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Identification of Fusarium Wilt Disease Causal Agents in Pistachio Fields and Determination of Efficacy of Some Fungicides Controlling of The Disease

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ABSTRACT

Pistachio (*Pistacia vera*) propagation is commonly carried out by seedlings and new orchards are mostly set by in situ grafting of rootstocks. Pathogenic and molecular characterization of wilt and desiccation agents observed in nurseries and young orchards were carried out and the causal species were identified as *Fusarium oxysporum* and *F.solani*. *In vitro* inhibition of mycelial development and conidia germination were carried out with seven fungicides selected against the disease. The plant protection product containing Prothioconazole+Spiroxamine was found to be 100% effective in mycelial growth trials in *F.oxysporum* isolates from 20 mgL^{-1} and in *F.solani* from 100 mL-1 at all increasing doses. . The fungicide containing phosphoric acid was found to be 18.3-33.3% effective on both species at 2.5 times the recommended dose. On conidial germination, Prothioconazole+Spiroxamine and Captan provided 100% inhibition even at the lowest doses in all isolates tested. The EC_{50} values of Prothioconazole+Spiroxamine, which was determined as the most effective chemical in mycelial development and conidial germination, were 3.67 ml and 3.72 ml in *F.oxysporum* isolates and 56.83 ml in *F.solani*, respectively.

Keywords: Pistachio, Fusarium wilting, Fungicides, *in vitro*

Antepfıstığı Alanlarında Görülen Fusarium Solgunluk Hastalığı Etmenlerinin Tanımlanması ve Mücadelesinde Bazı Fungusitlerin Etkinliklerinin Belirlenmesi

ÖZ

Antepfıstığı (*Pistacia vera*) fidan üretimi yaygın olarak çöğürlerle yapılmakta ve yeni tesis bahçeler çoğunlukla aşısız dikilen anaçların daha sonra yerinde aşılanmasıyla kurulmaktadır. Fidanlıklarda ve genç bahçelerde yapılan incelemelerde görülen solgunluk ve kuruma etmenlerinin patojenik ve moleküler karakterizasyonları gerçekleştirilerek etmen türlerin *Fusarium oxysporum* ve *F.solani* olduğu tespit edilmiştir. Hastalığa karşı seçilen yedi adet fungusitle *in vitro* düzeyde miseliyal gelişimin inhibisyonu ve konidi çimlenmesinin engellenmesi üzerine çalışmalar yürütülmüştür. Prothioconazole+Spiroxamine etkili madde içeren bitki koruma ürünü, miseliyal gelişim denemelerinde *F.oxysporum* izolatlarında 20 mgL^{-1}'den itibaren, *F.solani*'de ise 100 mgL^{-1}'den itibaren artan tüm dozlarda %100 etkili bulunmuştur. Fosforöz asidi içeren fungisit, tavsiye dozunun 2,5 katında her iki tür üzerinde %18,3-33,3 oranlarında etkili bulunmuştur. Konidiyal çimlenme üzerinde Prothioconazole+Spiroxamine ve Captan denemeye alınan tüm izolatlarda en düşük dozlarda bile %100 inhibisyon sağlamıştır. Miseliyal gelişim ve konidi çimlenmesinde en etkili kimyasal olarak belirlenen Prothioconazole+Spiroxamine'nin EC_{50} değerleri sırasıyla *F.oxysporum* izolatlarında sırasıyla 3,67 ml ve 3,72 ml ve *F.solani* izolatında ise 56,83 ml olarak tespit edilmiştir.

Anahtar Kelimeler: Antepfıstığı, Fusarium solgunluk hastalığı, Fungisit, *in vitro*

INTRODUCTION

Turkiye has suitable climatic conditions for the cultivation of Pistacia species and is located on one of the germplasms of Pistacia species. Türkiye has gained a certain share in pistachio production in the world in terms of domestic market and foreign market and pistachio has become a valuable product of increasing importance for our country [1, 2, 3]. Fusarium species cause serious problems in pistachio as in many plant species. While Verticillium wilt and Phytophthora diseases are known as the most important problems among soil-borne diseases in the world [4, 5, 6], in recent years, wilting disease caused by Fusarium species in pistachio has been reported from many countries in the world [7]. While root rot disease caused by *F.solani* was reported for the first time in irrigated orchards and nurseries in Tunisia [8],

