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Original Research Article

In vitro Efficacy of Fungicides and Bio Fungicides against *Pythium aphanidermatum* (Edson.) Fitzp. causing Rhizome Rot of Ginger

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Keywords

Bio fungicide, Fungistatic, Fungicide, Rhizome/soft rot, Systemic, Non systemic

A B S T R A C T

Five systemic, four non systemic, one combo fungicide, three fungal and one bacterial antagonist were evaluated *in vitro* against *P. aphanidermatum* causing Rhizome/soft rot of ginger. All fungicides and biofungicides were found fungistatic / fungicidal action against test pathogen. The systemic fungicides were evaluated at 1000 and 1500 ppm. The non-systemic and combo fungicides were evaluated at and at 2000 and 2500 ppm concentration. The Thiophnate methyl and Copper oxychloride were found to be most fungistatic and recorded significantly highest mean mycelia inhibition (93.28%) and least Propiconazole (35.05%).The percentage mean mycelia inhibition of the test pathogen was found to be increased with increase in concentration. Amongst the bio fungicide used *T. viride* was found most effective and showed significantly highest mycelia inhibition (94.97%) while the bacterial biofungicide *Pseudomonas fluorescens* showed least inhibition (54.76%).

Introduction

The pathogen *P. aphanidermatum* (Edson.) Fitzp. is one of the important fungal pathogen causing soft / rhizome rot.

It is rhizome as well as soil borne. Many workers have been worked on the management of this disease and reported the importance of chemicals and bio fungicides (Sharma *et al.*, 2005; Singh *et al.*, 2012 and Sterling *et al.*, 2009).

In order scrutinise the *in vitro* effect of some systemic, non-systemic, combo fungicides and bio fungicides against *P. aphanidermatum* (Edson.) Fitzp. of ginger the present studies were planed during the year 2016.

Material and methods

Source of pathogen, fungicides and bio fungicides

Typical diseased samples of rhizome rot were collected from farm. The isolations of pathogen from infected tissue were made on *Pythium* selective medium. The pathogen as was identified as *P. aphanidermatum* by microscopic and pathogenic studies.

The culture was multiplied on PDA for further studies. The systemic fungicides *viz.* Hexaconazole, Thiophanate methyl, Azoxystrobin, Fosetyl-AL, Propiconazole, four non systemic fungicides *viz.*, Copper oxychloride, Dimethomorph, Mancozeb, Propineb) and combo fungicide

Carbendazim 12 % WP + Mancozeb 63% WP were purchased from local market. The seven fungal antagonists *viz., T. viride, T. harzianum, T. hamatum, T. Longibrachiatum, T. koningii, T. virens, Aspergillus niger* and one bacterial antagonist *Pseudomonas fluorescens* were procured from the department of plant pathology, VNMKV, Parbhani.

In vitro evaluation of fungicides

For *in vitro* evaluation PDA was used as basal media and Poisoned Food technique method by Nene and Thapliyal, 1993 was used. On the basis of review 1000 and 1500 ppm of systemic and contact/non systemic fungicide 2000 and 2500 ppm concentrations were used.

100 ml PDA medium was poured in 250 ml capacity of sterile glass conical flask and sterilized at 15 psi for 15 minutes. Required quantity of test fungicides for 1000, 1500, 2000 and 2500 ppm was calculated and added to flasks containing the sterilized PDA medium separately and mixed thoroughly.

These media were poured (20ml/plate) in sterilized glass petriplates (90 mm. dia) and allowed to solidify at room temperature. The plates were inoculated by keeping 5 mm disc of one week old pure culture of *P. aphanidermatum* in the center of plate. Three plates per treatment per replication were maintained. Suitable control without pathogen was maintained. These plates were incubated at 27 + 2^0C temperature.

The observations on colony diameter were recorded after a week of incubation. Per cent inhibition of the test pathogen was calculated by using following formula (Vincent, 1927) and the data was statistically analyzed.

$$PI = \frac{C-T}{C} \times 100$$

Where,

PI=Per cent inhibition

C= Growth in control plates

T= Growth in plates treated with fungicides.

