

Turkish Journal of Agriculture - Food Science and Technology

Available online, ISSN: 2148-127X | www.agrifoodscience.com | Turkish Science and Technology Publishing (TURSTEP)

In Vitro Efficiency of Some Fungicides Against *Neoscytalidium dimidiatum* (Penz.) Crous and Slippers Causing Sudden Shoot Dry on Apricot Trees

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ARTICLE INFO	A B S T R A C T
Research Article	Turkey is known as a top producer and exporter of apricots, both fresh and dried. More than half of Turkey's apricot production is provided by Malatya province. There are many pests and diseases affecting apricots in addition to climatic factors such as front and hail. Partial shoet drying and
Received : 31/01/2021 Accepted : 06/03/2021	sudden drying in apricots in addition to enhance factors such as nost and half. Fatual shoet drying and sudden drying in apricot orchards have increased in recent years. In this study, it was aimed to determine the chemical control possibilities of <i>Neoscytalidium dimidiatum</i> (Penz.) Crous &Slippers, which is one of the fungal agents that cause partial branch drying and sudden drying in apricot orchards in Malatya province by in vitro studies. For this purpose, Kale 4-C isolate of <i>N. dimidiatum</i> collected from Malatya in a previous study was used. Mycelial growth of <i>N. dimidiatum</i>
<i>Keywords:</i> Apricot Neoscytalidium dimidiatum Fungicide Chemical control Botryosphaeriaceae	Kale 4-C isolate in PDA were observed in 0 (control), 0.01, 0.03, 0.1, 1, 3, 10, 30 ve 100 µg/mL concentrations of Azoxystrobin 250g/L, Trifloxystrobin %50, Tebuconazole %25, Floupyram 200 g/L+Tebuconazole 200 g/L, Cyprodinil+Fludioxonil %37.5+25, %70 Thiophanate-Methyl and 400 g/L Phosphorous acide fungicides applications. As a result of the study, Floupyram 200g/L+Tebuconazole 200g/L ve Cyprodinil+Fludioxonil %37.5+25 fungicides effectively inhibited the mycelial growth of the fungus.
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Introduction

Turkey has a location where climate and soil conditions are appropriate for cultivating several crops. Turkey is among important fruit cultivating countries throughout the World. One of the most grown fruit crops is apricot in Turkey (Ercisli, 2009).

Apricot (*Prunus armeniaca* L.) belongs to Rosales order, Rosaceae family, Prunoidae sub-family, Prunus genus, and Prunophora sub-genus (Janick and Moore, 1979). Centers of origin of apricot is a wide area from East Turkistan to China and the cultivation of it in Anatolia dates from 2000 years ago (Asma, 2000; Eriş ve Barut, 2000; Gülcan, 2001; Özçağıran ve ark. 2004; Sevindik et al., 2020). Apricot is widely cultivated in Elazığ-Erzincan-Sivas provinces, Mediterranean Region, Kars-Iğdır provinces, Agean Region, Central Anatolia Region, and Thrace Region in Turkey where it is widely grown in Malatya province with different cultivars and different intensities (Asma, 2000; Asma ve Kan, 2001; Durmuş ve Yiğit, 2003; Ercisli, 2004). Turkey supplies 20 % and 8590 % of fresh and dried apricot production of the world, respectively (Gezer et al, 2003).

Apricot production varies according to the years affected by climatic factors, primarily spring late frosts as well as diseases and pests. The reverse effects of these biotic and abiotic agents cause economically serious crop losses. The main diseases of apricot are *Monilinia laxa* Aderh et Ruhl and *Monilinia fructigena* Aderh et Ruhl, *Coryneum beijerinckii* Oudem), *Cytospora cincta* Sacc.) *Pseudomonas syringae* pv *syringae* Van. Hall, Plum pox virus) and root rot diseases such as *Armillaria mellea* (Vall) Quel. and *Rosellinia necatrix* Prill. (Asma, 2000, Kaygısız, 2000).

Besides these diseases, *N. dimidiatum* was detected as one of the most important agents responsible for the sudden drying of branches and shoots causing economic losses in apricot orchards (Oksal and Özer, 2020). The necessity for this study was that there were limited studies in the World and no research about the chemical control of this disease in apricot. It was aimed to determine the chemical control possibilities against *N. dimidiatum* in apricot and to prevent the unnecessary or excessive usage of chemicals within this study

Materials and Methods

The isolate used in the study is the most pathogenic isolate obtained from a previous study, *N. dimidiatum* Kale 4-C. Potato dextrose agar (PDA) was used to observe the mycelial growth and water agar (WA) was used to investigate conidia germination of *N. dimidiatum* 4-C isolate in efficacy determining studies of the isolate against fungicides. Other fungicides used in the study are shown in Table 1.

