International Journal of Life Science and Agriculture Research

ISSN (Print): 2833-2091, ISSN (Online): 2833-2105

Volume 04 Issue 03 March 2025

DOI: https://doi.org/10.55677/ijlsar/V04I03Y2025-02

Impact Factor: 7.88 , Page No : 167-173

Efficacy of Fungicides in Controlling *Pythium aphanidermatum* pathogenic of Root and stem rot and Their Impact on Plant Health and Productivity

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ABSTRACT

Pythium aphaneidermatium is one of the most destructive fungi to agricultural products, as it is a soil fungus that causes serious diseases that affect seedlings, such as root rot and seedling death, leading to huge losses in many plant families. Due to the great importance of this fungus and its negative impact on crops, the effect of a group of fungicides, both systemic and non-systemic, on its growth and control was evaluated in this study. The fungicides used in the study included: Propiconazole, Hexaconazole, Carbendazim, Mancozeb, Chlorothalonil, and Captan, where different concentrations of each fungicide were prepared, including (0, 100, 250, 500, and 1000 ppm). All fungicides showed high efficacy at a concentration of 1000 ppm, while the levels of effect varied at other concentrations, indicating the difference in the degree of sensitivity of the fungus to each fungicide and its concentration used. Despite the environmental and health damages that may result from the use of fungicides, the need to use them is still very necessary to protect agricultural crops from pathogens and reduce the economic losses in agricultural production. Therefore, it is important to continue to evaluate the effectiveness of these fungicides and study their residual effects to ensure a balance between effective control and environmental and health safety.

Published Online: March 05, 2025

KEYWORDS: *Pythium aphaneidermatium*, fungicides, inhibition.

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INTRODUCTION

The genus Pythium was first described and classified by Pringsheim in 1858, and belongs to the family Pythaceae and the order Pronosporales (Pringsheim, 1858; Van der Plaats-Niterink, 1981). Pythium is a microscopic fungus known to be a facultative parasite, and is classified as an oomycete in most classifications (Cheung et al., 2008; Bhalerao, et al., 2020). More than 60% of field-grown seedling mortality is attributed to pre- and post-emergence diseases caused by Pythium species. This effect is influenced by factors such as temperature, soil moisture, and rainfall. Root and ring rot diseases are common problems affecting vegetables, and are widespread worldwide. *Pythium aphanidermatum* (Edson) Fitz is a major cause of these diseases, especially for root and ring rot (Jadhav et al., 2007; Kamali et al., 2020).

Pythium aphanidermatum causes a variety of plant problems, including stunting, root rot, wilting, and leaf drop under favorable environmental conditions, and in severe cases can result in plant death. Traditionally, management of root rot caused by this fungus has relied on preventive applications of fungicides combined with strict sanitation practices. However, control of this disease is increasingly difficult due to long production seasons and common irrigation practices. (Lookabaugh et al., 2020; Moorman et al., 2002).

Fungicides are an effective and rapid means that most farmers rely on to control destructive pathogens. Since the introduction of Bordeaux mixture in the late 19th century, fungicides have witnessed a great development with the development of many compounds that target harmful plant pathogens (Iqbal and Mukhtar, 2020; Maher, 2021). The agrochemical industry is constantly striving to innovate new compounds that have higher efficacy, lower toxicity, and less harmful environmental impacts. Accordingly, these fungicides have been tested on various pathogens in laboratory and field conditions (Ayana and Gabrekerstos, 2022).

AIM OF STUDY

Testing the effectiveness of a group of fungicides available in local markets against the fungus *Pythium aphanidermatum* isolated from some vegetables infected with the fungus in the farms of Al-Muthanna Governorate in Iraq.

MATERIAL AND METHODS

Samples were collected from different areas in Muthanna Governorate after diagnosing infections in the plant stem, especially in areas close to the soil, and the appearance of black ulcers in cucumber and tomato plants grown in these areas. After observing field infections, a group of dead seedlings were taken in addition to infected plants, plant roots, and soil samples to grow the fungus in the microbiology laboratory in the Department of Life Sciences at the College of Science - Muthanna University.