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Fusarium species (*F.equiseti*, *F.oxysporum*, *F.proliferatum*) and Neocosmospora species (*N.falciformis*, *N.solani*) caused crown rot and stem cancer in trees grafted on UCB-1 rootstocks in the United States [9]. In detailed studies on soil-borne pathogens in pistachio fields in our country, Fusarium species (*F.solani*, *F.oxysporum*, *F.brachygibbosum*, *F.chlamydosporum*) and Macrophomina phaseolina were found to be the most common disease agents related to wilt and drying of trees [7]. On the other hand, the disease was defined as 'Fusarium wilt of pistachio' in the instructions for pest and disease control in Türkiye and the causal species were stated as a Fusarium complex (*F.solani*, *F.proliferatum*, *F.oxysporum* and *F.redolens*). In the same technical instruction, it is stated that the disease is most common in young plants and the severity of the disease increases after 4-5 years of age after the stress caused by inoculation and some cultural practices on the plant. There is no known effective fungicide against Fusarium wilt disease in pistachio [10]. However, some chemicals can be used in seedling production in annual plants against infections that may occur in the plant during seedling production [11, 12]. This study was carried out to determine the Fusarium species causing pistachio wilt disease in pistachio orchards and nurseries in Gaziantep and its surroundings and to determine the *in vitro* efficacy of some chemicals for its control.

MATERIAL AND METHODS

Plant Materials and Fungal Isolations

Pistacia vera L. (Pistachio) production areas in the Southeastern Anatolia Region, where pistachio production is the highest in Turkey, were sampled from plants showing wilt disease symptoms and from seedlings in nurseries. Samples were taken from Şanlıurfa-Bozova (28), Pistachio Research Institute seedling production greenhouses (14) and Şanlıurfa-Birecik producer seedling production locations (8). A total of 50 diseased plant samples were brought to Gaziantep University Biology Department laboratories and isolated according to the method described by Leslie and Summerell [13].

In the laboratory, 0.5-1 cm² of transverse sections were taken, including healthy tissue, taking into account the lesions seen especially in the conduction bundle tissues, washed in running tap water for 2-3 minutes, and then subjected to the following sterilisation procedures. 3% Sodium Hypochlorite solution was shaken for 1-5 minutes, washed 3 times in distilled water and left to dry on blotting papers for 4-5 hours and cultured in Potato Dextrose Agar (PDA) medium at 24°C and in dark conditions. After weekly controls, colonies showing morphological differences were isolated and purified. Single spore isolations were made from the purified isolates according to Katan [14].

For morphological characterization each isolate was grown on PSA and SNA media according to Leslie and Summerell [13].

Fungicides Used In in vitro Trials

In order to prepare studying doses of fungicides, stock solutions were prepared by diluting the fungicides. The prepared stock solutions were added to the media which were allowed to cool down to 48°C after autoclaving at the dosage specified for each fungicide (Table 1) with the help of a micropipette and shaken for homogenous mixing. The mixture was poured into each petri dish as 15 ml and kept for 30-60 min for solidification. For each dose, 4 petri dishes were used and 4 petri dishes without any fungicide were used as control. Samples taken from the tips of the fungal isolates with a 0.5 cm fungal disc cork borer were planted one in the centre of the petri dishes with and without fungicide (Control) and left to incubate at 24°C [15]. Measurements were made from four directions depending on the development and mycelial growth was measured with the average of the values obtained. Based on the 21st day measurements, the percentage effects according to doses were calculated according to Abbott formula [16]. The experiment was established according to the random plots experimental design with 4 replicates.

In order to determine the effects of fungicides on conidial germination, pure sterile water was added to 7-day-old Fusarium isolates, scraped with a spatula and the spores were allowed to pass into the water. The spore density was adjusted to 1×10^5 on Thoma cell counting chamber. After the prepared PDA media were cooled to 48 degrees in a water bath after autoclaving, fungicides at the determined doses were added to the media and homogenous mixing was ensured. PDAs were poured into petri dishes in a sterile cabinet and kept for 30-60 min for freezing. For each dose, 4 petri and 4 petri were prepared as control. Afterwards, 50 µl of the prepared spore suspension was added to each petri and spread on the surface with the help of a pipette. Circles with a diameter of approximately 1,5 cm were drawn by determining certain areas under the petri dishes. At the end of incubation under the specified conditions, 100 spores were counted for each area to determine the conidia germination rate (%). Measurements were

made at the end of the 4th day. EC_{50} values of each fungicide were calculated by probit analyses.

The active ingredients and ratios, formulation types, trade names and doses of the fungicides used in the trials are given in Table 1.