Determination of Effects of Fungicides on Mycelial Growth of N. Dimidiatum

PDA (Merck, Germany) was prepared for each concentration of the fungicides individually, autoclaved at 121°C for 20 min, and cool down to 40–45°C. Fungicides used in the trials were weighed and stock solutions and dilutions were prepared in sterile water.

Fungicides were performed with trials the concentrations of 0 (control) 0.01, 0.03, 0.1, 1, 3, 10, 30, and 100 µg/mL active substances to determine the inhibition rates of the mycelial growth of the isolate. These concentrations were prepared from the stock solutions from active substance concentration. Prepared PDA growth cultures were sterilized and cooled down to 45-55°C and fungicide concentrations prepared from stock solutions were added. Growth media were poured into 9 cm diameter sterile Petri dishes 20 mL for each. These growth media were dried for 1-2 days and later were inoculated with colony disks of fungicide-free isolates with the help of a 4 mm diameter cork-borer. The side of fungal growth of the disks were inoculated to growth media as 3 disks for each petri dish.

After isolation, the inoculated isolates were incubated in dark conditions for 7 days 25°C. Trials were performed as 5 repetitions for each isolate and for each concentration. Petri dishes were observed after the 4th day and it was noticed that colonies got close to the petri edge on the 7th day. So, the diameters of the colonies of control and fungicide concentration were measured in three different directions and arithmetic mean was calculated on the 7th day. The colony diameter of the control was admitted as 100 % and the growth rate of the fungicide concentration applied colonies were determined by comparing the diameters with the control.

Determination of Effects of Fungicides on Conidia Germination

Water agar (WA, 1.5 %) was prepared, sterilized, and cooled down to 40°C. In the meantime, fungicides were weighed and stock solutions and dilutions were prepared in sterile water. Fungicide concentrations of 0 (control), 0.1, 1, 3, 10, 30, and 100 μ g/mL prepared from the stock solutions were added to the growth media and poured to 9 cm diameter petri dishes 20 mL for each and let dry for one day. *N. dimidiatum* Kale 4-C isolate was grown in PDA at 25°C for 3-7 days in dark conditions for abundant sporulation to be used in the trials. 5-10 mL distilled water

was added to the petri dishes, conidia were harvested by sterile baget and filtered from a filter paper. The intensity of conidia suspension was adjusted to 1×10^6 conidia/mL by Thoma slide. 0.2 mL of this suspension was added to the growth media containing fungicide and control growth media and to agar surface via sterile bacteria diffuser. Petri dishes with conidia suspensions were incubated at 25°C in dark conditions for 24-48 hours. At the end of this period, conidia germination was controlled and conidias were counted when there was 90% germination in the control petri dishes. 100 conidia for each petri dish were counted under the microscope and conidia numbers and germination rates were calculated (Figure 1). Trials were performed for 5 repetitions for each concentration of the fungicide.

Statistical Analysis

Data obtained from the study were subjected to variance analyses via package software and the differences between the means were determined by the LSD test (P \leq 0.05). Percentage effects of fungicides were calculated according to the Abbott formula (Abbott, 1925).

Results and Discussion

Parameter values obtained from this study were shown in Table 2a, b. The study was repeated two times with 5 repetitions for each trial and both were evaluated together since there was no statistical difference between them. The statistical differences of the chemicals on the conidia germination and mycelial characteristics of *N. dimidiatum* were determined (Table 2, P<0.05).

It was determined that fungal growth of *N. dimidiatum* Kale 4-C isolate was completely inhibited in the Floupyram 200 g/L+Tebuconazole 200 g/L containing media on the 7th day beginning from the concentration of 10 μ g/mL. When % effects were analysed, the most effective concentrations were found to be 10, 30, and 100 μ g/mL with 100% effect following 3.1, and 0.1 μ g/mL concentrations with the rate of 90.56, 84.70, and 72.7%, respectively (Figure 2).

Fungal growth of *N. dimidiatum* Kale 4-C isolate was completely inhibited in the Cyprodinil+Fludioxonil 37.5%+25 containing media beginning with the concentration of $30 \ \mu\text{g/mL}$ on the 7th day. When % effects were analysed, the most effective concentrations were found to be 30 and 100 $\ \mu\text{g/mL}$ containing media with 100% inhibition effect. No inhibition effect was determined in 0.01 ve 0.03 $\ \mu\text{g/mL}$ concentrations (Figure 3).