The samples were washed with distilled water to remove impurities from the samples. More than one method was used according to the type of sample, as soil was added to distilled water, then dilutions were made, so that 10 grams of soil were added to 90 ml of distilled water, then 1 ml was transferred to 9 ml in a test tube, and from the second test tube, 1 ml was also transferred to 9 ml for the third tube. The process continued to 4 test tubes to obtain the required dilutions, then 1 ml was taken from tubes number two and four and added to a petri dish containing a culture medium.

As for the plant samples, they were cut into small pieces to contain signs of infection, then placed in a 2% solution of sodium hydroxide solution for 1 minute, then taken out and placed in distilled water for 1 minute as well to remove the remains of the sterilizing material, then placed on drying paper to remove the suspended moisture. After removing the water, they were placed in Petri dishes containing potato dextrose acre medium. Both samples, whether soil or plant parts, were placed in an incubator at a temperature of 25 degrees Celsius. After 7 to 10 days, fungal growth and the formation of fungal threads were observed. They were examined under a microscope to confirm the sample, and then the plant was examined at home for fungal infection of the plants to compare the symptoms. The result was positive with the appearance of symptoms similar to those that appeared in the field during the sample collection process. Fungal colonies were increased and preserved for use in future experiments. During the experiment, a group of local fungicides were used, mentioned in **Table (1)**, where different concentrations of the pesticide were prepared, which were (100, 250, 500 and 1000 ppm), in addition to the control sample free of the fungicides. During this experiment, the technique of poisoning the culture medium was used by adding 1 ml of the fungicides and mixing it well by stirring in the Petri dishes containing the culture medium, then leaving the culture medium to solidify, then taking a 5 mm disk from the fungal colony and placing it in the center of the culture medium treated with fungicides and leaving it in the incubator for 7 to 10 days, after which the growth of the fungus was calculated using a ruler. The amount of inhibition of fungal growth was calculated according to the following equation (Nawaz et al. 2018):

$$\text{Inhibition} = \frac{(A1 - A2) \times 100\%}{A1}$$

Where, A_1 = growth in control, A_2 = growth in treatment.

Table 1: List of fungicides used for in vitro evaluation of *Pythium aphanidermatum*

Sl. No.	Trade name	Common name	Chemical name	type of fungicide
2	Tilt 25 EC	Propiconazole	1-(2-(2, 4-D)-4-Propyl-1,3 diozolan-2yl methyl) IH-1, 2, 4 Triazole	Systemic
3	Contaf 5 EC	Hexaconazole	RS-2-(2, 4-D)-1-(1H-1, 2, 4 Trizole-1-yl) hezan 2-ol	Systemic
4	Bavistin50% WP	Carbendazim	2-(Methoxy-carbomyl)-benzimidazole	Systemic
1	Indofil M-45 75% WP	Mancozeb	Zinc-manganese ethylene bisdithiocarbamate	Non-Systemic
2	Kavach 75% WP	Chlorothalonil	Tetrachloride isopthalonitrile	Non-Systemic
3	Captaf 50% WP	Captan	N-trichloromethyl thio4-cyclohexene-1, 2-dicarboximide	Non-Systemic

RESULTS AND DISCUSSION

According to the results shown in **Tables** 2 and 3, it was found that most of the fungicides used in the experiment showed high efficacy in inhibiting the growth of *Pythium aphanidermatum*, although there were differences in their effect based on the fungicide concentration. At a concentration of 1000 ppm, all fungicides achieved 100% inhibition, indicating their high efficiency at this high concentration.

However, the efficacy of most fungicides gradually decreased with decreasing their concentration. However, some fungicides, such as Propiconazole, Cabendazim, and Captan, showed exceptional performance; they were able to achieve 100% inhibition across all concentrations included in the experiment (100, 250, 500, and 1000 ppm), reflecting their great efficacy even at low concentrations.