Table 1. Trademarks, formulations and active ingredient ratios of the chemicals used in the study

Active Ingredients and Ratios	Formulation	Trademark	Doses (mgL^{-1})
Captan 50%	WP	CAPTAN	20, 50, 150, 300, 500
Azoxystrobin+Metalaxyl-M+Fludioxanil 75+37,5+12,5 g/l	FS	DYNASTY	10, 50, 100, 250, 500
Fosforoz Asidi 400 g/l	SL	AGRIFOS	20, 100, 200, 400, 1000
Hymexazol 360 g/l	SC	KORGAREN	30, 100, 250, 500, 750
Metalaxyl-M+Mancozeb 4%+64	WG	RIDOZEB	10, 50, 100, 250, 500
Prothioconazole+ Spiroxamine 160 g/l+300 g/l	EC	INPUT	30, 100, 250, 500, 750
Propamocarb+Cymoxanil 400+50 g	SC	PROXANIL	10, 40, 100, 175, 350

DNA Isolation of Fusarium spp.

DNA isolation studies from the pure Fusarium isolates were performed by modifying the method developed by Peever et al. [17]. Accordingly, the isolates were incubated in PDB medium on a shaker at 75 rpm for 3-5 days. Developing fungal hyphae were washed with sterile water and prepared for DNA analyses, wrapped in foil and stored at -80°C. Developing hyphae were crushed in a porcelain mortar by adding liquid nitrogen. DNA isolation was performed using the method modified from Peveer et al. [17]. The crushed hyphae were treated with lysis baffle and then vortexed for 3 min. The buffer solution was then added 2 times with a 24:1 ratio of chloroform:isoamyl alcohol and centrifuged at 10.000 rpm for 5 min at 4°C. After this process, the supernatant was precipitated 2 times with cold 95% ethanol (2 volumes) and centrifuged at 14000 rpm for 5 min at 4°C. The pellet was washed with 70% ethanol and the DNA in eppendorfs was allowed to dry for approximately 45 minutes to remove the ethanol. Finally, the DNA was dissolved in 1×TE (Appendix 4) and stored at -20°C until use in PCR analyses. The concentrations of the DNAs obtained as a result of DNA isolation were measured using a Nanodrop device and necessary dilutions were made for concentrations greater than 20 ng/µl.

PCR Protocols

The PCR protocol was modified according to Zhang et al., [18]. For fungal DNA amplification, 1 µl DNA obtained from the fungus was added to 25 µl reaction volume. The reaction mixture consisted of 10× PCR buffer, $MgCl_2$ (2 mM), dNTP (2 mM), Primer F (0.4 mM), Primer R (0.4 mM), Taq DNA Polymerase (1.0 U) and dH_2O. FOF1 and FOR1 primers specific for *F.oxysporum* were used in the study [19] (Table 2).

Table 2. Specific primers for *Fusarium oxysporium*

Primers	Sequences (5'-3')
FOF1	5'-ACATACCACTTGTTGCCTCG-3'
FOR1	5'-CGCCAATCAATTTGAGGAACG- 3'

Firstly, PCR optimisation conditions were determined for Fusarium spp. molecular studies [18]. Accordingly, DNA was denatured at 94°C for the first 1 min. Then 25 cycles were set at 58°C for 25 cycles for primer binding and finally elongation was achieved at 72°C.

DNAs were loaded on agarose gel electrophoresis for visualisation of the bands. For the preparation of the gel, 1.5-2.0% agarose gel was dissolved in 1×TAE (Tris, Acetic acid, EDTA, given in Appendix 3) solution. 25 µl of ethidium bromide (0.5 µg/ml) was added to the agarose gel to visualise the DNA under UV light. The solution was allowed to cool down to 55-60°C and poured into the gel tank. After gel polymerisation, the combs were removed and the gel was transferred to the electrophoresis tank containing 1×TAE buffer. The first well of the gel was loaded with marker (1 kb or 50 bp DNA) and the other wells were loaded with 25 µl PCR product mixed with 4 µl loading buffer. Electrophoresis was performed in 1.5% agarose gel at 90 V/cm for 1.5 hours. Evaluation was made according to band formation. The bands formed in gel electrophoresis were visualised and photographed in a computerised gel imaging system.

RESULTS

Morphological and Molecular Analysis of Isolates

In the cultures of 50 diseased plant samples collected from the fields, 38 isolates showing characteristic Fusarium symptoms were obtained. Colony morphology, sporodochia, polyphialidia, macroconidia and microconidia structures of the isolates were analyzed on PSA and SNA media. In molecular studies, amplification was obtained from PCR studies of 6 samples using FOF1 and FOR2

primers (Figure 1). These samples were identified as *F.oxysporum* by matching the sequence results in NCBI.

