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EFFICACY OF A RANGE OF FUNGICIDES AGAINST FUNGAL DISEASES OF WALNUT IN THE SOUTHERN FRUIT-GROWING ZONE OF KAZAKHSTAN

Abstract

The most widespread and effective method of protecting walnut trees from diseases is through chemical treatments. With the increasing variety of fungal pathogens affecting walnut trees in the southern zone of fruit production in Kazakhstan, there is a need to study the effectiveness of fungicides against these pathogens. Therefore, laboratory and field studies were carried out to evaluate the effectiveness of several fungicides against the most common diseases of walnut trees caused by *Fusarium* and *Alternaria* fungi.

The effectiveness of fungicides with different mechanisms of action was studied for the first time. The fungicides evaluated were Score (difenoconazole, 250 g/l), Horus (cyprodinil, 750 g/kg), Stroby (kresoxim-methyl, 500 g/kg), Luna Tranquility (fluopyram, 125 g/l + pyrimethanil, 375 g/l) against walnut diseases in the southern zone of fruit cultivation in Kazakhstan. The results of both laboratory and field experiments showed that the most effective fungicide against fungal pathogens on walnut was Score (difenoconazole, 250 g/l). Stroby (kresoxim-methyl, 500 g/kg) also demonstrated over 80% biological efficacy against *Alternaria alternata*.

The results of this study will assist local growers in managing various fungal diseases in walnut plantations.

Keywords: walnut, fungal diseases, fungicides, antifungal activity, *Alternaria*, *Fusarium*, fungal isolates.

Introduction

Walnut (*Juglans regia* L.) is widely spread all over the world and with proper care can yield large quantities of this valuable crop. Walnut growing is a priority in agricultural development in the Republic of Kazakhstan [1]. The favorable climatic conditions in Kazakhstan for plant development also create favorable conditions for the development of various diseases. In previous studies conducted through laboratory microscopy and field monitoring, we found that *Alternaria* and *Fusarium* are the most commonly isolated fungi in the southern fruit-growing region of Kazakhstan [2]. Every year, without protective measures, a significant portion of our potential yield is lost due to diseases caused by these fungi [2]. Preventive and agrotechnical measures, when carried out correctly and in time against plant diseases, have a positive effect on reducing the population of pathogens. However, they are not sufficient in cases of mass and strong development of diseases. The most common and effective way to protect walnuts from diseases is through the use of chemical methods. The in vitro method allows for the quick assessment of the effectiveness of chemical treatments against pathogens [3-5]. However, the "List of pesticides allowed for use on the territory of the Republic of Kazakhstan" does not currently include fungicides for protecting walnuts from pests [6]. In spite of efforts to combat them, fungal pests continue to cause significant economic harm to walnut plants and therefore need to be constantly monitored. As a result, there has been a search for effective fungicide treatments to control fungal pathogens affecting walnuts. Fungicides containing active

substances such as difenoconazole, cyprodinil, kresoxim-methyl, and fluopyram + pyrimethanil, which have shown high efficacy in other countries [7-9], were selected for testing. These preparations were chosen based on the fact that these fungicides, with contact, systemic, and systemic-contact actions, have been authorized for use in the Republic of Kazakhstan against fruit crop diseases and are currently registered on the List of pesticides [6].

Literature analysis suggests that *Fusarium* pathogens may have high levels of resistance [10]. *Alternaria alternata* is a well-known pathogen of many agricultural crops, and some reports indicate that this fungus is also responsible for causing leaf spot disease in walnut trees [11-13]. Brown apical necrosis (BAN) is caused by common polyphagous organisms, with *Alternaria* and *Fusarium* being the major genera [14]. However, the epidemiological aspects of BAN disease are not fully understood, and there is limited information available [15]. As a result, there is a need for improved phytosanitary programs to control this disease.

The study focused on examining strains of fungi from the *Fusarium* and *Alternaria* genera, which were taken from diseased walnut leaves, stems, and fruits in the southern fruit-growing zone of Kazakhstan. The purpose of the research was to assess the effectiveness of several fungicides against the most common fungal diseases of walnuts in the conditions of the southern fruit-growing zone in Kazakhstan.

