Research Article

Sensitivity of Different Isolates of *Pythium aphanidermatum* to Old and Novel Fungicides

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Abstract | *Pythium aphanidermatum* is one of the most devastating soil-borne pathogens in the warmer climates of the world. It is more damaging in vegetables by causing seedling rots, root rot, pre- and post-emergence damping-off, cottony-leak, cottony blight, and stalk rot diseases. An advantageous combination of various factors makes the control of *Pythium aphanidermatum* difficult. However, synthetic fungicides provide quick and effective control. Therefore, we checked the sensitivity of different isolates of *P. aphanidermatum* to 17-old and novel fungicides. Against all isolates, the fungicides' main effect, concentration's main effect, and fungicides' × concentration's effect are highly significant. Generally, based on their effectiveness, all tested fungicides were divided into three groups i.e., highly effective, moderately effective, and completely ineffective. All isolates of *P. aphanidermatum* were grown at par with the control when exposed to different concentrations of mandipropamid and fluoxastrobin. Both fungicides completely failed to cause any negative effects on *in vitro* growth of all isolates. On the other hand, all isolates appeared extremely sensitive to 10 fungicides including azoxystrobin, copper oxychloride, difenoconazole, propiconazole, azoxystrobin+difenoconazole, trifloxystrobin+tebuconazole, hexaconazole, mancozeb+mefenoxam, myclobutanil, and flutolanil. All isolates except *Pa 12* (which grew only at 250 ppm of azoxystrobin) failed to tolerate 250-8000 ppm concentrations of these fungicides and produced no growth under *in vitro* conditions. To some extent, the tested isolates showed variable responses to fosetyl aluminium, iprovalicarb+propineb, thiophanate-methyl, cymoxanil+mancozeb, and propineb. The isolates grew well at lower concentrations, gradually inhibited, and failed to grow at higher concentrations. The findings of the present research will help to design an effective control strategy against *P. aphanidermatum* with effective fungicides under field conditions.

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Introduction

Among different groups of cultivated plant species, vegetables are the most sensitive to soil-borne pathogens due to their tender nature. They are frequently affected by many destructive diseases such as damping-off, wilt, and root rot, which are caused by species of genera *Fusarium, Pythium, Phytophthora,*

Verticillium, Macrophomina, and *Rhizoctonia* (Koike *et al*., 2003; Noble and Coventry, 2005; Persley *et al*., 2010). The species of *Pythium* (class Oomycetes, order Pythiales) are not true fungi and, therefore, are classified in a separate kingdom Straminopila (Alexopoulos *et al*., 1996; Dick *et al*., 1999). The family Pythiaceae contains the two most economically important genera, *Phytophthora* and *Pythium,* each consisting of more than 200 species (Hyde *et al*., 2014; Brasier *et al*., 2022). Their species are considered the most damaging phytopathogens, causing huge economic losses worldwide (van West *et al*., 2003; Alexopoulos *et al*., 1996). Within the genus *Pythium*, *P. aphanidermatum* (Edson) Fitzpatrick is distributed worldwide but more common in warmer regions. It has a wide host range, infecting vegetables, ornamentals, fruits, and field crops (Lodhi, 2007; Waterhouse and Waterston, 1964; Grisanapundha, 1987; Parveen *et al*., 2020). It caused different diseases in crop plants, varying from host to host, including seedling mortality, root rot, damping-off, stalk rot, cottony leak, and blight. Moreover, new hosts and diseases caused by this destructive pathogen are continuously reported elsewhere (Shah *et al*., 2021, 2022). In severe cases of damping-off and seedling rots, total crop loss can happen (Sharma *et al*., 2020). The number of factors including, rapid growth and fast multiplication, soil-borne, aquatic nature, and tolerance to high temperature makes the control of *P. aphanidermatum* difficult. In order to suppress its infection, various control measures, including synthetic fungicides, plant extracts, biocontrol agents, and cultural practices, were tested (Mani and Marimuthu, 1994; Triki and Priou, 1997; Syed *et al*., 2020). Fungicides provide quick and effective control and most farmers depend upon them to get rid of such destructive pathogens. Since the introduction of the Bordeaux mixture in the late nineteenth century, many fungicides were developed for the control of destructive plant pathogens. Agrochemical sectors have been continuously in search of novel compounds that will be more effective, less toxic, and environmentally friendly. Accordingly, these fungicides were tested against different pathogens under *in vitro* as well as *in vivo* conditions (Iqbal and Mukhtar, 2020; Mahr, 2021; Ayana and Gabrekiristos, 2022). However, over time, fungicidal resistance has been developed in some pathogenic species. Moreover, within the single species isolates of different origins would have different levels of fungicidal sensitivity (White *et al*., 1988; Mazzola *et al*., 2002; Feng *et al*., 2020). The present study was also planned to screen out all the novel fungicides available against different isolates of *Pythium aphanidermatum* isolated from various vegetables grown in Sindh, Pakistan.