In the species identification based on morphology, 9 samples were macroscopically and microscopically identified as *F.solani* (Figure 2). The other specimens were identified as different Fusarium species.

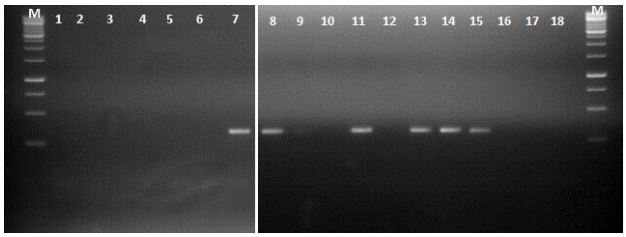

Figure 1. DNA bands of isolates on gel

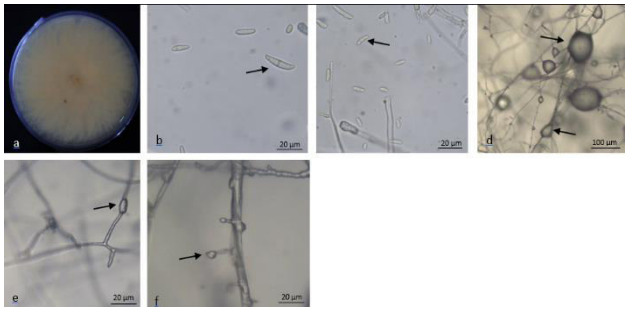

Figure 2. Morphology of K.2/5.1. isolate on SNA and PSA, a) Colony morphology on PSA, b) macroconidi, c) microconidi, d) sporodochia, e) chlamidospore, f) monophialide

Efficacy Levels of Fungicides in Mycelial Growth

Two isolates of *F.oxysporum* (63.111 and Fidan.1) and an isolate of *F.solani* (K.2/5.1) were selected to determine the efficacy of different fungicides on Fusarium isolates in pistachio. It was observed that different doses of fungicides had different effects on the inhibition of mycelial growth of Fusarium isolates and the results obtained are given in Figure 3 according to the fungicides.

Fungicide Dynasty™, the highest effect among the isolates was seen in Fidan.1 isolate with 79.1% at 250 mgL^{-1} dose. At the same dose, another *F.oxysporum* isolate 63.111 showed a similar effect with 76.2%, while *F.solani* isolate K.2/5.1 showed a lower effect with 66.9% compared to the other isolates. The highest effect was observed at 250 mgL^{-1} in isolates 1 and K.2/5.1. The lowest effect in all 3 isolates was observed at 10 mgL^{-1} dose as 22% in 63.111, 35.5% in K.2/5.1 and 48.2% in Fidan 1, respectively. In 63.111, while the percentage effects increased with increasing doses, the highest effect was 78.7% at 500 mgL^{-1}. In K.2/5.1 and in Fidan.1, the highest level of activity was found at 250 mgL^{-1} and more than 500 mgL^{-1}.

It was observed that Captan™ provided similar inhibition on Fusarium spp. at all doses. The highest effect was 50.6% at 300 mgL^{-1} and the lowest effect was 8.9% at 20 mgL^{-1} dose. In K.2/5.1, the highest effect was 62.8% at 500 mgL^{-1} and in Fidan 1, the highest effect was 55.8% at 150 and 300 mgL^{-1}. The lowest dose of 20 mgL^{-1} was not effective in both isolates.

In the trial with Ridozeb™, the highest effect value was observed at 250 mgL^{-1} dose in isolate 63.111 and approximate percentage values with 250 mgL^{-1} were found at 100 mgL^{-1}, 250 mgL^{-1} and 500 mgL^{-1}. The lowest value was recorded as 6.4% at 10 mgL-1 dose. In K.2 /5.1 isolate, the highest percentage effect value was recorded at 100 mgL^{-1} and it was observed that mycelial development was completely stopped. In the same isolate, no effect was observed at 10 mgL^{-1}. In another *F.oxysporum* isolate, Fidan.1, the highest effect was recorded at 500 mgL^{-1} and mycelial growth was completely stopped on this dose. In the same isolate, the lowest effect was 31.3% at the lowest dose of 10 mgL^{-1}.

In the trials with Agrifos™, the highest effect was observed at the highest dose of 1000 mgL^{-1} in all three isolates. While the inhibition rate in isolate 63.111 was 18.3% at 1000 mgL^{-1}, the highest effect in isolates K.2/5.1 and Fidan.1 was 25.2% and 33.3%, respectively. No inhibition was observed in isolate 63.111 up to 400 mgL^{-1}. While no effect was observed in isolate K.2 /5.1 at a dose of 20 mgL^{-1}, 17.5% effect was observed in isolate Fidan.1.

