives with acaricides at different doses (Hd, Rd, and Ld) (Abamectin, Etoxazole, and Abamectin with Etoxazole) and acaricide-free unadditive culture media (control group) were prepared and used. Spore suspensions were prepared from *A. alternata* pathogens, grown in 5 cm discs in the culture media for a total of 7 days, on the 3rd and 7th days when growth began in the incubator ($1x10^4$ spores/ml). Spore counts were performed using a Thoma slide (ISO Lab) under a light microscope (Boeco/Germany). The study trials were designed according to a randomized complete block design with 4 characters (3 different doses + control) and 10 replications. To determine the percentage effect of acarids on spore quantity, the colony diameter measurement in the formula from Deans and Svoboda (1990) was modified to represent the percentage effect of spore quantity and used in the study in the formulation given below:

$$SMEO(\%) = \left(\frac{Ks-Ms}{Ks}\right) X100 \quad (2)$$

Where, SMEO = Impact on the number of spores (%), Ks = Spore quantity in control (spores/ml), and Ms = Spore quantity in treatment (spores/ml).

2.5. Impact on the number of sports

The data obtained from the experiment were analyzed using the SPSS statistical package program. The comparison of means was determined using the "*Duncan multiple comparison test*" (SPSS, 2021).

3. RESULTS AND DISCUSSION

3.1. The effect of acaricides

3.1.1. On colony growth effect

The effects of acaricides (Abamectin and Etoxazole) on the colony growth of *A. alternata* under in vitro conditions are shown in Figures 1 and 2. In the tables and figures, high dose (Hd), recommended dose (Rd), low dose (Ld) and control dose (Cd) are used. It was found that the colony growth and physical appearance of the pathogen in acaricide-added (Abamectin and Etoxazole) culture media were significantly different from its morphological appearance in the control group. Especially, it was observed that the colony color took on white, reddish-white, and cream tones. This was interpreted as the acaricide having a mutation effect on the fungus in the culture medium. It was also observed that the mycelia transformed into aerial mycelial structures. The fungus in the control group was determined to exhibit the typical characteristics of *A. alternata*, as seen in Figure 1.

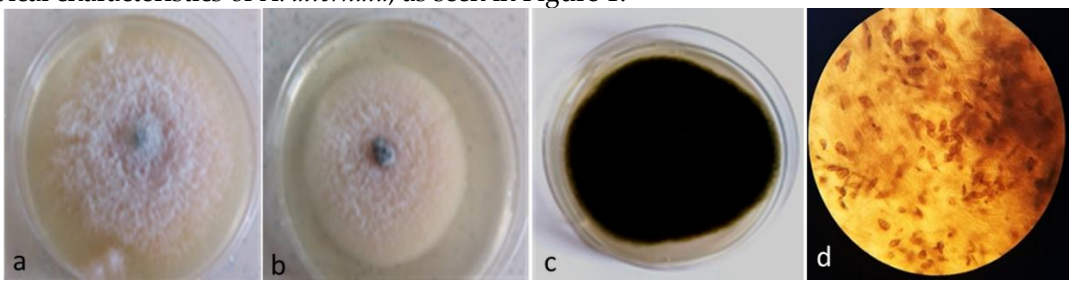

Figure 1. The colonial growth. a: Colonial growth of *A. alternata* in culture medium with etoxazole. b: Colonial growth of *A. alternata* in culture medium additives with Abamectin. c: Colonial growth of *A. alternata* in the control group. d: Microscopic image of *A. alternata* spores in the additive medium.

Four different treatments were used in the study, and no significant differences were found between the treatments. The statistical averages of the treatments were used. In this study, the effects of different acaricide doses on colony and mycelial growth of A. alternata were investigated; T-square tests were applied to the data, and a statistically significant difference was found between the groups; Duncan's test was also performed. Accordingly, it was determined that Abamectin, at a high dose, inhibited colony size to the greatest extent (41.90 mm), and this was statistically significant. Etoxazole, at a high dose, showed the highest inhibition (53.83 mm). The combined use of acaricides provided the highest pathogen suppression at high doses (48.19 mm). The study determined that Abamectin is an acaricide that significantly and effectively suppresses the growth of *A. alternata* colonies at high doses (Table 2 & Figure 2).