As for the rest of the fungicides, they showed variation in performance at the lowest concentration (100 ppm). Mancozeb recorded an inhibition rate of 74.07%, while Hexaconazole achieved 35.19%, and Chlorothalonil achieved 24.07%. With increasing concentration, the inhibition rates improved gradually and proportionally for all fungicides used, until they reached 100% at the maximum concentration (1000 ppm). These results illustrate the differences in the efficacy of fungicides depending on their concentration, indicating the importance of choosing the appropriate concentration to obtain the best performance in controlling *Pythium aphanidermatum* **fig (1,2).**

In this research, the ability of systemic and non-systemic fungicides to inhibit the growth of *Pythium aphanidermatum* fungus was tested, taking into account the control of environmental conditions, as the experiment was conducted in the laboratory and the technique of poisoning the culture medium with different concentrations of the fungicide was used. The use of fungicides in the treatment of various crops has become increasingly necessary to reduce soil-borne diseases, such as those caused by Pythium spp., thus contributing to enhancing crop health and improving crop productivity (Doherty and Roberts, 2022; Lookabaugh et al., 2021).

Many studies have focused on the effectiveness of pesticides against Pythium fungus, where (Emad Abd Atia et al., 2015) focused on the use of systemic and non-systemic pesticides against Rhizoctonia solani, the cause of root rot disease in tomatoes, and the results were effective for most of the pesticides used in the experiment, such as propiconazole, carbundezime, captan, and mancozeb.

Phosphonate-based fungicides are effective in controlling diseases caused by Oomycetes, according to Dann and McLeod (2021). Rashelle, et al., (2021) tested the sensitivity of different strains of Pythium against a group of fungicides (metalaxyl, azoxystrobin, ethaboxam, captan, and thiram) and the results were satisfactory in terms of inhibition of growth and spore formation.

Ghulam et al., (2023) tested a range of modern and old fungicides (azoxystrobin, copper oxychloride, difenoconazole, propiconazole, azoxystrobin + difenoconazole, trifloxystrobin + tebuconazole, hexaconazole, mancozeb + mefenoxam, myclobutanil and flutolanil) against *Pythium aphanidrnatum*. The fungus showed high sensitivity to a range of fungi and within different concentrations within a concentration range of 250 to 8000 ppm.

CONCLUSIONS AND RECOMMENDATIONS

The inhibition capacity of a group of fungicides, both systemic and non-systemic, was tested in laboratory conditions to control the fungus Pythium aphaneidermatium. The results showed variation in inhibition rates among the tested fungi, but the vast majority of them proved remarkably effective and achieved satisfactory results in reducing the spread of the fungus.

Despite the negative effects associated with the use of fungicides and insecticides, whether on the environment or public health, their use is still a basic necessity in the agricultural sector, due to their vital role in protecting agricultural crops and ensuring high productivity.

Therefore, we recommend conducting extensive studies and research to evaluate the effectiveness of fungicides, with a special focus on studying the residual effect of these pesticides within agricultural products, whether green leaves, fruits, or other crops, in order to ensure food safety and reduce potential risks to consumers.

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Table 2: Effect of Systemic and non-systemic fungicides on mycelia growth of *Pythium aphanidermatum*

Sl.No	Radial growth of mycelium (Cm)				Mean
	100 ppm	250 ppm	500 ppm	1000 ppm	
Propiconazole	0.00	0.00	0.00	0.00	0.00
Hexaconazole	5.83	4.33	2.97	0.00	3.28
Cabendazim	0.00	0.00	0.00	0.00	0.00
Chlorothalonil	6.83	5.67	0.00	0.00	3.13

Mancozeb	2.33	2.00	0.00	0.00	1.08
Captan	0.00	0.00	0.00	0.00	0.00
control	9.00	9.00	9.00	9.00	9.00
Mean	3.43	3.00	1.71	1.29	

Table 3: Effect of Systemic and non-systemic fungicides on mycelia growth of *Pythium aphanidermatum*

Sl.No	**Per cent inhibition of mycelia growth over control**				**Mean**
	100 ppm	**250 ppm**	**500 ppm**	**1000 ppm**	
Propiconazole	100 (90)*	100 (90)	100 (90)	100 (90)	100
Carbendazim	100 (90)	100 (90)	100 (90)	100 (90)	100
Captan	100 (90)	100 (90)	100 (90)	100 (90)	100
Mancozeb	74.07 (59.42)	77.78 (61.87)	100 (90)	100 (90)	87.9625
Chlorothalonil	24.07 (29.35)	37.04 (37.47)	100 (90)	100 (90)	65.2775
Hexaconazole	35.19 (36.37)	51.85 (46.06)	67.04 (54.96)	100 (90)	63.52
Mean	72.22	77.78	94.51	100.00	
S. Em±	1.31	1.01	0.15	0.00	
CD@1%	5.87	4.55	0.68	0.00	
CV%	3.66	2.64	0.32	0.00	

