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RESEARCH ARTICLE

Effectiveness of The Fungicide Pirisect Sl On Non-Target Organisms *Fusarium solani* and *Macrophomina phaseolina* Present in Iraqi Soils

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A B S T R A C T

This study aimed to determine the effect of the fungicide Pirisect SL on non-target organisms the two pathogenic fungi *Fusarium solani* and *Macrophomina phaseolina*, where the fungicide was used, which is used to combat soil fungal diseases, where the effect of the pesticide on the solid culture media (P.D.A.) of the fungi was measured. The results showed the superiority of the pesticide in inhibiting the two pathogenic fungi in solid culture medium P.D.A. The study showed that the two pathogenic fungi *M. phaseolina* and *F. solani* have a high pathogenicity of 100%. The results showed the efficiency of the fungicide Pirisect SL in controlling the fungi under study in the solid agricultural media P.D.A. The radial growth of the pathogenic fungi *M. phaseolina* and *F. solani* reached (5.00 and 5.05) cm, respectively, compared with the control treatment, which reached (9.00) cm for the two fungi. As for the liquid medium P.D.B., the percentage of inhibition was in relation to the wet weight and dry weight and for the two pathogenic fungi *F. solani*, *M. phaseolina*, it reached (47.50 and 44.72) %, respectively, for the wet weight, and it reached (45.02, 43.89) %, respectively, for the dry weight.

Keywords: Biodegradation, Inhibition, Non-target effects, *Fusarium solani*, *Macrophomina phaseolina*.

INTRODUCTION

Pesticides have made an effective contribution to reducing diseases transmitted to plants (Lengai *et al*., 2020). Some studies have shown that it is difficult to obtain economic production without using pesticides in the production process (Tudor *et al*., 2023) and that not using pesticides leads to a decrease in agricultural crop production by a rate ranging from 50-70% which comes in the forefront (Karem and Haidery, 2022; Raj *et al*., 2023). Fungal pathogens whose effects vary quantitatively and qualitatively depending on environmental conditions such as temperature, humidity and agricultural processes (Mangi *et al*., 2021). They are the largest and most important groups in plant pathogenicity. There are about 8,000 species of fungi that have the ability to parasitize plants, and they are widely and clearly diverse in their shape, life cycle and degree of complexity in infecting plants (Karem *et al*., 2019; Heldt and Piechulla, 2021). Sometimes they produce toxic compounds and the products become unusable (Mohamed *et al.,* 2024; Szczygieł *et al.,* 2024). The importance of pesticides may be represented in three aspects reducing losses resulting from plant diseases and increasing production in quantity and quality (Popp *et al*., 2013). Some plant products may

become less dangerous as a result of their exposure to toxic substances produced by fungi (Ragsdale *et al.,* 1991; Barceloux, 2012).

A heteroaryl hydroxy compound called hymexazol (HML) is used as a systemic fungicide to treat fungal infections in seeds and soil. According to Payne and William (1990), seed treatment with HML at 10.5–14 g/kg seed was advised which is a systemic fungicide and soil disinfectant that in acidic soil combines iron and aluminum ions to prevent disease spore development (Myresiotis *et al*., 2012). It has been used to manage Fusarium wilt in a variety of crops, including soybeans, cucumbers, watermelon and more (Fan *et al*., 2018). However, non-target organisms may be impacted by fungicides (Storck *et al*., 2017). Even after being exposed to half of the authorized dosage of hymexazol, studies have shown a decrease in the population of five predatory soil mite species (Alhewairini and Al-Azzazy, 2019).

The aim of the research is to evaluate the efficiency of the pesticide Pirisect SL on non-target organisms against the two pathogenic fungi. *Fusarium solani* and *Macrophomina phaseolina.*

MATERIALS AND METHODS

The pesticide used in the study was obtained from agricultural equipment offices. Fungicide: Pirisect SL**;** Active ingredient: Hymexazol 36%, Producing company: Turkish Agri Sciences with a new registration on 2/7/2020, recommended dose: 2 ml/L of water.