When Fusarium spp. isolates were examined in Korgaren™ commercial preparation containing Hymexazol active ingredient, similar levels of inhibition were achieved in isolates numbered 63.111 and K.2/5.1 according to the increasing doses of Korgaren™ commercial preparation. In the isolate numbered Fidan.1, 55.4% inhibition was observed at the lowest dose and 100% inhibition was achieved at a dose of 500 mgL^{-1}. In 63.111, inhibition was not achieved at the lowest dose, while in K.2/5.1, inhibition rate at the lowest dose was 1.2%. In 63.111, the highest effect was 83.33% at the highest dose of 750 mgL^{-1}, while the highest effect was 77.4% at the same dose in isolate no. K.2/5.1.

In the study conducted with Proxanil™ containing Propamocorp+Cymoxonail active substance, no activity was observed at any dose in isolates 63.111 and Fidan.1. In K.2/5.1, the highest effect was 55.0% at a dose of 350 mgL^{-1}, while no effect was observed at the lowest dose of 10 mgL^{-1} and 16.3% effect was observed at a dose of 40 mgL^{-1} (Figure 3).

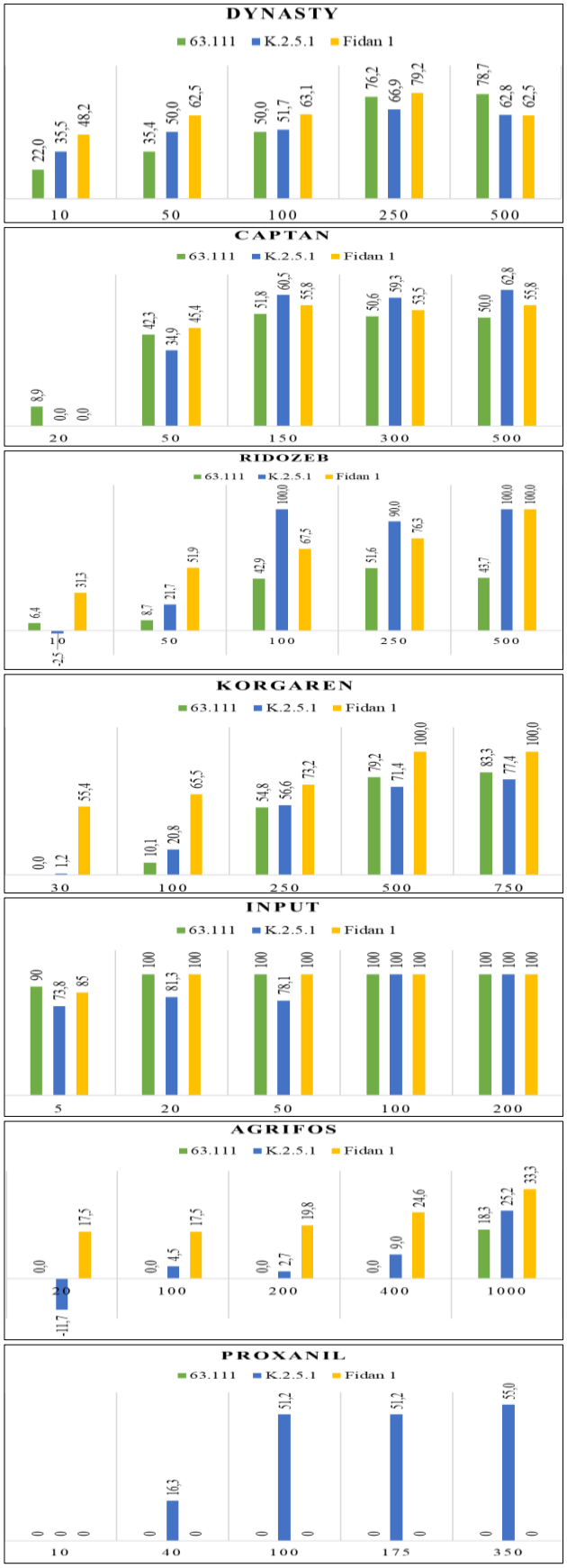

Figure 3. Inhibition of mycelial growth of Fusarium isolates by different fungicides used in the study

EC₅₀ Values

EC_{50} (the dose that inhibits mycelial growth by 50%) values were calculated for 7 fungicides for Fusarium isolates with the measurement results of the fungicides whose percentage effects were calculated. EC_{50} values according to the evaluations in probit analysis are given in Table 3.