Materials and Methods

Sample collection and isolation of Pythium aphanidermatum

Roots and rhizospheric soil samples of different vegetables were collected from different locations in district Hyderabad. The samples were labeled and brought to the laboratory for isolation. For isolation of the *P. aphanidermatum,* a slightly modified baiting technique (Harvey, 1925) was used. Soil paste was made by adding distilled sterilized water (DSW), one teaspoon of this paste was placed on one side of sterilized Petri plates, added 10-15 ml of DSW in plates without disturbing the soil. Further, 2-3 sterilized grass blades of 2-3 cm long were also placed on each Petri plate, one near the soil and the other opposite side of the soil. These plates were kept at room temperature (25-30°C). After 5-7 days these grass blades having some mycelial growth at edges were washed with DSW and placed in new Petri plates followed by the addition of fresh DSW and grass blades. After 2-3 days of incubation, these grass blades were transferred to PDA plates containing Nystatin @100,000 L^{-1} to check the undesired fungi. The emerging colonies were purified by subsequent hyphal tip transfer on fresh agar plates. The targeted pathogen was then identified based on sporangial and oogonial characters (Plaats-Niterink, 1981). Finally, four isolates of *P. aphanidermatum* isolated from different sources were maintained on agar medium for further studies.

Evaluation of different fungicides

Different commercial fungicides (Table 1) available in the market were evaluated against *P. aphanidermatum* by the poisoned food method (Singh and Milne, 1974). A wide range of concentrations of these fungicides *viz.*, 250, 500, 1000, 2000, 4000, 6000, and 8000 ppm was prepared and mixed in PDA before sterilization. PDA without fungicides served as control. After solidification of the medium, a 5 mm disc from 7 days old culture was placed in the center of the Petri plate. Similarly, the response of four selected isolates was evaluated against each fungicide and their diffident concentrations individually. These plates were incubated at 30°C till the control plates were

Table 1: *Details of fungicides used against Pythium aphanidermatum.*

Trade name	Active ingredient	Chemical group
Alliete	Fosetyl Aluminium	Ethyl Phosphonates
Dynasty	Azoxystrobin	Methoxyacrilates
Curzate M8	Cymoxanil + Mancozeb	Cyanoimidazole + Dithiocarbamates
Antracol	Propineb	Dithiocarbamates
Melody Duo	Iprovalicarb + Propineb	Valinamide carbamates and Dithiocarbamates
Copper oxychloride	Copper oxychloride	Inorganic
Topsin M	Thiophanate-methyl	Thiophanates
Score	Difenoconazole	Triazoles
Tilt	Propiconazole	Triazoles
Amistar Top	Azoxystrobin + Difenoconazole	Methoxyacrilates and Triazoles
Nativo	Trifloxystrobin + Tebuconazole	Oximino acetates and Triazoles
Contaf Plus	Hexaconazole	Triazole
Ridomil Gold	Mancozeb + Mefenoxam	Dithiocarbamates and Acylalanines
Revus	Mandipropamid	Mandelic acid amides
Systhane	Myclobutanil	Triazole
Moncut	Flutolanil	Benzamides
Evito	Fluoxastrobin	Dihydro-dioxazines

fully covered with the growth of *P. aphanidermatum*. The experiment was arranged completely randomized design (CRD) with six replications. The percent inhibition of fungus was calculated by using the formula given below:

$$PIRG = (R1 - R2)/R1 \times 100$$ (Vincent, 1947)

Where; R1: Colony growth of pathogen in control plate (without fungicide). R2: Colony growth of pathogen in the treated plate (with fungicide).

Statistical analysis

The *Statistix* 8.1 software was used to calculate the analysis of variance (ANOVA). Mean values were compared by least significant difference (LSD) test at $p< 0.05$ (Statistix, 2006).