Isolation and diagnosis of fungi: The pathogenic fungus *Fusarium solani* was isolated from the roots of tomato plants and the pathogenic fungus *Macrophomina phaseolina* was isolated from the roots of infected okra plants. The infected parts were washed with running water to remove dust from them, then dried on filter paper. They were then cut into small parts and planted in Petri dishes containing P.D.A. culture medium. It was sterilized and incubated in the incubator at a temperature of (25±2) °C. After seven days, the fungus was purified and diagnosed.

Media used this study: In the study, different culture media were used to isolate, grow, and diagnose fungi for the purpose of conducting experiments as follows: -

Potato Dextrose Agar (P.D.A.): It was prepared by dissolving 39 grams per liter of distilled water according to the instructions of the producing company and divided into beakers as needed, then sterilized in an autoclave at a temperature of 121 degrees C and a pressure of 15 pounds/inch2 for 20 minutes. This medium was used to classify some fungi as a standard medium.

Potato Dextrose Broth (P.D.B.): This culture medium was prepared in the same way as mentioned in paragraph A-1-4-3 without adding agar to it. It was then added to glass flasks in an amount of 150 ml for each flask, closed and sterilized in the same way, then stored in the refrigerator until use. This medium is used to obtain fungal filtrate and determine the biomass of the fungi used in the experiments mentioned in the study.

Pathogenicity test: The plates were filled with W.A. culture medium. Then it was planted with both *F. solani* and *M. phaseolina* from ten-day-old farms with three replicates for each, then the plates were incubated at a temperature of (25±2) ºC. After 48 hours, the plates were planted with radish seeds after sterilizing them with sodium hypochlorite at a concentration of 2% for two minutes, then washed with sterile distilled water twice in each time, the seeds were left in water for two minutes, then dried by placing them on a sterile filter paper. After that, the seeds were planted in petri dishes. 20 seeds in each dish, 1 cm from the edge of the dish, in a circular manner. After seven days, the percentage of rotten seeds, infected seedlings and healthy seedlings was calculated.

$$\text{Decay seeds Percentage} = \frac{\text{No. of emeraging seeds}}{\text{No. of total seeds}} \times 100$$

$$\text{Infected seedling Percentage} = \frac{\text{No. of infected seedling}}{\text{No. of total seedlings}} \times 100$$

$$\text{Healthy seedling Percentage} = \frac{\text{No. of healthy seedlings}}{\text{No. of total seedlings}} \times 100$$

Testing the effect of the fungicide Pirisect SL on the growth of the pathogenic fungi *F. solani* and *M. phaseolina* in the two-culture media, Potato Dextrose Agar and Potato Dextrose Broth, at a temperature of 25 ± 2°C, as follows: -

Growing the fungi in solid medium (Potato Dextrose Agar) P.D.A.: Thawed the culture medium P.D.A. It is divided across three 250 ml flasks, each holding 100 ml of P.D.A. medium. The flasks were left

to cool and before they reached the solidification stage at a temperature of 45 ^{0}C, the pesticide used in the study, Pirisect SL was added in the recommended concentrations. The second treatment was the control treatment without using the pesticide with three replicates for each treatment, then it was poured into petri dishes. As for the control treatment, untreated medium was poured. It was poisoned and the dishes were planted with the two fungi used in the study *F. solani* and *M. phaseolina* each separately. A newly formed colony was taken and planted in the center of the dish separately. The dishes were incubated at a temperature of 25 ± 2 degrees C. The national growth of the fungi under study was measured by taking the rate. Two perpendicular diameters from the back of the plate pass through the center of the disc during a period of seven days.

Culturing fungi in liquid media (Potato Dextrose Broth) P.D.B.: In this test, liquid culture medium P.D.B. was used. The sterilizer was distributed in 250 ml beakers, each containing 100 ml. The beakers were inoculated with three tablets of each of the two fungi (*F. solani* and *M. phaseolina*) with the medium poisoned with the pesticide Pirisect SL and the non-poisoned medium, in three replicates for each treatment, then incubated at a temperature of 25 ± 2 °C. For a period of 30 days, making sure to shake the flasks every 2-3 days. After that, the biomass was extracted with sterile forceps and placed on blotting paper to get rid of free water. Then its fresh weights were taken with a sensitive balance and then it was dried in the oven at a temperature of 70°C for 48 hours, that is until the weight was constant and its dry weights were taken. It was preserved. The percentage of inhibition was calculated according to the equation of Abbott (1925) mentioned by Shaaban and Al-Mallah (1993):

$$\% \text{ inhibition} = \frac{\text{Mean of myclia growth in control} - \text{Mean of myclia growth in treatment}}{\text{Mean of myclia growth in control}} \times 100$$

STATISTICAL ANALYSIS

A completely randomized design (C.R.D.) was used for laboratory studies, and the Least-Significant Differentiation (L.S.D.) was used to compare the means. According to Mahmoud and Muhammad (2000), each figure in the following tables is the average of three replicates at a 0.05 probability level.