*Figures in parenthesis are arcsine transformed values

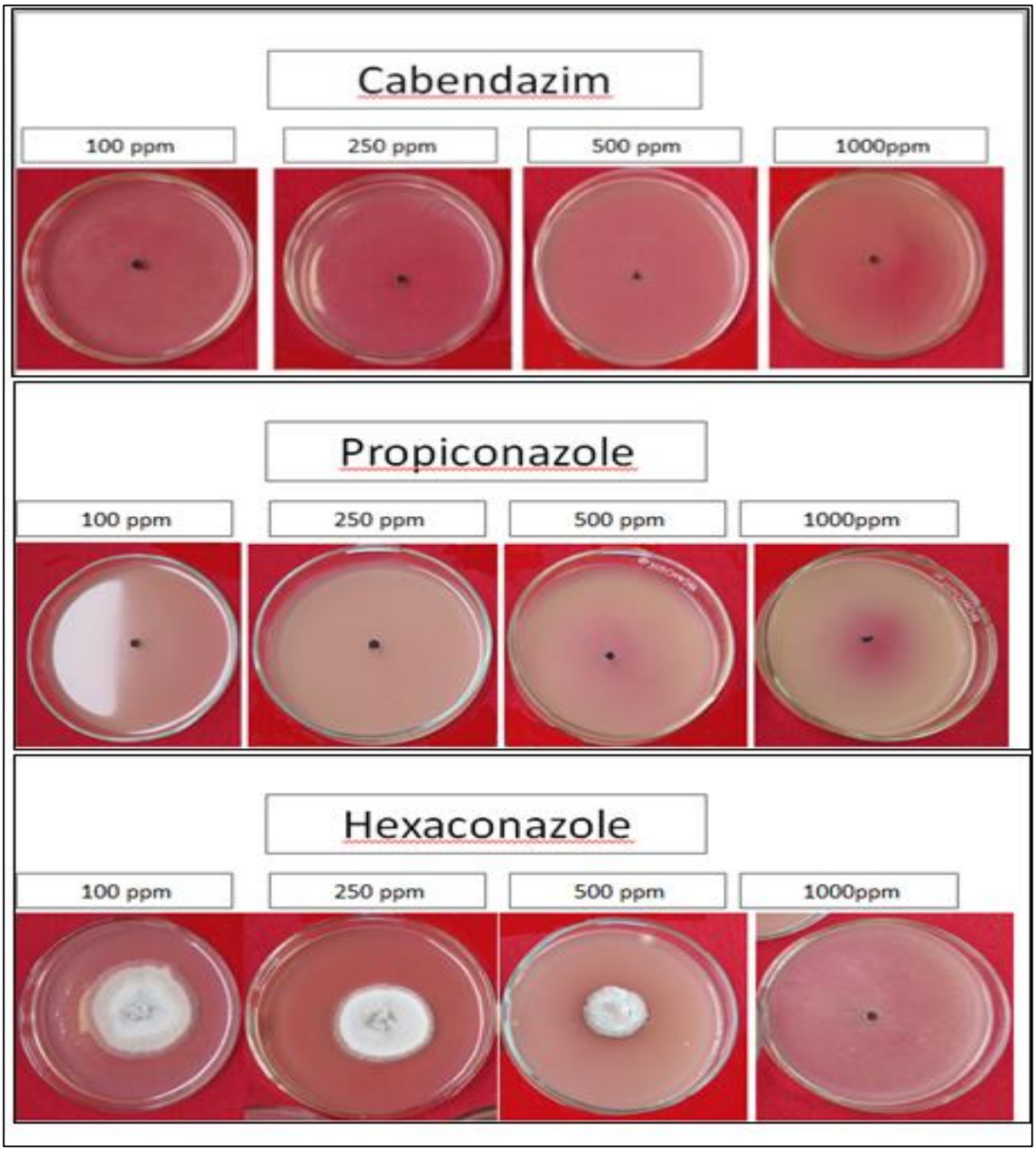

Figure 1: **Effect of Systemic fungicides on mycelia growth of *Pythium aphanidermatum***