Table 3. EC_{50} values of the fungicides ($p<0.05$)

Isolates	Fungucides						
	Dynasty™	Captan™	Ridozeb™	Agrifos™	Korgaren™	Proxanil™	Input™
63.111	147,72	261,45	384,87	2758,86	266,46	NA*	3,67
K.2.5.1	58,99	175,64	63,28	2069,06	259,13	180,29	56,83
Fidan.1	19,53	203,5	103,92	4159,10	250,12	NA*	3,72

*Not applicable: It could not be calculated statistically since it has no effect.

As given in Table 3, EC_{50} values were calculated for each isolate. The EC_{50} value of the commercial preparation named Dynasty™ was calculated as 147.22 ml for isolate 63.111, 58.66 ml for isolate K.2/5.1 and 19.53 ml for isolate Fidan.1. In Captan™, EC_{50} value for isolate 63.111 was 261,45 g; EC_{50} value for isolate K.2/5.1 was 175,64 g; EC_{50} value for isolate Fidan.1 was 203,5 g. EC_{50} values for Ridozeb were 384.87 g for isolate 63.111; 63.28 g for isolate K.2/5.1; 103.92 g for isolate Fidan.1. When it is looked at EC_{50} values for Agrifos™, it was calculated as 2758.86 g for isolate 63.111; 2069.06 g for isolate K.2/5.1; 4159.1 g for isolate Fidan.1. In Korgaren™, EC_{50} values were recorded as 266.46 ml for isolate 63.111; 259.13 ml for isolate K.2/5.1; 250.12 ml for isolate Fidan.1. The efficacy level of Proxanil™ was found to be low in the percentage calculations and accordingly, EC_{50} values could not be calculated for isolates 63.111 and Fidan.1. EC_{50} value for isolate K.2 /5.1 was calculated as 180,29 ml. In Input™, the EC_{50} value for isolate 63.111 was 3.67 ml, for isolate K.2/5.1 EC_{50} value was 56.83 ml and for isolate Fidan.1 the EC_{50} value was 3.72 ml.

Efficacy of Fungicides on Spore Germination

Spore germination was evaluated according to 4th day measurements. Observations were made in 4 different ocular fields of the microscope in each petri dish and germinated and non-germinated spores were counted in the ocular field since the spores were not homogeneously distributed. It was observed that all spores germinated even at the first measurements and at the highest dose of Proxanil™, Korgaren™, Agrifos™, Ridozeb™. When the effects of fungicides named Input and Captan™ on spore germination

were examined microscopically, it was observed that there was no germination even at the lowest dose of the preparations, while in the preparation named Dynasty™, germinated and non-germinated spores in the determined areas could be counted and calculated as a percentage. Dynasty™ spore germination percentage values for isolates are given in Table 4.

Table 4. Spore germination (%) ratio of isolates for Dynasty™

Doses (mgL^{-1})	Means of Spore Germination (K.2/5.1)	Means of Spore Germination (63.111)	Means of Spore Germination (Fidan.1)
10	0	0	0
50	0	0	0
100	0	0	96,52
250	93,35	98,50	100
500	100	100	100

DISCUSSION

Pistachio is an important strategic product for our country. Türkiye, which is one of the oldest shelled fruit species with high nutritional value and rich substances in its composition, is one of the origin regions of pistachio and ranks 3rd in the world in terms of production [20]. Pistachio is becoming an increasingly important product of increasing importance for our country. In pistachio cultivation, just like all other plants, plant protection problems that restrict cultivation are encountered. Especially in new plant gardens, plant diseases that occur during the development of plants, including fungal diseases, can cause serious problems. The increase in plant diseases may be caused by some inaccuracies in cultivation. The most extensive study on soilborne pathogens in pistachio in our country was carried out by Aydın et al. [7] and it was reported that the causal species of the disease were mostly Fusarium spp. species (*Fusarium solani*, *F.oxysporum*, *F.brachygibbosum* and *F.chlamydosporum*). *Fusarium* spp. have recently been reported in other pistachio growing countries in the world [8].

Captan 50% (Captan™), Azoxystrobin+ Metalaxyl-M+Fludioxanil 75+37,5+12,5 g/l (Dynasty™), Phosphorose Acid 400 g/l (Agrifos™), Hymexazol 8360 g/l) (Korgaren™), Metalaxyl-M (4%)+Mancozeb (64%) (Ridozeb™), Prothioconazole (160 g/l) + Spiroxamine (300 g/l) (Input™), Propamocarb (400 g/l) + Cymoxanil (50 g/l) (Proxanil) were used for *Fusarium oxysporum* (63. 111, Fidan.1) and *F.solani* (K.2 /5.1) isolates obtained in the study.