The experiment was carried out with 3 replications and 11 treatments in Completely Randomized Design.

Treatments

T_1: Hexaconazole (Contaf 5 EC) - Systemic

T_2: Thiophanate methyl (Topsin M 70 WP) - Systemic

T_3: Azoxystrobin (Amistar 23 SC) - Systemic

T_4: Fosetyl – AL (Aliette 80 WP) - Systemic

T_5: Propiconazole (Tilt 25 EC) - Systemic

T_6: Copper oxychloride (Blitox 50 WP) - Contact

T_7: Dimethomorph (Acrobat 50 WP) - Contact

T_8: Mancozeb (Diathane M-45 WP) - Contact

T_9: Propineb (Antracol 70 WP) - Contact

T_{10}: Carbendazim 12 % WP + Mancozeb 63 % WP (SAAF 75 WP) - Contact

T_{11}: Control (untreated)

In vitro evaluation of biofungicides

The seven fungal antagonistic *viz., Trichoderma viride, T. harzianum, T. hamatum, T. longibrachiatum, T. koningii, T. virens, A. niger* and one bacterial antagonist *P. fluorescens* was evaluated *in vitro* tested dual culture technique (Dennis and Webster 1971) on PDA medium.

Autoclaved and cooled PDA medium was poured (@ 20 ml/plate) in petriplates (90 mm) and allowed to solidify. The plates were inoculated with 5 mm disc of 7 days old culture of biocontrol agents as well as culture of *Pythium aphanidermatum* at equidistance and exactly opposite with each other. The bacterial antagonist was streaked with the help of sterilized inoculating needle at one end of the PDA petriplate and after 24 hrs. of incubation just opposite to the bacterial streak a 5 mm disc of test pathogen was placed. Suitable control without inoculating any biofungicide was kept. All these treatments were carried in triplicate and were incubated at 27 + 2^0C in incubator. Observations on radial mycelial growth of the fungal pathogen and biocontrol agents was measured and per cent inhibition of the test fungus (*P.aphanidermatum*) was calculated by applying formula given by Arora and Upaddyay (1978) as follows.

$$\text{Percent inhibition (PI)} = \frac{\text{Colony growth in Control plate} - \text{Colony growth in intersecting plate}}{\text{Colony growth in control plate}} \times 100$$

The experiment was carried out with 3 replications & 9 treatments in Completely Randomized Design.

Treatments

T_1: *T. viride*
T_2: *T. harzianum*
T_3: *T. hamatum*
T_4: *T. longibrachiatum*
T_5: *T. koningii*
T_6: *T. (Gliocladium) virens*
T_7: *A. niger*
T_8: *P. fluorescens*
T_9: Control (untreated)

Results and Discussion

Systemic Fungicides at 1000 ppm and non-systemic at 2000 ppm concentration (Table 1), the radial mycelia growth of the test pathogen was ranged from 6.53 mm (Thiophnate methyl and Copper oxychloride) to 61.16 mm (Propiconazole). However, maximum radial mycelial growth was recorded with Propiconazole (61.16mm). This was followed by Dimethomorph (55.53), Fosetyl-Al (41.79mm), Azoxystrobin (38.5mm), Propineb (29.06mm), Hexaconazole (25.24mm), Carbendazim 12% WP+ mancozeb 63% WP (23.84mm), Mancozeb (13.58mm), Significantly least mycelial growth was recorded with Thiophnate methyl and Copper oxychloride (6.53 mm).

Systemic fungicides at 1500 ppm and non-systemic fungicides at 2500 concentration and radial mycelial growth of the test pathogen was ranged from 5.55 mm (Thiophnate methyl and Copper oxychloride) to 55.74mm (Propiconazole).