Table 2. The effects of Abamectin and Etoxazole acaricides at different doses on the *in vitro* colony growth of *A. alternata*.

Acaricides	Application	Doses (ml)	*A.alternata* (ALT128) Colony Diameter (mm)*
18g/l Abamectin	Hd	0.525	41.90 d
	Rd	0.350	42.98 c
	Ld	0.175	46.64 b
	Cd	Control	70.04 a
110g/l Etoxazole	Hd	0.375	53.83 d
	Rd	0.250	58.96 c
	Ld	0.125	59.75 b
	Cd	Control	70.86 a
18g/lAbamectin with 110g/lEtoxazole	Hd	0.525+0.375	48.19 d
	Rd	0.350+0.250	59.59 c
	Ld	0.175+0.125	61.75 b
	Cd	Control	70.57 a

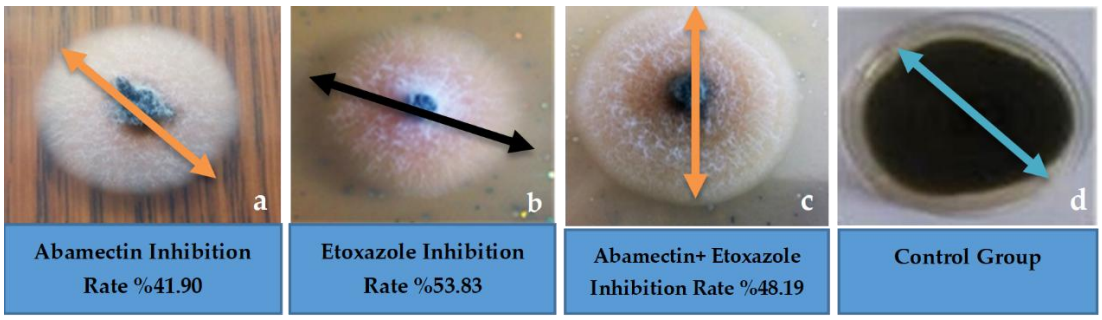

Figure 2. The colonial growth inhibition. a: High-dose inhibitory effect in abamectin-supplemented culture medium (41.90%). b: High-dose inhibitory effect in etoxazole-supplemented culture medium (53.83%). c: High-dose inhibitory effect in Abamectin with Etoxazole additives culture medium (48.19%). d: Control group.

3.1.2. Inhibition Percentage Rate

It was determined that the Abamectin-based acaricide inhibited colony and mycelial growth by a high of 40.18% and a low of 33.41% at different doses applied against the fungal pathogen (Table 3). High-dose abamectin administration was found to be statistically significantly more effective than normal and low-dose administrations. The Etoxazole-based acaricide inhibited colony and mycelial growth by a high of 24.04% and a low of 16.38% at different doses applied against the fungal pathogen (Table 3). High-dose Etoxazole administration was found to be statistically significantly more effective than normal and low-dose administrations. The Abamectin with Etoxazole mixture inhibited colony growth by a high of 43.90% and a low of 32.77% at different doses applied against the fungal pathogen (Table 3). High-dose Abamectin with Etoxazole administration was found to be statistically significantly more effective than normal and low-dose administrations.

Table 3. The effects of Abamectin and Etoxazole acaricides at different doses on the in vitro colony and mycelial growth of *A. alternata*.

Application Doses	Abamectin Doses	Etoxazole Doses	Abamectin with Etoxazole Doses
Hd	40.18^{a}	24.04^{a}	31.93^{a}
Rd	38.63^{b}	16.81^{b}	15.56^{b}
Ld	33.41^{bc}	16.38^{bc}	12.50^{bc}
Cd	00.00^{d}	00.00^{d}	00.00^{d}

Legend: *Differences between means with the same letter in the same column were found to be insignificant according to Duncan's test (P< 0.05).

3.2. Effect of acaricides on spore numbers

The percentage effects of acaricides on the spore count of *A. alternata* are also given in Table 4. Accordingly, it was determined that abamectin additives in culture media inhibited the pathogen's spore count by 36.86% at high doses. The lowest inhibition was found at 28.00% in the low-dose application (Table 4). Since there was no statistically significant difference between the applications, the mean values of the application data were used. Duncan's test was applied to these data, and the differences between means in the same row and with the same letter were found to be insignificant. When the percentage effect rates of Abamectin were examined in the study, it was also determined that the high dose was statistically significant compared to other doses.

