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In-Vitro Efficacy of Fungicides and Bioagents for the Management of Soft Rot of Ginger Caused by *Pythium aphanidermatum*

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A B S T R A C T

Ginger soft rot caused by *Pythium aphanidermatum* is the most destructive and widespread fungal disease of ginger. It has drastic effect on yield causing upto 50% loss under favorable conditions. *In vitro* experiment was conducted for selection of superior fungicides for the management of ginger soft rot. Different non systemic, systemic and combi fungicides were evaluated against the *Pythium aphanidermatum* pathogen in *in-vitro* condition with three different concentrations, among non systemic fungicides, Mancozeb found best effective fungicide which shows 100 per cent inhibition of fungus growth at all three concentrations,. In systamic fungicides, the Carbendazim exhibited 100% fungal growth inhibition at all three concentration. Among six different combifungicides were evaluated, Carbendazim 12% + Mancozeb 63% WP and Metalaxyl M 8%+ Mancozeb 64% WG showed the maximum inhibition (100 %, 100 % and 100 %) of the test fungus at all the three concentrations (0.1, 0.2 and 0.3 %) respectively. Among the bio agents tested aginst *P. aphanidermatum*, *T. harzianum* (Th-4) inhibited maximum mycelia growth of the test fungus (70.40 %).

Introduction

Ginger (*Zingiber officinale* Rose) is one of the most important spices crops in India. It is grown throughout the country. In northern part of the country, the farmers cultivate it as a cash crop. Ginger has special significance for tropical countries, where it is produced and consumed in large quantities (Burkill, 1966 and Purseglove *et al.*, 1981). It has medicinal value too. At present ginger is also being used for chewing purpose. In India, ginger is cultivated with an area of 1,68,000 ha, with the production of 10,76,000 MT and productivity of 6.4 MT/ha (Anonymous, 2017-18).

Materials and Methods

In-vitro evaluation of fungicides

Efficacy of seven non systemic fungicides, eight systemic and six combi fungicides was evaluated *in vitro* at various concentrations against *P. aphanidermatum*, applying Poisoned food technique (Nene and Thapliyal, 1993) and using Potato dextrose agar (PDA) as basal culture medium.

Based on active ingredient, requisite quantity of the test fungicides was calculated, mixed separately thoroughly with autoclaved and cooled (40 ^{o}C) PDA medium in conical flasks to obtain desired concentrations. This PDA medium amended separately with the test fungicides was then poured (20 ml / plate) aseptically in Petri plates (90 mm dia.) and allowed to solidify at room temperature. For each of the test fungicide and its desired concentrations, three plates / treatment / replication were maintained.

After solidification of the PDA medium, all the plates were inoculated aseptically by placing in the centre a 5 mm culture disc obtained from actively growing 7 days old pure culture of *P. aphanidermatum* and incubated in an inverted position at 28 ± 2 ^{o}C. Petri plates filled with plain PDA (without any fungicide) and inoculated with the pure culture disc of *P. aphanidermatum* were maintained as untreated control.

Observations on radial mycelial growth / colony diameter were recorded at an interval of 24 hours and continued till untreated control plates were fully covered with mycelial growth of the test pathogen. Per cent inhibition of the test pathogen with the test fungicides over untreated control was calculated by applying following formula (Vincent, 1927).

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

Where I= Per cent inhibition
C= Growth of fungal plant pathogens in control (mm)
T= Growth of fungal plant pathogens in treatment (mm)

In vitro evaluation of bioagents

Different fungal and bacterial bioagents were evaluated *in vitro* against *P. aphanidermatum*, applying Dual Culture Technique (Dennis and Webster, 1971). Seven days old cultures of the test bioagents and test pathogen (*P. aphanidermatum*) grown on PDA were used for the study.

Two 5 mm culture discs, one each of the test pathogen and test bioagents were cut out with sterilized cork borer and placed at equidistance, exactly opposite to each other on autoclaved and solidified PDA medium in Petri plates and three plates were incubated at 27 ± 2 ^{o}C. PDA plates inoculated alone with pure culture disc (5 mm) of the test pathogen were maintained as untreated control.