The most effective inhibition in Trifloxystrobin %50 containing media was found to be 100 μ g/mL concentration on the 7th day whereas the most effective concentrations were found to be 100 and 3 μ g/mL concentrations following them with 30, 10, 1, ve 0.03 μ g/mL concentrations against *N. dimidiatum* Kale 4-C isolate. No inhibition effect was observed in 0.01 μ g/mL concentration.

The most effective inhibition against *N. dimidiatum* Kale 4-C isolate was determined to be 100 μ g/mL concentrations in Azoxystrobin 250 g/L containing media. The most effective concentrations were found to be 100 and 30 μ g/mL concentrations following 10 μ g/mL concentration. No inhibition effect was observed in 3, 1, 0.1, 0.03 and 0.01 μ g/mL concentrations.

Table 1. General information about fungicides used in this study

0		2		
Active substance and quantity	Ft	Application dose/100L water	Fungicide group	Commercial name
Azoxystrobin 250 g/L	SC	50 mL-75 mL-100 mL	Strobilurin	QUADRİS®
Trifloxystrobin 50%	WG	10 g-12.5 g-15 g	Strobilurin	FEATURE [®]
Tebuconazole 25%	WP	40 g-60 g-80 g	Triazole	FOLİCUR®
Floupyram 200 g/L+Tebuconazole 200 g/L	SC	15 mL-25 mL-35 mL	Benzamide + Triazole	LUNA EXPERİENCE®
Cyprodinil +Fludioxonil 37.5%+25	WG	30 g-40 g-50 g	Pyrimidine + Pyrrole	SWİTCH®
%70 Thiophanate-Methyl	WP	40 g-60 g-80 g	Benzimidazole	SUMİTOP®
400 g/L Phosphorous Acide	SL	300 mL-400 mL-500 mL		AGRI-FOS®

Ft: Formulation type

 Table 2a. The effects of chemicals with different concentrations on mycelial growth and conidia germination of Neoscytalidium dimidiatum

	Effect against mycelial growth			Effect against conidia germination			
Actives	Doses	Average mycel	%	Doses A	verage germinated conidi	a %	
	μg/mL	diameter (mm)	Effect	µg/mL	(number)	Effect	
	0.01	6.25 ^a	0.00	-	-	-	
	0.03	6.25 ^a	0.00	-		-	
	0.1	6.25 ^a	0.00	0.1	92.80 ^b	4.33	
	1	6.25^{a}	0.00	1	89.60°	7.63	
Azoxystrobin 250g/l	3	6.25 ^a	0.00	3	88.40°	8.87	
	10	1.77°	71.68	10	85.20 ^d	12.16	
	30	1.44 ^c	76.90	30	81.20 ^e	16.29	
	100	1.18 ^a	81.06	100	74.60 ¹	23.09	
	Control	6.25ª	0.00	Control	97.00 ^a	0.00	
	LSD	0.14	0.00	LSD	2.40		
	0,01	6.25 ^a	0.00	-	-	-	
	0.03	2.20°	64.80	-		-	
	0.1	2.22°	64.48	0.1	92.20°	4.95	
	1	1.04°	/3./0	1	88.20°	9.07	
Trifloxystrobin 50%	5	1.10 ⁻ 1.20 ^{de}	82.40 70.20	5 10	87.80 ⁻ 82.80d	9.40	
	20	1.50 ^m	79.20 81.76	20	05.00° 75.20°	15.01	
	100	1.14 0.88f	85.02	100	73.50 [°]	22.47	
	Control	0.00 6.25ª	0.00	Control	24.20 97.00ª	0.00	
	LSD	0.25	0.00	LSD	1 97	0.00	
	0.01	6.25ª	0.00	-	-	_	
	0.01	6.25 ^a	0.00	_	_	_	
	0.05	6.25 ^a	0.00	0.1	93 60 ^a	3 51	
	1	1.62ª	74.05	1	80.00 ^b	17.53	
	3	1.42 ^a	77.31	3	82.00 ^b	15.46	
Tebuconazole 25%	10	1.10^{b}	82.34	10	91.80 ^c	5.36	
	30	0.63 ^b	89.92	30	76.40^{d}	21.24	
	100	0.00^{b}	100.00	100	79.40^{d}	18.14	
	Control	6.25 ^a	0.00	Control	97.00 ^a	0.00	
	LSD	9.93		LSD	3.73		
	0.01	1.87 ^b	70.11	-	-	-	
	0.03	2.08°	66.66	-	-	-	
	0.1	1.70^{d}	72.74	0.1	8.60 ^b	7.63	
	1	0.96 ^e	84.70	1	74.60 ^c	23.09	
Floupyram 200	3	0.59 ^e	90.56	3	40.60 ^d	58.14	
g/L+Tebuconazole 200 g/L	10	0.00 ^r	100.00	10	56.80 ^e	41.44	
	30	0.00^{1}	100.00	30	9.60 ¹	90.10	
	100	0.001	100.00	100	7.001	92.78	
	Control	6.25 ^a	0.00	Control	97.00 ^a	0.00	
	LSD	0.10	0.00	LSD	5.83		
	0.01	6.25 ^a	0.00	-	-	-	
Cuprodinil - Eludiovonil	0.03	0.25°	0.00	-	- -	-	
	0.1	0.49	92.21	0.1	20 80°	60.28	
	1	0.31	95.04	2	29.80 17.60 ^d	09.20 81.86	
$37.5\% \pm 25$	10	0.24 0.12d	90.10	10	9 20e	01.00 00.52	
51.570725	30	0.12	100.02	30	9.20 8 20e	90.52	
	100	0.00	100.00	100	6.80°	92.99	
	Control	6.00 6.25ª	0.00	Control	97.00	0.00	
	LSD	0.11	0.00	LSD	5.45	0.00	