In Azoxystrobin+Metalaxyl-M+Fludioxanil (Dynasty™), the highest effect was observed in *F.oxysporum* (Fidan.1) with a rate of 79,17% at the dose of 250 mgL^{-1} recommended by the company. A similar effect was observed in another *F.oxysporium* isolate (63.111), at 500 mgL-1 with a rate of 78.66%. In *F.solani* (K.2/5.1), an effect of approximately 10% less than *F.oxysporum* isolates was observed. *In vitro* mycelial growth experiments carried out with the preparation named Dynasty™, it can be said that the effective dose is 250 mgL^{-1} recommended by the company. In the spore germination study, high percentage inhibition was observed in all 3 isolates. Gökalp et al. [21], in a study on Fusarium species on grass seeds, similarly found the plant protection product Dynasty™ to be 82.3% effective. In the present this study, an interesting result emerged when the EC_{50} values of the preparation were analysed. In *F.oxysporum* (Fidan.1), a low value of 19.53 ml was encountered, but in another *F.oxysporum* (63.111), the EC_{50} value was 147.22 ml, which was approximately 7 times higher. It is thought that this may be due to the difference in age, location or pathotype of the plants from which the two isolates were isolated.

In Captan™, the highest mycelial inhibition in all 3 isolates was observed on 150 mgL^{-1}, which is half of the dose recommended by the company. Among the applied preparations, the highest inhibition effect was observed in *F.solani* (K.2/5.1) with an average of 62.79%. Inhibition of mycelial growth remained approximately the same at doses after 150 mgL^{-1}. *F.oxysporum* isolates were similar with Fidan.1 55,81% and 63.111, 51,79% for mycelial inhibition. No spore germination was observed until the end of the 21st day for all 3 preparations even at the lowest dose. This shows that the effect of the preparation with 50% diameter active ingredient on conidia germination is 100%. When looking to EC_{50} values, approximate values are observed in both *F.oxysporum* isolates. Zang et al. [22] also observed that the preparation was effective for *in vitro* tests against damping-off in vegetables and increased seed germination by 52.1% and also increased field emergence in maize plants. Yaman [23], *in vitro* study on *F.oxysporum*, *F.solanum*, Rhizoctonia solani AG+ causing root rot in kiwis, observed that mycelial growth was completely inhibited with Captan at 2% concentration of organic 21 salt.

The commercial preparation Ridozeb containing Metalaxyl-M+Mancozeb showed different effects between two Fusarium species. Starting from the dose of 100 mgL^{-1}, which is a lower dose of the recommended dose, 100% inhibition was achieved in *F.solani* and mycelial growth was completely inhibited. At 500 mgL^{-1}, which is the highest dose in the experiment, 100% inhibition was again obtained by Fidan.1, while 51.59% inhibition was observed in

another *F.oxysporum* (63.111) at the recommended dose (250 mgL^{-1}). It is thought that the difference in the effect between Fusarium species is related to the age of the isolated plant and it is recommended that this factor should be taken into consideration in the trials of this preparation. In a study on the mycelial development of *F.oxysporum* f.sp. *dianthi*, similarly, Metalaxyl+Mancozeb was found to be 58.77% effective [24]. In the spore germination trials of fungicides, conidia were germinated in all isolates. When evaluating EC_{50} values of fungicides, a very low value of 63.28 g was recorded for *F.solani* isolate. When *F.oxysporum* isolates were compared in terms of EC_{50} value, it was calculated that the EC_{50} value of isolate 63.111 (384.87 g) was approximately 3 times higher than the EC_{50} value of isolate Fidan.1 (103.92 g).

Lower inhibition was observed in all doses of Agrifos™, a plant protection product with the active ingredient Phosphorose acid, when comparing other fungicides. The highest effect in all isolates was observed at 1000 mgL^{-1}, which is the highest dose studied. Among all isolates, the highest effect was observed in *F.oxysporum* (Fidan.1) with 33.33%, while no inhibition was observed in another *F.oxysporum* (63.111) in 1000 mgL^{-1} dose and very low inhibition was observed at 1000 mgL^{-1} dose with 18.25%. In *F.solani* (K.2/5.1), the highest mycelial inhibition ratio was 25.23%. In spore germination experiments, similar low effect results were obtained with mycelial development and germination was found to be 100% in all petri dishes. When the EC_{50} values for the preparation were analysed, 4159.1 g in Fidan.1, 2758.86 g in and 2069 g in K.2/5.1 respectively. As a result, fungicide containing phosphorose acid was found to be low *in vitro* effects for *F.solani* and *F.oxysporum* species.