During our field study, we observed the symptoms of the disease caused by *Alternaria alternata* fungus in late spring, when the leaves were fully unfolded, and also in early summer, about a month after flowering. The primary symptoms of the pathogen included the development of dark brown spots on the leaves, which gradually increased in size and coverage on the plant. On the fruit, the symptoms varied from slightly indented light brown spots to dark brown circular areas on the outer surface. These fungi thrive in air temperatures ranging from 25-30 °C, and within this temperature range, the disease actively spread throughout the orchard.

Methods and materials

In 2022-2023, studies were conducted on the effectiveness of fungicides in field conditions at "Integration – Turgen" LLP in the Almaty region. Laboratory studies were also carried out at "Kazakh Research Institute for Fruit and Vegetable Growing" LLP.

In the laboratory experiment, pathogens were isolated from infected walnut leaves and fruits and identified using microbiological and molecular-genetic methods [2]. Four strains of phytopathogenic fungi commonly found on walnut, including *Fusarium solani*, *Fusarium proliferatum*, *Fusarium graminearum*, and *Alternaria alternata* were used as test pathogens.

The efficacy of four fungicides was tested against the pathogens under aseptic conditions using the poison food technique [3]. The study investigated the effects of fungicides from three different chemical groups, each with a different mechanism of action on fungi.

- 1) Triazole - Score 250, EC (difenoconazole, 250 g/l);
- 2) Anilinopyrimidine - Horus 750, WDG (cyprodinil, 750 g/kg);
- 3) Anilinopyrimidine - Luna Tranquility, SC (fluopyram 125 g/l + pyrimethanil 375 g/l);
- 4) Strobilurins - Stroby 50%, WG (kresoxim-methyl, 500g/kg).

The fungicide solutions were prepared according to the manufacturer's recommendations [16] as follows: 120 µl of Luna Tranquility fungicide (fluopyram, 125 g/l + pyrimethanil, 375 g/l), 35 µl of Score fungicide (difenoconazole, 250 g/l), 20 µl of Stroby fungicide (kresoxim-methyl, 500g/kg), and 25 µl of Horus fungicide (cyprodinil, 750 g/kg) were added to 100 ml of sterile water. The aqueous solutions of these preparations were made on the day of the experiment and used immediately. Distilled water was used as a control. A seven-day pure culture of the pathogen, grown on potato-dextrose agar (PDA) medium, was used to seed both the control and experimental variants of the nutrient medium.

In each Petri dish containing PDA, 300 µl of an aqueous solution of the drug or water was applied and evenly distributed with a sterile glass spatula over the surface of the nutrient agar. The agar block with fungal mycelium was placed in the center of a Petri dish and incubated at 25 °C in a thermostat. Starting from the seventh day of incubation, the diameter of growing colonies was

measured until the complete overgrowth of the medium in the control cup. The experiments were conducted in triplicate, including a control variant without fungicides [3].

The efficiency of the composition was evaluated by measuring the rate of plant pathogen growth inhibition using the formula [17].

$$BE = \left(\frac{K - E}{K} \right) * 100, (1)$$

Where BE - biological efficiency, expressed as a percentage; K - colony diameter in the control, measured in centimeters; E - colony diameter in the experiment, measured in centimeters.

The level of damage to tree organs is assessed using the following empirical scales [18]:

0 - no lesions (immunity),

1 - up to 1% of organs, leaf area, or shoot surface area affected (high resistance),

2 - 1-10% of organs or leaf area, shoot surface affected (high resistance),

3 - 11-25% of organs or their surface area affected (medium resistance),

4 - 26-50% of organs or their surface area affected (increased susceptibility),

5 - more than 50% of organs or their surfaces affected (high susceptibility).