RESULTS

Testing the pathogenicity of the two pathogenic fungi *F. solani* and *M.phaseolina* and their effect on the percentage of seed rot and death of radish seedlings seven days after planting them on P.D.A. culture medium at a temperature of (25±2) °C.

The results of Table (1) showed that the highest percentage of rotten seeds was in the *F. solani* treatment. Followed by the *M. phaseolina* treatment, it amounted to (98.00 and 50.32) %, respectively, as it produced highly significant differences compared to the control treatment, in which the percentage of rotten seeds was 0.00%. As for the infected seedlings, the highest percentage appeared in the *M. phaseolina* treatment, as it reached (49.68) %. With high significant differences compared to the control treatment. It was 0.00%, and as for healthy seedlings, the highest result appeared in the control treatment. It was 100.00) %.

Table 1. Pathogenicity of the two pathogenic fungi *F.solani* and *M.phaseolina*. Their effect on the percentage of seed rot and seedling death seven days after planting them on P.D.A. culture medium at a temperature of (25±2) ºC.

	Percentage (%)		
Treatments	Decay seeds	Infected seedlings	Healthy seedling
Control	0.00	0.00	100.00
F.solani.	98.00	2.00	0.00
M.phaseolina	50.32	49.68	0.00
L.S.D.0.05	11.54	12.45	

Testing the effect of the fungicide Pirisect SL on the growth of the two pathogenic fungi under study, *F.solani* and *M.phaseolina*, at a temperature of 25 ± 2°C on solid culture medium P.D.A.

According to the results of Table (2), the highest radial growth for pesticides occurred in the control treatment and the lowest radial growth occurred in the Pirisect SL pesticide treatment, reaching (05 and

9.00.1) cm, respectively. As for the two fungi, the highest radial growth occurred in *F. solani*. The lowest radial growth occurred in the *M. phaseolina* treatment, reaching (5.05 and 5.00) cm, respectively. As for the interaction between the pesticide and the fungicide, we find the highest radial growth rate occurred in the control treatment of the two pathogenic fungi *F. solani* and *M. phaseolina*. With the pesticide, the lowest radial growth occurred in the *F. solani*. The fungicide reached (9.00 and 1.00) cm respectively, with significant differences for the treatments compared to the control treatment.

Table 2. Effect of the fungicide Pirisect SL on the growth of the two pathogenic fungi under study, *F. solani* and *M. phaseolina* at a temperature of 25 ± 2°C on the solid culture medium P.D.A.

Treatments	Control	Pesticide	Effect of Treatments
F.solani	9.00	1.00	5.00
M.phaseolion	9.00	1.10	5.05
Mean	9.00	1.05	
L.S.D. 0.05	Treatments = 1.06	Fungi = 3.20	

The wet and dry weight of biomass

The results obtained from Table (3) indicate that the highest rate of inhibition for the two fungi appeared in the *M. phaseolina* treatment, and the lowest rate of inhibition appeared in the *F. solani* treatment, where it reached (47.50, 44.72) %, respectively. As for the highest rate of inhibition for the pesticide Pirisect SL, the lowest rate of inhibition appeared in the *F. solani* treatment. It appeared in the control treatment, where it reached (92.22, 0.00) % respectively. As for the interaction, the highest rate of inhibition appeared in the treatment of the pesticide Pirisect SL + the fungus *M. phaseolina*, and the lowest rate of inhibition appeared in the two control treatments of the two fungi (*R. solani*, *M. phaseolina*), where It reached (0.00, 95.00) % respectively.