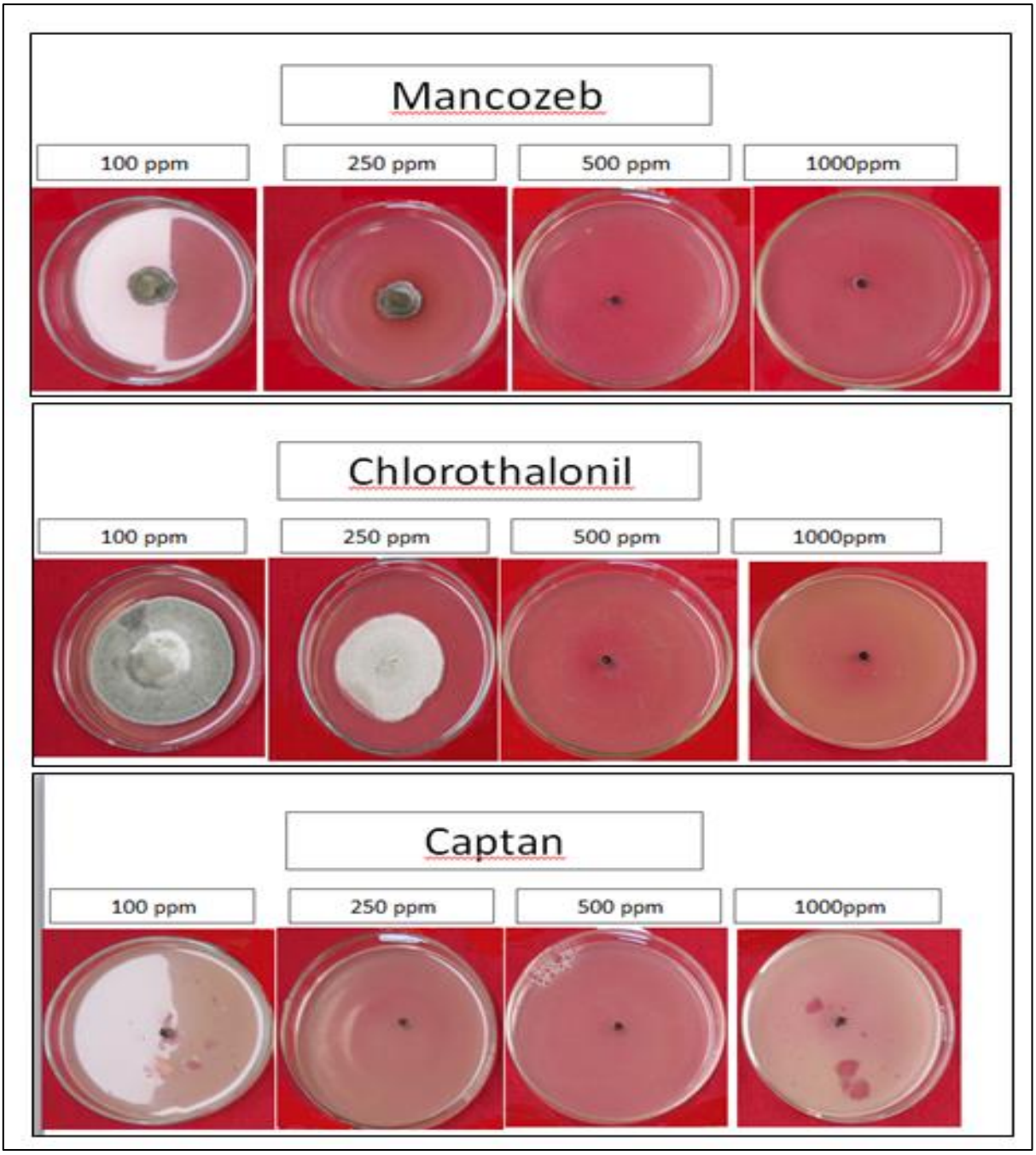

Figure 2: Effect of non-Systemic fungicides on mycelia growth of *Pythium aphanidermatum*