The spread of disease and the degree of disease development were calculated using the established VIZR (All-Russian Institute of Plant Protection) formulas [19]. The biological efficacy of fungicides was calculated using the empirical formula:

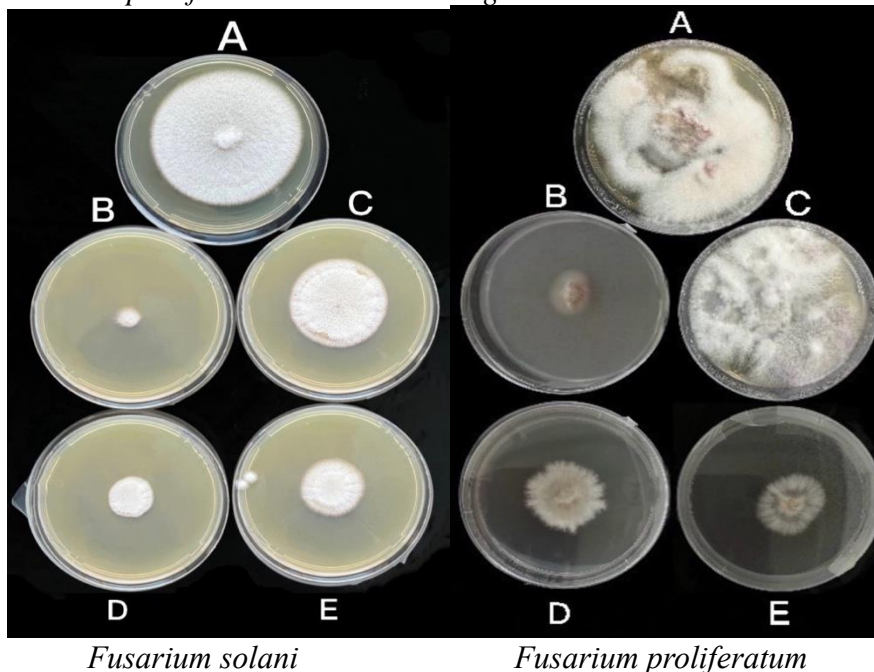
$$B(\%) = \frac{(Ik - Io)}{Ik} * 100, (2)$$

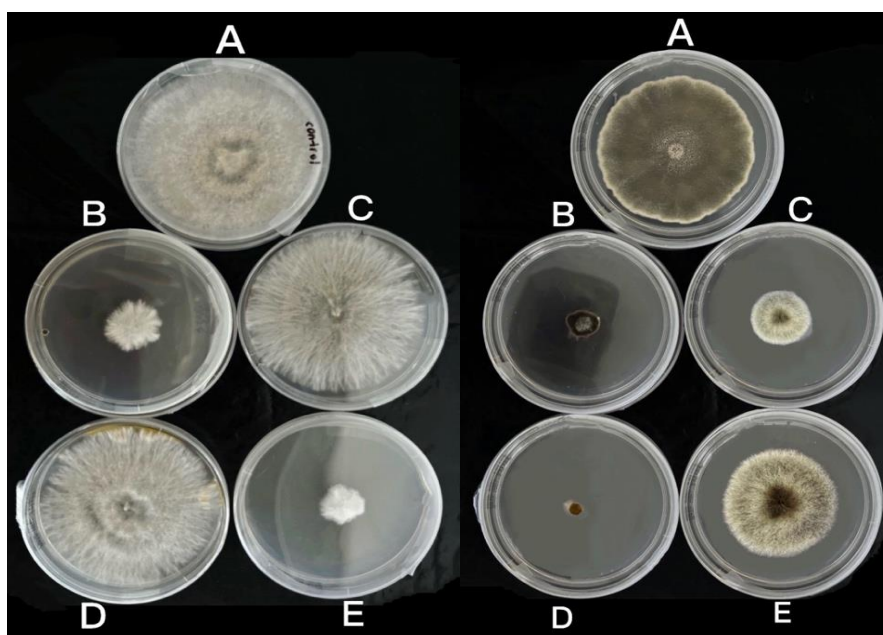
where B - biological efficiency, %; Ik - disease development in the control; Io - disease development in the experiment.