	Effect against mycelial growth			Effect against conidia germination		
Actives	Doses	Average mycel	%	Doses	Average germinated	%
	µg/mL	diameter (mm)	Effect	µg/mL	conidia (number)	Effect
	0.01	6.25 ^a	0.00	-	-	-
	0.03	6.25 ^a	0.00	-	-	-
	0.1	6.25 ^a	0.00	0.1	96.80 ^a	0.21
	1	1.74 ^b	72.10	1	95.20ª	1.86
700/ Thiophopota Mathul	3	1.04 ^c	83.39	3	92.20 ^b	4.95
70% Thiophanate-Methyl	10	1.38 ^d	77.98	10	88.80 ^c	8.45
	30	1.26 ^e	79.87	30	76.00 ^d	21.65
	100	0.81^{f}	87.01	100	25.00 ^e	74.23
	Control	6.25 ^a	0.00	Control	97.00 ^a	0.00
	LSD	0.08		LSD	2.65	
	0.01	6.25	0.00	-	-	-
	0.03	6.25	0.00	-	-	-
	0.1	6.25	0.00	0.1	97.00 ^a	0.00
	1	6.25	0.00	1	96.60 ^a	0.41
400 c/l Phasmharous Asida	3	6.25	0.00	3	92.40 ^b	4.74
400 g/1 Phosphorous Acide	10	6.25	0.00	10	95.60 ^b	1.44
	30	6.25	0.00	30	95.20°	1.86
	100	6.25	0.00	100	94.20 ^d	2.89
	Control	6.25	0.00	Control	97.00 ^a	0.00
	LSD			LSD	1.47	

 Table 2b. The effects of chemicals with different concentrations on mycelial growth and conidia germination of Neoscytalidium dimidiatum

The most effective inhibition in Thiophanate-Methyl 70% containing media was found to be in 100 μ g/mL concentration against *N. dimidiatum* Kale 4-C isolate on the 7th day. 100 ve 3 μ g/mL concentrations were found to be the most effective concentrations in % effect followed by 30 μ g/mL concentration. No inhibition effect was observed in 0.1, 0.03 and 0.01 μ g/mL concentrations.

The most effective inhibition was recorded in 100 μ g/mL concentration in Tebuconazole 25% containing media against *N. dimidiatum* Kale 4-C isolate on the 7th day. No mycelial growth was observed in this concentration. The most effective inhibition was recorded in 100 and 30 μ g/mL concentrations followed by 10 μ g/mL concentration. No inhibition effect was observed in 0.1, 0.03, and 0.01 μ g/mL concentrations against the isolate.

No inhibition effect on mycelial growth of the isolate was observed when colony diameters were measured on the 7th day in phosphorus acid-containing media (Figure 4).

Similar results were obtained in conidia germination effects of the fungicides against the pathogen in vitro. Floupyram 200 g/L+Tebuconazole 200g/L and Cyprodinil+Fludioxonil 37.5%+25 were found to be effective inhibiting conidia germination of the fungus the least inhibition effect was obtained in phosphorus acid-containing media.