Table 4. The effect of Abamectin and Etoxazole acaricides on sports amounts of *A. alternata* after use (%)

Acaricide	Impact of Application Doses on Sports Amounts (%)			
	Hd	**Rd**	**Ld**	**Cd**
Abamectin	36.86^{a}	34.94^{ab}	28.00^{b}	00.00^{c}
Etoxazole	22.15^{a}	17.50^{b}	10.61^{bc}	00.00^{c}
Abamectin with Etoxazole	39.06^{a}	35.21^{ab}	28.68^{b}	00.00^{c}

Legend: * Differences between means with the same letter in the same row were found to be significant according to the Duncan test (P< 0.05).

In culture media, Etoxazole was added; it was determined that high doses inhibited the spore count of the pathogen by 22.15%. The lowest inhibition rate was observed at low doses, at 10.61% (Table 4). Differences between means with the same letter in the same row were found to be insignificant according to the Duncan test. When the percentage effect rates of etoxazole were examined in the study, it was determined that the high dose was statistically significantly more effective than other doses.

It was determined that the combination of Abamectin and etoxazole suppressed the spore count of the pathogen in the culture medium by 39.06% at a high dose. It had the lowest effect on the spore count of the fungal pathogen, inhibiting it by 28.68% (Table 4). Differences between means in the same row and with the same letter were found to be insignificant according to the Duncan test. When the percentage effectiveness rates of the combined use of Abamectin and etoxazole were examined in the study, it was determined that the high dose was statistically significantly more effective than the other doses.

In the study concluded that Abamectin was the most effective acaricide inhibition and in reducing the spore count of *A. alternata*. Furthermore, it was found that the combined use of acaricides suppressed the spore count of the pathogen. It was also determined that the use of etoxazole acaricide alone had a low effect on the spore count of the pathogen.

Generally, there is no clear or closely related study with which we can compare the data that we obtained in our study. Therefore, the discussion section was created using in vitro studies of different fungal pathogens and pesticides with active ingredients against pathogenic fungi. The data obtained in these studies support our study. The data we obtained under laboratory conditions consist of the effects of Abamectin and Etoxazole acaricides on colony growth and spore number. In addition, the extent to which Etoxazole acaricide, a chitin inhibitor, affects the colony development and spore number of the fungal pathogen was also determined.

Regarding the effect of Acaricides on colony growth, the selection of etoxazole for this study was significantly influenced by its status as a chitin synthesis inhibitor and the presence of chitin in fungal cell walls. Chitin is a stable polysaccharide found in the cell walls of arthropods and fungal organisms. Etoxazole is a commonly used acaricide that disrupts arthropod developmental processes by inhibiting chitin synthesis. However, etoxazole does not exhibit antifungal activity despite the presence of chitin in fungal cell walls. Studies emphasize that this is due to fundamental biochemical and structural differences between the two biological systems (Merzendorfer, 2013; Demaeght et al., 2014; Brain et al., 2025). Therefore, this study discusses the effect of etoxazole on the colonial development and spore number of *A. alternata*.

Pesticides (herbicides, insecticides, acaricides, and fungicides) are specifically designed to affect or inhibit certain enzyme activities to restrict the living spaces of the target population. It has also been reported that this potential effect can lead to a decrease in microbial diversity and a reduction in soil fertility (Johnsen et al., 2001). In our study, the highest inhibitory percentage effect was determined as a result of high-dose application; Abamectin inhibited the colonial growth of *A. alternata* by 41.90%, and the combined use of Abamectin and Etoxazole was 48.19%. This finding partially differs from López-Manzanares et al. (2022), who reported that etoxazole did not directly inhibit conidial germination or mycelial growth of *B. bassiana*. This difference may be related to the fungal species, pesticide concentration, and experimental conditions. Since we determined that Etoxazole inhibited the colonial growth of *A. alternata* by 53.83% in our study, it is consistent with the aforementioned study. It has been observed that etoxazole does not completely inhibit chitin in the fungal cell wall; therefore, colony growth continues. It has also been found that Abamectin, etoxazole, and the combination of Abamectin and etoxazole do not completely stop colony growth.