Results and discussion

Laboratory experiment

In Figure 1, the efficacy of various fungicides applied against isolated pathogenic fungi is demonstrated. All the fungicides tested inhibited the aerial mycelial growth of the *Fusarium* and *Alternaria* species to different extents. In the control group without fungicides, colonies of *Fusarium solani* and *Alternaria alternata* covered the surface of the nutrient medium in a Petri dish by day 9, while colonies of *Fusarium proliferatum* and *Fusarium graminearum* covered the medium by day 7.





Fusarium graminearum

Alternaria alternata

Figure 1 - Effect of different fungicides on mycelial growth of *Fusarium solani*, *Fusarium proliferatum*, *Fusarium graminearum*, and *Alternaria alternata* (seventh day after sowing). A- Control; B- Score (250 g/l difenoconazole); C- Horus (750 g/kg cyprodinil); D – Stroby (500g/kg kresoxim-methyl); E- Luna Tranquility (125 g/l fluopyram + 375 g/l pyrimethanil).

The efficacy of each fungicide applied against isolated pathogenic fungi is detailed in Table 1.

Table 1 - Effect of fungicides on vegetative growth of isolates of the fungi *Fusarium solani*, *Fusarium proliferatum*, *Fusarium graminearum*, and *Alternaria alternata*

Isolate	Colony diameter, mm				
	Score (250 g/l difenoconazole)	Horus (750 g/kg cyprodinil)	Stroby (500g/kg kresoxim-methyl)	Luna Tranquility (125 g/l fluopyram + 375 g/l pyrimethanil)	Control
	7th day				
<i>Fusarium solani</i>	0,5	5,7	2	3	7,5
	0,4	6	1,5	2,9	6,7
	0,7	4,9	2	3,4	6,9
Average value	0,53	5,53	1,83	3,10	7,03
Growth inhibition of isolate relative to control, %	92,42	21,33	73,93	55,92	
LSD ₀₅	0,73				
<i>Fusarium proliferatum</i>	1,8	8,9	3,8	3	9
	1,2	8	4,2	1,9	8
	2,3	8,9	4,3	2,7	9
Average value	1,77	8,60	4,10	2,53	8,67
Growth inhibition of isolate relative to control, %	79,62	0,77	52,69	70,77	
LSD ₀₅	0,62				
<i>Fusarium graminearum</i>	2,8	8,5	8,9	2	9
	2,6	7,2	7,1	1,8	8,6
	2,3	8	7,3	2,5	8,9
Average value	2,57	7,90	7,77	1,67	8,83
Growth inhibition of isolate relative to control, %	70,94	10,57	12,08	81,13	
LSD ₀₅	0,88				
	1,2	2,7	0,5	5	8

<i>Alternaria alternata</i>	0,8	2,9	1,2	4,7	7,7
	1,7	1,5	1,2	4,5	7,9
Average value	1,23	2,37	0,97	4,73	7,87
Growth inhibition of isolate relative to control, %	84,32	69,92	87,712	39,83	
LSD ₀₅	0,95				

The table indicates the results of laboratory experiments on antifungal activity against *Fusarium solani*. The fungicide Score (250 g/l difenoconazole) showed the highest effectiveness, inhibiting isolate growth by 92.42%, followed by Stroby (500g/kg kresoxim-methyl) at 73.93%. Luna Tranquility (125 g/l fluopyram + 375 g/l pyrimethanil) was less effective at 55.92%, while fungicide Horus (750 g/kg cyprodinil) showed low antifungal activity with an efficiency of 21.33%. In the control group, the colony diameters ranged from 6.7 to 9 mm in the experimental variants.

For isolates of *Fusarium proliferatum*, Score (250 g/l difenoconazole) demonstrated a high fungitoxic effect at 76.92%, while Luna Tranquility (125 g/l fluopyram + 375 g/l pyrimethanil) showed 70.77% effectiveness. Stroby fungicide (500g/kg kresoxim-methyl) reduced mycelial growth by 52.69%. Horus (750 g/kg cyprodinil) showed minimal inhibitory effect on isolate colonies, with a growth reduction of only 0.77%.