Results and Discussion

Efficacy of fungicides against P. aphanidermatum 11 (Pa 11)

The fungicides main effect (DF= 16, F= 339741, P= 0.00), concentration's main effect (DF= 6, F= 112554, P= 0.00) and fungicides × concentration's effect (DF= 96, F= 19665.0, P= 0.00) are highly significant (Table 2). Among 17 fungicides, Copper oxychloride, Score, Tilt, Amistar Top, Nativo, Contaf Plus, Ridomil Gold, Systhane, and Moncut appeared highly effective, but the tested isolate failed to grow at all concentrations, even at 250 ppm. In contrast, fungicides like Revus and Evito appeared effective, their highest concentration i.e., 8000 ppm completely failed to check the growth of *Pa 11*. The remaining fungicides were ineffective at lower concentrations but became highly effective at higher concentrations. The 250-1000 ppm of Alliete, Antracol, and Melody Duo completely failed to inhibit the growth of the test pathogen, while higher concentrations (4000-8000 ppm) checked the pathogen growth. In the case of Dynasty, the pathogen growth was completely inhibited by all concentrations except 250 ppm, which completely failed to inhibit the growth. In the case of Curzate, the pathogen successfully grew at 250-4000 ppm but was completely inhibited at 6000 and 8000 ppm. In the case of Topsin M, the growth of *Pa 11* gradually reduced with increasing concentrations from 250 to 2000 ppm and completely stopped at 4000 to 8000 ppm (Table 3).

Table 2: *Analysis of variance of fungicides and concentrations for P. aphanidermatum 11.*

Source	DF	SS	MS	F	P
Replications	5	1	0.2		
Concentrations	6	114539	19089.8	112554	0.000
Fungicide	16	921952	57622.0	339741	0.000
Concentrations* Fungicides	96	320189	3335.3	19665.0	0.000
Error	590	100	0.2		
Total	713	1356780			

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Table 3: *Response of P. aphanidermatum 11 to different concentrations of various fungicides.*

Fungicides	Concentrations (ppm)						
	250	500	1000	2000	4000	6000	8000
	Growth inhibition (%)						
Alliete	0.00 j*	0.00 j	0.00 j	55.56 f	100.0 a	100.0 a	100.0 a
Dynasty	0.00 j	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a
Curzate M8	0.00 j	0.00 j	0.00 j	0.00 j	0.00 j	100.0 a	100.0 a
Antracol	0.00 j	0.00 j	0.00 j	9.25 i	44.44 g	83.33 b	100.0 a
Melody duo	0.00 j	0.00 j	0.00 j	66.66 d	77.77 c	100.0 a	100.0 a
Copper oxychloride	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a
Topsin M	0.00 j	20.37 h	64.44 e	77.77 c	100.0 a	100.0 a	100.0 a
Score	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a
Tilt	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a
Amistar top	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a
Nativo	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a
Contaf Plus	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a
Ridomal Gold	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a
Revus	0.000 j	0.00 j	0.000 j	0.000 j	0.00 j	0.000 j	0.00 j
Systhane	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a
Moncut	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a
Evito	0.00 j	0.00 j	0.00 j	0.00 j	0.00 j	0.00 j	0.00 j

Each value is a mean of six replications. Values with different letters differ significantly at $p < 0.05$ as determined by DMRT.

Table 4: *Response of P. aphanidermatum 12 to different concentrations of various fungicides.*

Fungicides	Concentrations (ppm)						
	250	500	1000	2000	4000	6000	8000
	Growth inhibition (%)						
Alliete	0.00 i*	0.00 i	0.00 i	100.0 a	100.0 a	100.0 a	100.0 a
Dynasty	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a
Curzate M8	0.00 i	0.00 i	44.44 g	70.37 d	77.77 d	100.0 a	100.0 a
Antracol	0.00 i	0.00 i	33.33 h	100.0 a	100.0 a	100.0 a	100.0 a
Melody duo	0.00 i	0.00 i	0.00 i	77.77 c	88.88 b	100.0 a	100.0 a
Copper oxychloride	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a
Topsin M	0.00 i	0.00 i	46.66 f	62.22 e	100.0 a	100.0 a	100.0 a
Score	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a
Tilt	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a
Amistar top	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a
Nativo	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a
Contaf plus	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a
Ridomal Gold	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a
Revus	0.00 i	0.00 i	0.00 i	0.00 i	0.00 i	0.00 i	0.00 i
Systhane	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a
Moncut	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a
Evito	0.00 i	0.00 i	0.00 i	0.00 i	0.00 i	0.00 i	0.00 i