As for dry weight, the highest rate of inhibition for the two fungi appeared in the *M. phaseolina* treatment and the lowest rate of inhibition appeared in the *F. solani* treatment reaching (45.02, 43.89) %, respectively. As for the highest rate of inhibition of the pesticide, it appeared in the pesticide treatment Pirisect SL and the lowest rate of inhibition appeared in the treatment. The control reached (88.91, 0.00) % respectively. As for the interaction, the lowest rate of inhibition appeared in the two control treatments for the two fungi (*M. phaseolina* and *F. solani*) where it reached (0.00.) % and the highest percentage of inhibition appeared in the Pirisect SL+ pesticide treatment. The fungus *M. phaseolina* reached (90.04) %.

Table 3. Effect of the fungi *F.solani* and *M.phaseolina* Analysis of the pesticide Pirisect SL under study at the recommended dose on the liquid culture media in P.D.B. A temperature of 25 ± 2 °C by measuring the wet weight and dry weight of the biomass (weight in milligrams) for 30 days by calculating the percentage of inhibition.

Fungi	Media with pesticides (wet weight)		Mean of	Media with pesticides (dry weight)		Mean of
	Control	Pirisect SL	Fungi	Control	Pirisect SL	Fungi
F.solani	0.00	89.45	44.72	0.00	87.78	43.89
M.phaseolina	0.00	95.00	47.50	0.00	90.04	45.02
Mean	0.00	92.22		0.00	88.91	
L.S.D.0.05	Fungi =2.89		Treatments =6.81		Interaction=9.89	

DISCUSSION

It is the fungus *F. solani* that is responsible for the rise in the percentage of rotting seeds. Its parasitic nature may be the cause of this, as it attacks seeds of various plant families, causing them to rot or not germinate. It does this by secreting a number of enzymes that break down cellulose, chitin, and protein, which causes seed rot, as well as by releasing some toxic compounds that kill embryos. It is regarded as one of the main reasons why seed rot and seedling death occur. Both in Iraq and globally (Rasmussen *et al.,* 1994; Agrios, 2005) The release of plant-pathogenic enzymes and toxins, many of which have been found and isolated, including phenyl acetic acid and its derivatives, is another characteristic (Gupta and Thind, 2018) as well as several carbohydrates that are composed of glucose, mannose,

N-acetyl galactosamine, or N-acetyl glucosamine. Acid is secreted by mushrooms. Plant cells are killed by oxalic acid, which also encourages the production of carbohydrates and amino acids. One of the fungi that kills the host the quickest is *F. solani*. Pectinase, Pectin methyledterase, Cellulase, and Phosphatase are among the enzymes associated with the fungus that aid in breaking down cell walls, according to research on this feature conducted in a lab (Dillard, 1987).

The fungus in order to facilitate seed decomposition and enable the fungus to propagate through plant tissues, *F. solani* uses a number of potent enzymes to break down plant cell walls (Sharma, 2021). Cellulases break down cellulose, which impairs the cell walls' structural integrity and increases the likelihood that the cells may collapse (Sela-Buurlage, 1996). Pectinases degrade pectin, which prevents seeds from germinating by separating plant cells and dissolving tissues (Durrands, 1986). By breaking down the chitin in fungal cell walls, chitinases facilitate the fungus's invasion and enable it get past protective barriers (Sela-Buurlage, 1996). Phosphatases cause organic substances to release phosphate, which interferes with essential cellular functions and causes cell structure to collapse. When combined, these enzymes provide *F. solani* the ability to effectively infiltrate and degrade plant tissue (Skiada, 2019; Chen *et al*., 2024).

It is possible that many chemical pesticides kill the organism by interfering with the process of cellular respiration and energy production in the mitochondria or by altering the biological packaging of certain compounds necessary for the organism's life, such as proteins and nucleic acids (DNA, RNA), which affects the Krebs cycle, oxidative phosphorylation in the mitochondria, and glycolysis in the cytoplasm. This could explain the results obtained by calculating the radial growth of two fungi (Shaaban and AL- Mallah, 1993). The fungi that were severely affected by pesticides may be due to the disruption of the work of some enzymes necessary in the nutritional process and this is consistent with what was mentioned by (Koller *et al.,* 1982) that some pesticides work to inhibit the action of the enzyme Cutinase and the enzyme Phosphatase or that pesticides affect growth through their effect on DNA synthesis and cell division or by inhibiting some important enzymes in mitochondria (Shaaban and AL- Mallah, 1993).