Luna Tranquility (125 g/l fluopyram + 375 g/l pyrimethanil) exhibited 81.13% inhibition, and Score (250 g/l difenoconazole) showed 70.94% antifungal activity against *Fusarium graminearum*. However, Horus (750 g/kg cyprodinil) and Stroby (500g/kg kresoxim-methyl) displayed low sensitivity with growth reductions of 10.57% and 12.08%, respectively.

Against *Alternaria alternata*, Score (250 g/l difenoconazole) and Stroby (500g/kg kresoxim-methyl) demonstrated high antifungal activity with growth reductions of 84.32% and 87.71%, respectively. Horus (750 g/kg cyprodinil) had the lowest efficacy at 69.92%, and Luna Tranquility (125 g/l fluopyram + 375 g/l pyrimethanil) showed poor efficacy at 39.83%.

Field experiment

A study was conducted from 2022 to 2023 to assess the effectiveness of fungicides against the *Alternaria alternata* pathogen on Liaohu variety trees at the «Integration – Turgen» LLP in the Almaty region.

The evaluation of the fungicides was carried out in field conditions suitable for the cultivation of the crop, with a natural infectious background.

The fungicide application scheme was as follows:

1. Score 250, EC (250 g/l difenoconazole) - 0.15 - 0.2 l/ha
2. Horus 750, WDG (750 g/kg cyprodinil) - 0.15 - 0.2 kg/ha
3. Stroby 50%, WG (500g/kg kresoxim-methyl) - 0.15 -0.2 l/ha
4. Luna Tranquility, SC (125 g/l fluopyram + 375 g/l pyrimethanil) - 0.8 - 1.2 l/ha
5. Control plants were sprayed with water for comparison purposes.

Fungicides were applied three times during the season, taking into account weather conditions and disease development - at full leaf opening, at the end of flowering, and 30 days after the second treatment. The rate of liquid used was 1000 liters per hectare. Each treatment was repeated three times during the experiment. Four surveys were conducted during the season: before applying fungicides, and 25, 50, and 80 days after the first survey. The presence and severity of disease development were visually assessed on four labeled branches on each tree, located on all four sides, and all the leaves were examined.

The intensity of plant infestation by *Alternaria alternata* pathogens during their peak development periods was determined as a result of monitoring walnut plantations in the study areas. In the southern zone of fruit growing in Kazakhstan, the fungal pathogenic complex of walnut plantations in 2022 - 2023 was mainly created by the *Alternaria alternata* pathogen, with a development intensity ranging from 55% to 73%.

Table 2 contains the recorded data on disease development and the effectiveness of various fungicides against *Alternaria alternata*.

Table 2 - Biological efficacy of a number of fungicides against *Alternaria Alternata* on walnut variety Liaohe (Almaty region, Enbekshikazakh district, 2022-2023).

№	Variant	Rate of consumption, litres, kg/ha	Development of disease, %			Biological efficiency, %		
			2022	2023	average	2022	2023	average
1	Control (without treatment)		31,5	39,47	35,47	-	-	-
2	Score (250 g/l difenoconazole)	0,15	3,60	2,80	3,2	88,53	92,85	90,7
3	Score (250 g/l difenoconazole)	0,2	2,93	2,53	2,73	90,60	93,51	92,1
4	Horus (750 g/kg cyprodinil)	0,15	11,80	19,40	15,6	62,32	50,02	56,2
5	Horus (750 g/kg cyprodinil)	0,2	8,33	11,40	9,9	73,69	71,02	72,4
6	Luna Tranquility, (125 g/l fluopyram + 375 g/l pyrimethanil)	0,8	18,80	26,20	22,5	39,82	32,80	36,31
7	Luna Tranquility, (125 g/l fluopyram + 375 g/l pyrimethanil)	1,2	14,47	19,00	16,7	53,94	51,42	52,7
8	Stroby (500g/kg kresoxim-methyl)	0,15	8,07	11,27	9,7	73,54	70,65	72,1
9	Stroby (500g/kg kresoxim-methyl)	0,2	6,33	5,53	5,9	79,19	86,23	82,7
	LSD 05	5,33						