However, maximum radial mycelia growth was recorded with Propiconazole (55.74 mm) and was followed by Dimethomorph (52.92 mm), Fosetyl- Al (36.56), Azoxystrobin (34.01), Propineb (22.84), Hexaconazole (20.86 mm), Carbendazim 12% WP + Mancozeb 63% WP (19.8 mm), Mancozeb (11.5 mm).

Average radial mycelia growth was recorded with all the fungicides tested (Systemic

fungicides @ 1000, 2000 ppm and non-systemic 1500 and 2500 ppm) was ranged from 6.04 mm (Thiophnate methyl and Copper oxychloride) to 58.45 mm (Propiconazole). However, highest mean radial mycelial growth was recorded with Propiconazole(58.45 mm) which was followed by Dimethomorph (54.22 mm), Fosetyl-Al (39.17 mm), Azoxystrobin (36.25 mm), Propineb (25.95 mm), Hexaconazole (23.2 mm), Carbendazim 12% WP + Mancozeb 63% WP (21.82 mm), Mancozeb (12.54 mm). Significantly least mean mycelial growth was recorded with Thiphnate methyl and Copper oxychloride (6.04 mm).

Mycelia inhibition

Results revealed that all the fungicides tested (Systemic @ 1000 and 2000 ppm and Non systemic@ 1500 and 2500 ppm) significantly inhibited mycelia growth of the test fungus over untreated control. Further, it was found that per cent mycelia inhibition was increased with the increase in concentration of the fungicides tested. Systemic fungicides at 1000 and non-systemic 2000 ppm concentration, per cent mycelial growth inhibition was ranged from (32.03%) Propiconazole to (92.74%) Thiphnate methyl and Copper oxychloride. However highest mycelial inhibition was recorded with Thiphanate methyl and Copper oxychloride (92.74%). This was followed by the fungicides, Mancozeb (84.91%), Carbendazim 12% WP+ Mancozeb 63% WP (73.51%), Hexaconazole (71.62%), Propineb (67.71%), Azoxystrobin (57.22%), Fosetyl-AL (53.56%), Dimethomorph (38.3%), Propiconazole (32.04%).

Systemic fungicides at 1500 and non-systemic 2500 ppm concentration, similar trend of mycelial growth inhibition with the test fungicides was recorded and it was ranged from (38.06%) Propiconazole to (93.82%) Thiphnate methyl and Copper oxychloride. However, highest percentage mycelial inhibition was recorded with Thiophante methyl and Copper oxychloride (93.83%), this was followed by Mancozeb (87.22%), Carbendazim 12% WP+ Mancozeb 63% (78.00%), Hexaconazole (76.82%), Propineb (74.62%), Azoxystrobin (62.21%), Fosetyl-Al (59.37%), Dimethomorph (41.2%), Propiconazole (38.06%).

Mean percentage mycelial inhibition recorded with all the fungicides tested (Systemic@ 1000 and 2000 ppm and Non systemic @ 1500 and 2500 ppm) was ranged from (35.05%) Propiconazole to (93.28%) Thiophanate methyl and Copper oxychloride. However, Thiophnate methyl and Copper oxychloride were found to be most fungistatic which recorded significantly highest mean mycelia inhibition of 93.28 per cent. This was followed by Mancozeb (86.06%), Carbendazim 12% WP + Mancozeb 63% WP (75.75 %), Hexaconazole (74.22%), Propineb (71.16), Azoxystrobin (59.71%), Fosetyl-Al (56.46%), Dimethomorph (39.75%) and Propiconazole (35.05%). Propiconazole was found least effective against the test pathogen which recorded the mean mycelia inhibition (35.05%).

Peshney *et al.,* (1990) reported Copper oxychloride, completely inhibited mycelia growth of *Pythium* species. Ram and Thakore, (2009) evaluated systemic fungicides, Mancozeb, Thiophanate Methyl against *P.aphanidermatum.* Result showed that Thiophanate methyl completely inhibited the mycelium growth of *P. aphanidermatum* similar work earlier by several workers (Alam *et al.,* 1998, Sagar, 2006 and Lalfakawma *et al.,* 2014).