***** *Each value is a mean of six replications. Values with different letter differ significantly at $p < 0.05$ as determined by DMRT.*

Efficacy of fungicides against P. aphanidermatum 12 (Pa 12)

There was a great variation in the efficacy of different fungicides against *Pa 12*. Two fungicides i.e., Revus and Evito failed to cause any negative effects on colony growth of *Pa 12*, the highest used concentration of these fungicides (8000 ppm) produced the growth identical to the control treatment (w/o fungicide). *Pa 12*, showed tolerance to 250-1000 ppm of Alliete, in which test pathogen grew vigorously, but completely inhibited at 2000-8000 ppm. In Curzate, 250 and 500 ppm failed to produce any inhibition, while, 1000, 2000, and 4000 produced 44.44, 70.37, and 77.78% inhibition, respectively, and completely inhibition at 6000 and 8000 ppm. In Antracol, 250 and 500 ppm produced no inhibition, 1000 ppm caused 33.33% inhibition and 2000-8000 ppm caused 100% inhibition. The 250, 500, and 1000 ppm of Melody Duo failed to cause any reduction, 2000-8000 ppm caused 77.78-100% gradual inhibition in the colony growth of the test pathogen. Other remaining fungicides *viz*., Dynasty, Copper oxychloride, Score, Tilt, Amistar Top, Nativo, Contaf Plus, Ridomil Gold, Systhane, and Monocot were found highly effective, and their all concentrations produced 100% inhibition (Table 4). On an overall basis, fungicides showed highly significant impact (DF= 16, F= 341178, P= 0.00). The fungicide concentration's effect was also highly significant (DF= 6, F= 131659, P= 0.00). The interactive effect of fungicides and concentration also appeared highly significant (DF= 96, F= 21200.2, P= 0.00) (Table 5).

Table 5: *Analysis of variance of fungicides and concentrations for P. aphanidermatum 12.*

Source	DF	SS	MS	F	P
Replications	5	5	1.0		
Concentrations	6	116256	19376.0	131659	0.000
Fungicide	16	803371	50210.7	341178	0.000
Concentrations* Fungicides	96	299521	3120.0	21200.2	0.000
Error	590	87	0.1		
Total	713	1219241			

Efficacy of fungicides against P. aphanidermatum 14 (Pa 14)

In terms of effectiveness against *Pa 14*, the 17 fungicides were divided into three groups i.e., highly effective fungicides at all concentrations, moderately effective-not effective at lower concentrations but effective at higher concentrations, not effective at all concentrations. The overall impact of fungicides was highly significant (DF= 16, F= 602459, P= 0.00). The overall response of concentrations to pathogen inhibition was also highly significant ($p < 0.001$). The 'fungicides × concentrations' to inhibition percentage also found highly significant (DF= 96, F= 41491.6, P= 0.00) (Table 6). The first group comprises Dynasty, Copper oxychloride, Score, Tilt, Amistar Top, Nativo, Contaf Plus, Ridomil Gold, Systhane, and Monocot, the pathogen failed to grow at all tested concentrations of these fungicides. The second group consists of Alliete, Curzate, Antracol, Melody Duo, and Topsin M, which appeared completely or partially ineffective at 250-1000 ppm, but completely inhibited the pathogen growth at 2000-8000 ppm. The third group consists of Revus and Evito, which completely failed to check the growth of *Pa 14* at all concentrations (Table 7).

Table 6: *Analysis of variance of fungicides and concentrations for P. aphanidermatum 14.*

Source	DF	SS	MS	F	P
Replications	5	2	0.3		
Concentrations	6	130672	21778.7	269252	0.000
Fungicide	16	779689	48730.5	602459	0.000
Concentrations*-Fungicides	96	322185	3356.1	41491.6	0.000
Error	590	48	0.1		
Total	713	1232595			

Efficacy of fungicides against P. aphanidermatum 16 (Pa 16)