Based on the data in Table 2, the fungicides Score (250 g/l difenoconazole) at a rate of 0.2 litres/ha showed the highest biological efficacy against *Alternaria alternata*, with 92.1% efficacy, and Stroby (500g/kg kresoxim-methyl) at a rate of 0.2 litres/ha showed 82.7% efficacy. The disease development in these variants was reduced by 6.6-12.9 times compared to the control. The biological efficiency of Luna Tranquility (125 g/l fluopyram + 375 g/l pyrimethanil) was 52.7% at a rate of 1.2 kg/ha, and that of Horus (750 g/kg cyprodinil) was 72.4% at a rate of 0.2 l/ha. When evaluated for phytotoxicity, they were found not to cause leaf scorch.

Conclusions

Results from in vitro studies indicate that the fungicide Score (250 g/l difenoconazole) effectively inhibits the growth of all tested pathogens. On the 7th day, mycelial growth inhibition ranged from 68.68% to 92.42%, and on the 14th day, it ranged from 59.25% to 91.71%. Fungicide Luna Traquility (125 g/l fluopyram + 375 g/l pyrimethanil) exhibited strong fungicidal effects against *Fusarium* (70.77% to 81.13%), but weaker effects against *Alternaria* (39.83%). Meanwhile, fungicide Horus (750 g/kg cyprodinil) demonstrated high effectiveness against *Alternaria* (69.92%) but weaker effects against *Fusarium* (0.77% to 10.57%). *Fusarium graminearum* showed low sensitivity to the fungicide Stroby (500 g/kg of kresoxim-methyl) at 12.08%, while other fungi displayed moderate to strong suppression when exposed to this fungicide.

The field evaluation of fungicides against the common fungal disease caused by the fungus *Alternaria* showed that fungicides Score (250 g/l difenoconazole) and Stroby (500 g/kg of kresoxim-methyl) effectively protected walnut leaves and fruits from the disease with a biological efficacy of over 90% and 80%, respectively.

The tests, conducted in both laboratory and field experiments, found that Score (250 g/l difenoconazole) was the most effective fungicide against fungal pathogens on walnuts. Additionally, Stroby (500 g/kg of kresoxim-methyl), demonstrated more than 80% biological efficacy against *Alternaria alternata*. This evaluation marks the first time the effectiveness of fungicides with different mechanisms of action against walnut fungal diseases in the southern zone of fruit growing in Kazakhstan.

Two-year field trials to determine the biological efficacy of several fungicides against fungal diseases on walnut conducted in three regions of Uzbekistan in 2018 and 2019 also showed that Score (250 g/l difenoconazole) showed the highest efficacy (90% to 100%). The efficacy of lower rates Cresoxin 50% WDG (kresoxim-methyl) was from 74.7% to 88.8% [8].

Score (difenoconazole) is a systemic fungicide with long-term preventive and therapeutic effects, designed to combat fungal diseases in fruit crops. It has the advantage of preventing resistance and minimizing the risk of phytotoxicity when used according to recommended guidelines. Stroby (kresoxim-methyl) is a next-generation fungicide that effectively targets a wide range of plant fungal pathogens. Unlike other systemic fungicides, Stroby (kresoxim-methyl) can be applied to wet foliage and is effective in low temperatures (1-4°C), which are common during early spring accompanied by prolonged rains. The findings of this study will be valuable for local growers and will help in developing effective protective measures against diseases in walnut crops [16].