From the previous results, we notice a sharp decrease in biomass after drying, especially for media containing the pesticide compared to the control treatment for each of the fungi under study. The high fluid content in the mycelium, which results from the fungal hyphae's increased surface area, may be the cause of this mass decrease. This increases contact between the pesticide molecules and the fungal cells, speeding up the process of removing the compounds into the mycelium and storing them in liquid form (Hatakka and Hammel, 2011; Deborah and Thatheyus, 2018).

Another explanation could be that the control treatment's rapid depletion of the medium's nutrients, which aid in the development of reproductive structures, resulted in a lower weight change after drying than the treatments with more reproductive structures. Although the dense or weak growth of the two fungi may not give a specific idea of the extent of biodegradation of the fungal isolates, there are other indicators of the extent of decomposition, such as a change in color and even a change in smell. However, overall, the dense growth provides a useful picture of the fungus's capacity for biodegradation, particularly because a larger surface area of the fungal hyphae the fungus forms increases the contact between the pesticide molecules and the fungus cells, which speeds up the process of removing compounds for analysis from the organism and increases the amount of enzymes the fungus secretes to the outside. cells, which raises the biodegradation level (Fatriasari and Hermiati, 2016; Deborah and Thatheyus, 2018).

This is in line with several studies that found a favorable association between enhanced growth and hydrocarbon compound biocracking in the majority of situations (Okerentugba and Ezeronye, 2000; Deborah and Thatheyus, 2018).

According to the present research, *M. phaseolina* and *F. solani* are highly pathogenic and significantly increase seed rot and seedling mortality in radish crops. These diseases' development was well inhibited by the fungicide Pirisect SL, suggesting its potential use in integrated pest control techniques. These findings highlight how crucial it is to create resistant crop types and early detection techniques in order to lessen the effects of these fungus. Furthermore, a balanced approach to disease control and environmental sustainability is required due to the ecological effects of fungicide use. Overall, the study supports all-

encompassing approaches to improve agricultural yield and health in the face of fungal challenges.

Future Implications: For a better understanding of Pirisect SL's influence on fungal cellular processes, future studies should investigate the molecular pathways by which it produces its fungicidal effects. Additionally, the fungicide's safety and effectiveness in a range of environmental circumstances might be evaluated by extended field experiments. Examining the possible emergence of fungal resistance to Pirisect SL is also essential for long-term disease control. Moreover, combining Pirisect SL with other farming techniques like crop rotation and biological control approaches may improve crop protection plans and lessen the need for chemical fungicides, thereby improving agricultural sustainability in general.

CONCLUSION

This study shows the fungicide Pirisect SL's inhibitory effectiveness as well as the notable pathogenic effects of *Macrophomina phaseolina* and *Fusarium solani* on radish seeds and seedlings. The results showed that *M. phaseolina* had a greater influence on seedling infections (49.68%) but *F. solani* produced the highest proportion of seed rot (98%). There was no seed rot or seedling death in the control treatment, highlighting the lack of pathogenic effect under untreated circumstances. Both fungi's radial growth was markedly inhibited by the application of Pirisect SL with *M. phaseolina* showing the most suppression. These results were corroborated by the biomass analysis, which revealed significant drops in the wet and dry weights of the fungicide-treated samples. The fungicide's impairment of essential cellular functions including respiration, enzyme activity and nutrient absorption is probably what caused the reduced biomass. Overall, the investigation confirms the effectiveness of Pirisect SL as a fungicidal agent and highlights the aggressive pathogenicity of *F. solani* and *M. phaseolina*. These findings advance our knowledge of fungal management in agricultural environments and imply that certain fungicidal treatments can successfully lessen the effects of these infections. To maximize the application of Pirisect SL and related compounds in crop protection measures, future studies should investigate the biochemical pathways behind fungicidal activity.

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Salwan A. Z. J. Allobawi	:	Basic idea of research, with some laboratory work, and writing parts of the manuscript.
Adeeb K. A. Z. Al-Shafiee	:	Field work with laboratory experiments.
Malik H. Karem	:	Statistical analysis of experiments with writing some parts of the manuscript.