Lin et al. (2017b) investigated inhibition effects of cyprodinil + fludioxonil, azoxystrobin + difenoconazole, metiram, trifloxystrobin, pyraclostrobin, azoxystrobin, iminoctadine, and tebuconazole fungicides on the mycelial growth and conidia germination of *N. dimidiatum*, the agent of pitaya cancer disease. According to the Petri trial results, cyprodinil + fludioxonil, azoxystrobin + difenoconazole, and tebuconazole chemicals inhibited the mycelial growth of the fungus. The results of this study are parallel with our study results.

XiaoYong et al. (2018), investigated the effects of pyraclostrobin, azoxystrobin, tebuconazole, and hexaconazole against pitaya cancer in China. Fungicides used in Petri trials were found to be effective whereas pyraclostrobin, azoxystrobin and tebuconazole were the most effective chemicals against the fungus in vivo trials. Azoxystrobin and tebuconazole Petri trials show similarity with our study.

Kılınç and Güldür (2020) investigated the effects of five chemicals in vitro conditions against *N. dimidiatum* in pistachio. According to the study results, Azoxystrobin + Propiconazole was found to be the most effective fungicide with 88.49% effect, whereas Thiophanate methyl + Tetraconazole was the second effective one with 85.51% effect, the third effective was Pyraclostrobin + Fluaxapyroxad with the effect of 77.77%. The fifth and the sixth effective fungicides were Metrafenone and Phosphorous acid with the effects of 8.73 % and 4.56%, respectively. Within the fungicides used, Phosphorous acid did not Show any inhibitor effect in our study, but only mycelial growth was investigated in the mentioned research.

Inhibition effects of 7 different fungicides against mycelial growth and conidia germination of *N. dimidiatum*, which is an important disease agent in apricot trees were investigated within the study. Especially fungicides with Floupyram 200g/L + Tebuconazole 200g/L and Cyprodinil + Fludioxonil 37.5% +25, which were found to be effective inhibiting mycelial growth of the fungus were hopeful for future studies. But none of the fungicides in the trials inhibited the conidia germination in 100% percent effect.

N. dimidiatum has been detected in apricot, grapevine, willow, walnut, tomato and potato in Turkey until now (Dervis et al., 2019, Turkölmez et al., 2019, Turkölmez et al., 2020, Oksal et al., 2020, Oksal et al., 2020).



Figure 1. Cultural and morphological characteristics of Neoscytalidium dimidiatum

(A) Coloni on PDA (7 days of incubation at 25°C); (B) Maturity stages of arthroconidia; (C) Germinated and non-germinated conidia.





a. Control





c. 30 µg/mL d. 100 µg/mL Figure 2. In vitro efficacy of Floupyram 200g/L+Tebuconazole 200g/L against Neoscytalidium dimidiatum;

(A) Control; (B) 10 μ g/mL concentration; (C) 30 μ g/mL concentration; (D) 100 µg/mL concentration.



a. Control



c. 30 µg/mL



b. 10 μg/mL



d. 100 µg/mL

Figure 3. In vitro efficacy of Cyprodinil+Fludioxonil %37,5+25 against Neoscytalidium dimidiatum; (A) Control; (B) 10 µg/mL concentration; (C) 30 µg/mL concentration; (D) 100 µg/mL concentration





a. Control b. 10 μg/mL Figure 4. In vitro efficacy of Phosphorus acid against Neoscytalidium dimidiatum; (A) Control; (B) 100 µg/mL concentration.

Symptoms of *N. dimidiatum* can be characterized by branch and leaf dryness, bark lesions, discoloration of xylem tissues, longitudinal wood necrosis and extensive gumming. General dieback signs were also observed leading to complete defoliation of leaves and ultimately death of trees in advanced stages. N. dimidiatum is becoming a new danger to agricultural production.

Since producers do not carry out cultural practices, hygiene and quarantine precautions are not being applied properly and because of the negative effects of global climate changes on the diseases, fungal diseases cause serious problems lately in apricot.

There are limited studies on biology, epidemiology, and control of the causal agent in different hosts. Field trials are needed to be performed to reveal the accurate effects and to determine the field performances of the fungicides. Field trials will also help to choose the fungicides to be applied in the orchard and nurseries but also fungicide inhibition effects as well as the resistance of the agent against these fungicides should be taken into consideration.

It is thought that study about in vitro trials of fungicides against N. dimidiatum will be helpful for future studies since there are limited studies on this subject.

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