Analysis of variance showed that for pathogen inhibition, there is a highly significant difference between fungicides (DF=16, F=360762, P=0.000), among concentrations (DF= 6, F= 133770, P= 0.000) and the interactive response of fungicides and concentrations (DF= 96, F= 22555.4, P= 0.000) (Table 8). The response of *Pa 16* to fungicides to almost similar to those of *Pa 14*. Among 17 fungicides, Revus and Evito were found to be ineffective; the higher concentration (8000 ppm) produced the same growth of pathogen that recorded in the control (w/o fungicides). On the other hand, 10 fungicides (Dynasty, Copper oxychloride, Score, Tilt, Amistar Top, Nativo, Contaf Plus, Ridomil Gold, Systhane, and Monocot) appeared highly effective, the test isolated failed to grow even at 250 ppm. The remaining five fungicides showed a mixed response to the test pathogen.

Table 7: *Response of P. aphanidermatum 14 to different concentrations of various fungicides.*

Fungicides	Concentrations (ppm)						
	250	500	1000	2000	4000	6000	8000
	Growth inhibition (%)						
Alliete	0.00 f*	0.00 f	0.00 f	100.0 a	100.0 a	100.0 a	100.0 a
Dynasty	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a
Curzate M8	0.00 f	0.00 f	22.22 d	100.0 a	100.0 a	100.0 a	100.0 a
Antracol	0.00 f	0.00 f	33.33 c	100.0 a	100.0 a	100.0 a	100.0 a
Melody duo	0.00 f	0.00 f	0.00 f	100.0 a	100.0 a	100.0 a	100.0 a
Copper oxychloride	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a
Topsin M	0.00 f	16.66 e	44.44 b	100.0 a	100.0 a	100.0 a	100.0 a
Score	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a
Tilt	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a
Amistar top	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a
Nativo	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a
Contaf plus	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a
Ridomil gold	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a
Revus	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f
Systhane	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a
Moncut	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a
Evito	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f

Each value is a mean of six replications. Values with different letter differ significantly at $p < 0.05$ as determined by DMRT.

Table 8: *Analysis of variance of fungicides and concentrations for P. aphanidermatum 16.*

Source	DF	SS	MS	F	P
Replications	5	5	1.0		
Concentrations	6	110647	18441.2	133770	0.000
Fungicide	16	795742	49733.9	360762	0.000
Concentrations*-Fungicides	96	298506	3109.4	22555.4	0.000
Error	590	81	0.1		
Total	713	1204982			

Alliete became completely ineffective at 250, 500, and 1000 ppm, but highly effective at 2000 ppm and additional concentrations. Similarly, no inhibition occurred at 250-1000 ppm of curzate, 2000 ppm produced 55.56% inhibition; 4000 ppm produced 77.79%, and no growth at 6000 and 8000 ppm. Antracol also successfully inhibited complete growth at 4000-8000 ppm, melody duo and topsin M brought 100% inhibition at concentrations of 2000-8000 ppm (Table 9).

Response of different isolates to fungicides

All isolates of *P. aphanidermatum* were successfully grown when exposed to different concentrations of Revus and Evito. Both fungicides completely failed to cause any negative effects on *in vitro* growth of all the four isolates. Their growth rate at a very higher concentration of 8000 ppm was almost equal to those of control (w/o fungicides). In contrast to Revus and Evito, all isolates appeared extremely sensitive to 10 fungicides including Dynasty, Copper oxychloride, Score, Tilt, Amistar Top, Nativo, Contaf Plus, Ridomil Gold, Systhane, and Monocot. All isolates except *Pa 12* (which grew only at 250 ppm of Dynasty) failed to tolerate 250-8000 ppm concentrations of these fungicides and produced no growth under *in vitro* conditions. To some extent, the tested isolates showed variable responses to Alliete, Melody Duo, Topsin M, Curzate, and Antracol. In the case of Alliete, all isolates grew well at 250-1000 ppm and completely stopped growing at 2000-8000 ppm, except Pa 11 which produced moderate growth at 2000 ppm. In the case of Melody Duo, *Pa 11* and *Pa 12* showed tolerance up to 4000 ppm; while, Pa 14 and *Pa 16* grew up to 1000 ppm. In the case of Topsin M, *Pa 11* and *Pa 12*, grew up to 2000 ppm, but *Pa 14* and *Pa 16* only grew up to 1000 ppm. Such type of variable response of four isolates was also found in the case of Curzate and Antracol (Table 10).