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ҚАЗАҚСТАННЫҢ ОҢТҮСТІК ЖЕМІС ӨСІРУ АЙМАҒЫНДАҒЫ ГРЕК ЖАҢҒАҒЫҢ САҢҒЫРАҚ АУРУЛАРЫНА ҚАРСЫ БІРҚАТАР ФУНГИЦИДТЕРДІҢ ТИІМДІЛІГІ

Аңдатпа

Қолданыстағы грек жаңғағының ауруларымен күресу әдістерінің ішінде олардан қорғаудың ең кең таралған және тиімді әдісі химиялық әдіс болып табылады. Қазақстанда жеміс өсірудің оңтүстік аймағында жаңғақ саңырауқұлақтарының қоздырғыштарының түрлік құрамының артуы саңырауқұлақ қоздырғыштарына қарсы фунгицидтердің тиімділігін зерттеуді өзекті етеді. Осыған байланысты *Fusarium* және *Alternaria* тектес саңырауқұлақтар тудыратын кең таралған грек жаңғағының ауруларына қарсы бірқатар фунгицидтердің тиімділігін анықтау мақсатында зертханалық және далалық зерттеулер жүргізілді.

Қазақстанның оңтүстік жеміс өсіру аймағында жаңғақ ауруларына қарсы алғаш рет әсер ету механизмдері әртүрлі фунгицидтердің тиімділігі бағаланды: Скор (дифеноконазол, 250 г/л), Хорус (ципродинил, 750 г/кг), Строби (крезоксим - метил, 500г/кг), Луна Транквилити (флуопирам, 125 г/л + пириметанил, 375 г/л). Зертханалық және далалық тәжірибелерде жүргізілген сынақтардың нәтижелері бойынша Скор (250 г/л дифеноконазол) препараты грек

жаңғағының саңырауқұлақ ауруларының қоздырғыштарына қарсы ең тиімді болып шықты. Строби (500г/кг крезоксим - метил) препараты да *Alternaria Alternata* қарсы ең жоғары (80%-дан астам) биологиялық тиімділікті көрсетті.

Бұл зерттеудің нәтижелері жергілікті өндірушілерге грек жаңғағы плантацияларында әртүрлі саңырауқұлақ ауруларымен күресуде пайдалы болады.

Кілт сөздер: жаңғақ, саңырауқұлақ аурулары, фунгицидтер, саңырауқұлақтарға қарсы белсенділік, *Alternaria*, *Fusarium*, саңырауқұлақ изоляттары.

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ЭФФЕКТИВНОСТЬ РЯДА ФУНГИЦИДОВ ПРОТИВ ГРИБНЫХ БОЛЕЗНЕЙ ГРЕЦКОГО ОРЕХА В ЮЖНОЙ ЗОНЕ ПЛОДОВОДСТВА КАЗАХСТАНА

Аннотация

Среди существующих методов борьбы наиболее распространенным и эффективным способом защиты грецкого ореха от болезней является химический метод. С увеличением видового состава грибных возбудителей на грецком орехе в южной зоне плодводства Казахстана, остро встал вопрос об изучении эффективности фунгицидов против них. В связи с этим, были проведены лабораторные и полевые исследования на определение эффективности ряда фунгицидов против самых распространенных болезней грецкого ореха, вызываемыми грибами рода *Fusarium* и *Alternaria*.

Впервые проводилась оценка эффективности фунгицидов с различным механизмом действия: Скор (дифеноконазол, 250 г/л), Хорус (ципродинил, 750 г/кг), Строби (крезоксим – метил, 500г/кг), Луна Транквилити (флуопирам, 125 г/л + пириметанил, 375 г/л) против болезней грецкого ореха в южной зоне плодводства Казахстана. По результатам проведенных испытаний, как в лабораторном, так и в полевом опытах самым эффективным против возбудителей грибных болезней на грецком орехе оказался препарат Скор (250 г/л дифеноконазола). Наиболее высокую (более 80%) биологическую эффективность против *Alternaria Alternata* проявили также препарат Строби (500г/кг крезоксим - метила).

Результаты этого исследования будут полезны местным производителям в борьбе с различными грибными болезнями на плантациях грецкого ореха.

Ключевые слова: грецкий орех, грибные болезни, фунгициды, антифунгальная активность, *Alternaria*, *Fusarium*, изоляты грибов.