In Korgaren™, containing Hymexazol active ingredient, the highest inhibition effect was observed in Fidan.1 (*F.oxysporum*) isolate at 500 mgL-1 dose recommended by the company and 100% inhibition was observed at this dose. The effect increased with increasing doses and at the highest dose of 750 mgL-1, 63.111 (*F.oxysporum*) with 83.33% and K.2/5.1 (*F.solani*) 77.38%. The fungicide was found highly effective in mycelial growth inhibition. In spore germination experiments, 100% germination rate was observed in all petri dishes and Korgaren was found ineffective. EC_{50} values were obtained parallel to mycelial inhibition. The EC_{50} values of the preparations were calculated as 259,46 ml for 63.111 isolate, 259,13 ml for K.2/5.1 isolate and 250,12 ml for Fidan.1 isolate, respectively.

Proxanil™ containing Propamocarb+Cymoxanil did not provide inhibition against *F.oxysporum* (63.111, Fidan.1) in *in vitro* mycelial growth, while it showed approximately the same percentage effect (51.16%) against *F.solani* isolate at 3 doses (100 mgL^{-1}, 175 mgL^{-1}, 350 mgL^{-1}) starting from 100 mgL^{-1} dose. In spore germination trials, it was observed that inhibition was not achieved in both species. Considering the EC_{50} values of the preparation, EC_{50} values could not be calculated for *F.oxysporum* isolates due to the percentage effect values and EC_{50} value for *F.solani* isolate was calculated as 180.29 ml.

In the *in vitro* study carried out with Input™ containing Prothioconazole+Spiroxamine, it was observed that the highest inhibition was achieved on *F.oxysporum* and *F.solani* from the lowest dose experimented (5 mgL^{-1}). Even at 20 mgL^{-1}, which is one fifth of the dose recommended by the company, it was observed that inhibition was obtained in all *F.oxysporum* isolates. In *F.solani*, 100% inhibition was achieved at 100 mgL^{-1}, which is the dose recommended by the company. The same success was recorded in spore germination trials and conidia germination could not be observed in all isolates. EC_{50} values calculated based on the percentage effect were quite low. 3.72 ml for Fidan.1 and 3,67 ml for 63.111 and 56.83 ml for K.2/5.1 were recorded. Sanssene et al. [25] found that prothioconazole active ingredient was effective at a good level between 60% and 70% within 7 days in a study against Septoria in wheat. Küçükkaya [26] found that Prothioconazole+ Tebuconazole (250 g/L+150 g/L) had a MIC (minimum inhibitory concentration) value of 40 µg/ml and 95.78% efficacy with a MIC value of 40 µg/ml in a study on *Fusarium oxysporum* f.sp. *radicis-lycopersici*, a soil-borne pathogen.

CONCLUSIONS

Fusarium pathogens are prominent among soil-borne pathogens in pistachio. Since the disease causes more problems in young plants and nurseries, it is very important to produce healthy plants at the nursery production stage. First of all, studies on disease resistant individual breeding should be prioritised. Since Fusarium wilt disease has become widespread in our country in recent years, it is obvious that this issue should be addressed in breeding studies.

In this study, the *in vitro* effects of some selected fungicides on the fungal isolates obtained were investigated. Fungicides licensed against fungal diseases on some other plants were used in the determination of plant protection products.

In conclusion, it is first studied for controlling of Fusarium spp., which are becoming increasingly common in young pistachio orchards. *In vitro* experiments, Input™ containing Prothioconazole+ Spiroxamine inhibited mycelial growth and conidial germination of *F.oxysporium* and *F.solani* isolates by almost 100% and it is thought that it can be a very effective preparation in vivo studies to control of the disease. Although there were slight differecences of Korgaren containing Hymexazol and Dynasty containing Azoxystrobin+Metalaxyl-M+Fludioxanil on *F.oxysporum* isolates among experimented doses, the efficacy level of inhibition of all isolates was found to be quite higher. In the trials with Metalaxyl-M+Mancozeb (Ridozeb™), the efficacy against *F.solani* was found to be 90%, but the efficacy level against *F.oxysporum* isolates remained around 76%. The efficacy levels of Propamocarb+Cymoxanil (Proxanil) and Phosphorose Acid (Agrifos) were very low. The effective doses for each fungicide were determined in the studies, which can guide further studies and prevent excessive chemical usage.

In line with the data obtained as a result of the researches, especially in vivo trials should be carried out and the results should be shared and be recommend to producers for Fusarium wilting disease, which is a significant problem on pistachio.

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