Table 9: *Response of P. aphanidermatum 16 to different concentrations of various fungicides.*

Fungicides	Concentrations (ppm)						
	250	500	1000	2000	4000	6000	8000
	Growth inhibition (%)						
Alliete	0.00 h*	0.00 h	0.00 h	100.0 a	100.0 a	100.0 a	100.0 a
Dynsaty	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a
Curzate	0.00 h	0.00 h	0.00 h	55.55 e	77.77 b	100.0 a	100.0 a
Antracal	0.00 h	0.00 h	66.66 c	77.77 b	100.0 a	100.0 a	100.0 a
Melody duo	0.00 h	0.00 h	11.11 g	100.0 a	100.0 a	100.0 a	100.0 a
Copper oxychloride	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a
Topsin M	0.00 h	38.88 f	61.11 d	100.0 a	100.0 a	100.0 a	100.0 a
Score	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a
Tilt	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a
Amistar top	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a
Nativo	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a
Cuntoff plus	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a
Ridomal Gold	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a
Revus	0.00 h	0.00 h	0.00 h	0.00 h	0.00 h	0.00 h	0.00 h
Systane	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a
Monocut	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a
Evoto	0.00 h	0.00 h	0.00 h	0.00 h	0.00 h	0.00 h	0.00 h

Each value is a mean of six replications. Values with different letter differ significantly at $p < 0.05$ as determined by DMRT.

Table 10: *Comparative sensitivity of different isolates of P. aphanidermatum to different fungicides.*

Fungicide	Mean growth inhibition (%) of different isolates			
	Pa 11	Pa 12	Pa 14	Pa 16
Alliete	50.79 d*	57.14 d	57.14 e	57.14 e
Dynsaty	85.71 b	100.0 a	100.0 a	100.0 a
Curzate	28.57 g	56.08 e	60.31 d	47.61 f
Antracal	33.86 f	61.90 b	61.90 c	63.49 c
Melody duo	49.20 e	52.38 f	57.14 e	58.73 d
Copper oxychloride	100.0 a	100.0 a	100.0 a	100.0 a
Topsin M	66.08 c	58.41 c	65.87 b	71.42 b
Score	100.0 a	100.0 a	100.0 a	100.0 a
Tilt	100.0 a	100.0 a	100.0 a	100.0 a
Amistar top	100.0 a	100.0 a	100.0 a	100.0 a
Nativo	100.0 a	100.0 a	100.0 a	100.0 a
Cuntoff plus	100.0 a	100.0 a	100.0 a	100.0 a
Ridomal gold	100.0 a	100.0 a	100.0 a	100.0 a
Revus	0.00 h	0.00 g	0.00 f	0.00 g
Systane	100.0 a	100.0 a	100.0 a	100.0 a
Monocut	100.0 a	100.0 a	100.0 a	100.0 a
Evoto	0.00 h	0.00 g	0.00 f	0.00 g
LSD (0.05)	0.1765	0.1644	0.1219	0.1591

Each value is a mean of six replications. Values with different letter differ significantly at $p < 0.05$ as determined by DMRT.

Use of fungicide for commercial seeds treatment of various crops for the management of soil-borne diseases such as caused *Pythium* spp. increasingly become crucial (Doshi and Mathur, 1987; Mocioni