Table.1 *In vitro* efficacy of fungicides against *P. aphanidermatum*

Tr. No.	Fungicides	Colony diameter (mm)* at conc.		Average Mean col. dia. (mm)	Inhibition % at conc.		Average Inhibition (%)
		a* conc.	b* conc.		a* conc.	b* conc.	
T_1	Hexaconazole	25.54	20.86	23.2	71.62 (45.73)	76.82 (50.18)	74.22 (47.90)
T_2	Thiophanate methyl	6.53	5.55	6.04	92.74 (68.04)	93.83 (69.76)	93.28 (68.88)
T_3	Azoxystrobin	38.5	34.01	36.25	57.22 (34.89)	62.21 (38.46)	59.71 (36.65)
T_4	Fosetyl –Al	41.79	36.56	39.17	53.56 (32.38)	59.37 (42.79)	59.71 (37.40)
T_5	Propiconazole	61.16	55.74	58.45	32.04 (18.68)	38.06 (22.36)	35.05 (20.51)
T_6	Copper oxychloride	6.53	5.55	6.04	92.74 (68.04)	93.83 (69.76)	93.28 (68.88)
T_7	Dimethomorph	55.53	52.92	54.22	38.30 (22.50)	41.2 (24.32)	39.75 (23.41)
T_8	Mancozeb	13.58	11.5	12.54	84.91 (58.11)	87.22 (60.70)	86.06 (59.38)
T_9	Propineb	29.06	22.84	25.95	67.71 (42.63)	74.62 (48.25)	71.16 (45.37)
T_{10}	Carbendazim 12% wp + Mancozeb 63% WP	23.84	19.8	21.82	73.51 (47.31)	78.00 (51.24)	75.75 (48.66)
T_{11}	Control	90.00	90.00	0.00	0.00 (00.00)	0.00 (00.00)	0.00 (00.00)
	SE	**0.31**	**0.32**	**0.21**	**0.35**	**0.36**	**0.36**
	CD @ 1%	**0.93**	**0.95**	**0.63**	**1.03**	**1.08**	**1.08**

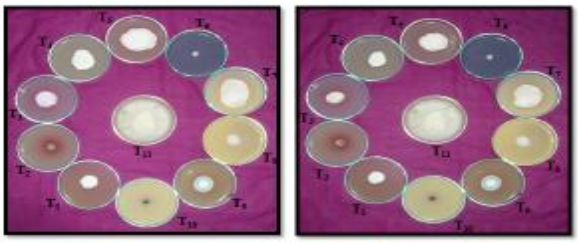
* Mean of three replications. Figure in parenthesis are angular transformed value.
a* Conc.=Systemic at 1000 ppm + non and combo systemic fungicides at 2000 ppm.
b* Conc.= Systemic at 1500 ppm + non systemic and combo fungicides at 2500 ppm.

Table.2 Efficacy of the bioagents antagonists against *P. aphanidermatum*

Tr. No.	Treatments	Mean col. Dia. (mm)*	Inhibition (%)
T_1	*T.viride*	4.52	94.97 (71.77)
T_2	*T. harzianum*	7.86	91.26 (65.87)
T_3	*T.hamatum*	14.91	83.43 (56.53)
T_4	*T. longibrachiatum*	23.58	73.80 (47.55)
T_5	*T. koningii*	22.67	74.81 (48.41)
T_6	*T. virens*	16.78	81.35 (54.43)
T_7	*A. niger*	40.71	54.76 (33.20)
T_8	*P.fluorescens*	49.24	45.28 (26.92)
T_9	Control	90.00	00.00 (00.00)
SE±		**0.31**	**0.37**
CD @ 5%		**0.93**	**1.12**

* Mean of three replications, Figure in parenthesis are angular transformed values.