Sebestyén et al. (2021) investigated the effect of Abamectin on *Phaeomoniella chlamydospora* and *Phaeoacremonium minimum*, two important pathogens involved in grapevine stem disease, in the laboratory. In their study, they found that Abamectin, added to a streptomycin-free culture medium and sterilized with a filter at concentrations of 62.5-312 µg/mL, showed limited inhibition of fungal growth on solid agar. While the addition of filter-sterilized streptomycin did not show a significant effect on fungal growth for *P. chlamydospora*, they found that for *P. minimum*, a small but significant reduction in growth occurred with streptomycin when abamectin concentrations were >62.5 mg/ml. This is consistent with our study. This study is consistent with our previous data regarding fungal growth resulting from the use of Abamectin and abamectin-ethoxazole together at high and recommended doses.

Vidal et al. (2025) investigated the effects of anthelmintic pesticides (Albendazole, Abamectin, and Levamisole) on the colony growth of nematophagous fungi (*Arthrobotrys musiformis, Duddingtonia flagrans,* and *Pochonia chlamydosporia*). In the study, diluted solutions of Albendazole and Abamectin were added to culture media at concentrations of 1, 10, 100, and 1000 ppm under in vitro conditions. The applied doses inhibited colony growth of *A. musiformis, D. fla-*

grans, and *P. chlamydosporia*. This study is consistent with our findings regarding the effect of Abamectin on fungal growth. Cole and Batson (1975) found that diphenamide affected the colony growth of *Rhizoctonia solani* and *Pythium aphamidermatum* in media prepared under in vitro conditions, reducing their density and the occurrence of rot in tomato seedlings. These studies and our study showed that diphenamide inhibited the fungi to a certain extent depending on the high dose used. This study is consistent with the data we obtained regarding fungal growth from the combined use of Abamectin and abamectin-etoxazole.

Regarding the effect of Acaricides on spore numbers, researchers have found that pesticides negatively affect the total number of bacteria, yeasts, and fungi when applied to soils. In our study, it was determined that the combined use of Abamectin and Abamectin with Etoxazole acaricides inhibited the spore count of *A. alternata* by 38.70% to 39.26% at high doses, but did not completely stop it.

Shi et al. (2019) investigated the effect of Abamectin with Fludioxonil at a dose of 375 g ha^{-1} on *Fusarium oxysporum* under greenhouse conditions. The study reported that the simultaneous application of Abamectin with Fludioxonil was effective in controlling the spore density of the *F. oxysporum* disease complex in the soil of cucumber production areas. This study is consistent with ours. Anderson (1978) stated that the negative effects of the MCPA pesticide on the densities of soil microorganisms were of low significance when applied at the proposed dose. Roger et al. (1994) found that pesticides applied directly to the soil had a different adverse effect on microbial populations and activities. Heinonen-Tanski et al. (1986) reported an increase in the total number of microorganisms in pesticide-treated soils. Dickinson (1973) reported that different concentrations of commonly used pesticides affect microbial densities (bacteria, actinomycetes, and fungi) and the vital processes they perform (soil respiration, nitrification, nodule formation, etc.).

4. CONCLUSION

The fact that the inhibition percentages of Abamectin and Etoxazole were close to each other at the high and recommended doses used in the study is considered promising. Considering that high-dose field applications may have a phytotoxic effect on tomato and eggplant plants, it is thought that there will be no risk of phytotoxicity at the recommended dose. It is also thought that, when applied together with fungicides to control the two-spotted spider mite (*Tetranychus urticae* Koch), they may provide additional treatment potential to support chemical control against *Alternaria alternata* in the field. Specifically, this study evaluated whether different doses of acaricides can affect the spread of the pathogen to varying degrees or reduce it to certain percentages. In this study, it is thought that acaricides containing Abamectin and Etoxazole, used in vitro against the leaf spot disease agent, may be promising in chemical control against pathogenic fungi. However, further advanced antifungal studies in living organisms, as a continuation of this research, are of great importance. The main conclusions of the study may be presented in a short Conclusions section.

Ethical Statement

Not Applicable.

Conflicts of Interest

The authors declare no competing interests.

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