Links Researchers

et al., 2003; Gerik, 2005; Ahmadzadeh and Sharifi-Tehrani, 2006; Carmona *et al*., 2018; Lookabaugh *et al*., 2021; Doherty and Roberts, 2022). Metalaxyl is being used successfully for its management. However, the rise of *Pythium*-resistant strains resistant to widely used Metalaxyl-M has emphasized the need for other technologies to fungicides for disease control (Cohen *et al*., 1999). Discovery and evaluation of alternative novel compounds that are more effective, less toxic, and environmentally friendly are highly desirable. The developed compound needs to check against the available isolates of candidate species. With this aim present study deal with the *in vitro* evaluation of 17 systemic and non-systemic fungicides against 4 isolates of the prominent *Pythium* species (*P. aphanidermatum*) causing diseases to many crops. All isolates appeared extremely sensitive to Dynasty (azoxystrobin), Copper oxychloride (copper oxychloride), Score (difenoconazole), Tilt (propiconazole), Amistar Top (azoxystrobin and difenoconazole), Nativo (trifloxystrobin and tebuconazole), Contaf Plus (hexaconazole), Ridomil Gold (mancozeb and mefenoxam), Systane (myclobutanil) and Monocot (flutolanil), Alliete (fosetyl aluminium), Melody Duo (iprovalicarb and propineb), Topsin M (thiophanate methyl), Curzate (cymoxanil and mancozeb) and Antracol (propineb). Several studies focus on the evaluation of different fungicides against *P. aphanidermatum*. Such studies are important as all the effective fungicides could be used alternatively to reduce the development of fungicide resistance (Suleiman, 2011). Azoxystrobin and mefenoxam were sensitive to *Pythium* spp., (Matic *et al*., 2019). The fungicides Benlate (benomyl), Ridomil, and mancozeb at 50-200 ppm significantly reduced *in vitro* mycelial growth of *P. aphanidermatum* (Suleiman, 2011). For *P. aphanidermatum* and *P. deliense,* Ridomil, Galben M, Warmine, Orthocide + Difolatan and Teroxin effectively reduced the growth of colony at low concentrations (Singburaudom *et al*., 1981). Ethaboxam (benzamide) anti-oomycete chemical (Noel *et al*., 2019). Phosphonic acid-based fungicides (phosphonates) are efficient versus diseases caused by oomycetes (Dann and McLeod, 2021). Fluazinam also was effective in the inhibition of *P. aphanidermatum* (Cohen *et al*., 1999).

Sensitivity to fungicides also varied from species to species within the genus *Pythium* as well as within the different isolates of the same species. Against 21 isolates of *P. aphanidermatum*, the Ec_{50} of metalaxyl was ranging from 1.19 to 3.12 µg ml-1 and 0.05 to 1.30 µg ml-1 for *P. ultimum* (Brantner and Windels, 1998). In the present investigation, different *P. aphanidermatum* isolates showed variable growth responses to different used doses. All isolates except *Pa 12* appeared resistant against 250-8000 ppm concentrations of these fungicides except dynasty and produced no growth under *in vitro* conditions. different isolates may exhibit variation in fungicidal sensitivity because of different origins. A study conducted in Oman indicated that out of 27, only one isolate of *P. aphanidermatum* was found resistant to hymexazole (Al-Balushi *et al*., 2018). Mostly the isolates of the same origin have less variation against fungicides, but differences to fungicides become more visible if a large and diverse population were tested (Al-Sa'di *et al*., 2008). Discussing the same point Matic *et al*. (2019) observed that azoxystrobin was equally effective against different isolates of six *Pythium* spp., with no azoxystrobin-resistant isolate. However, the same complex showed variable response to mefenoxam. Moreover, some of these isolates showed resistance to mefenoxam as well. In another study, out of 194 isolates belonging to four genera of peronosporalean lineage, 16% were insensitive to Ethaboxam (benzamide). Discovering the mechanism of insensitivity, they found mutations in the 239th codon in beta-tubulin as benzamides are hypothesized to bind to beta-tubulin (Noel *et al*., 2019).

Conclusions and Recommendations

A great variation in fungicides efficacy has been observed. Not all but only specific fungicides are effective against *P. aphanidermatum*. Different *P. aphanidermatum* isolates exhibited slight variation in fungicidal sensitivity. In areas of hot spots, the infection of *P. aphanidermatum* can be controlled by the application of azoxystrobin, copper oxychloride, difenoconazole, propiconazole, azoxystrobin + difenoconazole, trifloxystrobin + tebuconazole, hexaconazole, mancozeb + mefenoxam, myclobutanil and flutolanil. The present study also reveals a slight variation among *P. aphanidermatum* population, therefore, either varietal evaluation studies and/or testing different control measures presence of distinct isolates should be considered.

Novelty Statement

The present study reveals the response of four

different isolates of destructive and widespread soil-borne pathogens *Pythium aphanidermatum* to 17 old and novel synthetic fungicides.

Author's Contributions

Ghulam Sarwar Shah: Investigation and data curation and drafted part of it.
Maqsood Anwar Rustamani and Rab Dino Khuhro: Reviewed, editing, and approved the manuscript.
Rehana Naz Syed: Analyzed the data and drafted part of the manuscript.
Abdul Mubeen Lodhi: Analyzed, edited and approved the manuscript.
All authors have read and agreed to the published version of the manuscript.

Conflicts of interest
The authors have declared no conflict of interest.

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