PLATE 1

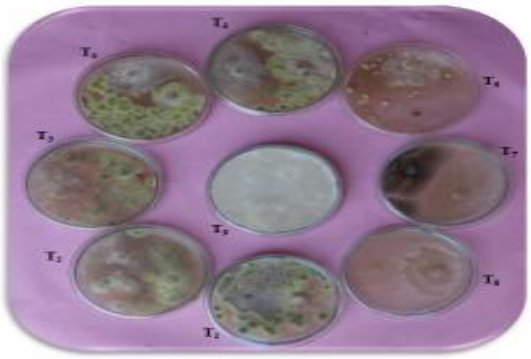


A. 1000 & 2000 ppm B. 1500 & 2500 ppm

In vitro efficacy of systemic fungicides at 1000 & 2000 and non-systemic fungicides 1500 & 2500 ppm against *P. aphanidermatum*

T_1 : Hexaconazole (Contaf 5 EC)
T_2 : Thiophanate methyl (Topsin M 70 WP)
T_3 : Azoxystrobin (Amistar 23 SC)
T_4 : Fosetyl –Al (Aliette 80 WP)
T_5 : Propiconazole (Tilt 25 EC)
T_6 : Copper oxychloride (Blitox 50 WP)
T_7 : Dimethomorph (Acrobat 50 WP)
T_8 : Mancozeb (Diathane 75 WP)
T_9 : Propineb (Antracol 70 WP)
T_{10} : Carbendazim 12% wp + Mancozeb 63% WP (SAAF 75 WP)
T_{11} : Control

PLATE 2



T_1 : *Trichoderma viride*
T_2 : *T. harzianum*
T_3 : *T. hamatum*
T_4 : *T. longibrachiatum*
T_5 : *T. koningii*
T_6 : *T. virens*
T_7 : *Aspergillus niger*
T_8 : *Pseudomonas fluorescens*
T_9 : Control

In vitro Efficacy of the bioagents against *P. aphanidermatum*

Efficacy of bioagents

Seven fungal antagonists *viz., T. viride, T. harzianum, T. hamatum, T. longibrachiatum, T. koningii, T. virens, A. niger* and one bacterial antagonist *P. flurescens* were evaluated *in vitro* against *P. aphanidermatum,* applying dual culture technique (Dennis and Webster, 1971) using PDA as basal medium and result obtained are presented in Table 2.

Result revealed that, all the bioagents evaluated exhibited fungistatic/antifungal activity against *P. aphanidermatum* and significantly inhibited its mycelial growth over untreated control of the antagonist tested, *T. viride* was found most effective and recorded significantly least mycelial growth (4.52 mm) with highest mycelial inhibition (94.97%) of the test pathogen over untreated control (00.00%).

The second and third best antagonists found were *T. harzianum* and *T.hamatum* which recorded mycelial growth of 7.86 mm and 14.91 mm, respectively and inhibition respectively of 91.26 and 83.43 per cent.

This was followed by *T. virens* and *T. koningii* with colony growth respectively of 16.78 mm and 22.67 mm and corresponding growth inhibition of (81.35%) and (74.81%), *T. longibrachiatum and Aspergillus niger* with mycelial growth of 23.58 mm and 40.71 mm respectively and inhibition respectively of (73.80%) and (54.76%) and *P. fluorescens* was found comparatively less effective with both 49.24 mm colony diameter and (45.28%) inhibition of the test pathogen.

Thus all the fungal and bacterial antagonists/bioagents evaluated *in vitro* were found fungistatic/antifungal against *P. aphanidermatum* and caused significant reduction in the linear mycelial growth of the test pathogen over untreated control.

Sharma (1998) reported the *T. harzianum, Trichoderma viride* were most effective in reducing mycelia growth of *P.*

aphanidermatum causing rhizome rot of ginger. Rani and Satheesh (2007) reported that *Trichoderma viride* effective against *P.aphanidermatum* which recorded maximum inhibition of 71.85 per cent. Similar work earlier by several workers (Usha rani and Satheesh, 2007; Muthukumar *et al.*, 2011; Singh *et al.*, 2012; Khasto and Tiameren, 2013).

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