The experiment is designed in CRD and all treatments replicated thrice. Observations on linear mycelial growth of the test pathogen and test bioagent were recorded when untreated control plates were fully covered with mycelial growth of the test pathogen.

Per cent inhibition of the test pathogen with the test bioagent, over untreated control by using the formula given below by Vincent (1947).

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

Where,
I = Per cent inhibition
C = Growth of fungal plant pathogens in control (mm)
T = Growth of fungal plant pathogens in treatment (mm)

Results and Discussion

In vitro evaluation of non systemic fungicides

The studies on *in vitro* evaluation of fungicides against *P. aphanidermatum*, revealed that the most effective fungicides was mancozeb, with a mean inhibition of fungal growth of 100 per cent. While cuprous oxide (29.57) was found to be least effective fungicide.

The next best fungicide was captan, which exhibited mean inhibition of 92.99 per cent within the treatments. Highest concentration (0.3 %) of all fungicides tested significantly inhibited the fungal growth than lower concentrations of 0.1 per cent. Mancozeb gave 100 per cent inhibition of growth of test fungus at all three concentrations tested. The results are depicted in Table 1 and Fig. 1.

These results very much supported results of Das *et al.,* (1990). where they studied efficacy of three different fungicides *i.e.,* Captan, Captafol, Dithane M-45 (mancozeb) for seed treatment to control the rhizome rot of ginger.

The captafol treatment at 0.2 % for 30 minutes was also effective than Captan. Dithane M- 45 (Mancozeb) at 0.3 % was almost as effective as Captan and Captafol. It was found that, Captan was more effective against rhizome rot of ginger.

The Sarma (1994) recommended Dithane M-45 was used as seed treatment and soil drench for controlling the rhizome rot of ginger and turmeric. Mancozeb contains dialkine dithiocarbamate which acts as strong chelating agent, which binds with the metal bound protein ions of the fungus hence normal metabolic function of the fungus gets affected.

In vitro evaluation of systemic fungicides

The different of fungicides *viz.,* Fosetyl-Al (80WP), Carbendazim (50WP), Tridemorph (75EC), Azoxystrobin (23SC), Tebuconazole (25EC), Triadimefon (20EC), Hexaconazole (5EC), Carboxin (75WP) were evaluated for both *P. aphanidermatum* and *F. oxysporum* at 0.05, 0.1 and 0.15 per cent concentrations.

All tested systemic fungicides were capable of inhibiting the mycelial growth to the extent of 50.00 per cent except fosetyl-al.

In case of *P. aphanidermatum* results revealed that carbendazim was resulted 100 per cent inhibition at all the three concentrations tested (0.05, 0.1 and 0.15 %) and found significantly superior over the other fungicides which is on far with carboxin (88.50 %).

Tebuconazole (68.61 %, 69.78 % and 72.22 %), Hexaconazole (63.35 %, 72.24 % and 74.44 %) were also equally effective and least inhibition was observed in fosetyl-al (42.14 %) at all the three concentrations was tested respectively. The results are depicted in Table 2 and Fig. 2.

The results are seen similar with results of Thakore *et al.,* (1988) evaluated six non-systemic and four systemic fungicides against rhizome rot with respective fungicides before planting. Of these, Mancozeb, Captafol, Ziride, Captan and Metalaxyl decreased rhizome rot incidence besides increased germination and yield. Chawan *et al.,* (2017) observed that Metalaxyl and Carbendazim gave cent per cent (100 %) mycelial inhibition against *P. aphanidermatum*.

Table.1 Efficacy of non systemic fungicides against *P. aphanidermatum*

Sl. No.	Fungicides	Per cent inhibition			
		Concentration (%)			
		0.1	0.2	0.3	Mean
1	Mancozeb (75WP)	100.00* (90.00)**	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
2	Captan (50WP)	85.84 (67.90)	93.14 (74.82)	100 (90.00)	92.99 (74.64)
3	Cuprous oxide (75WP)	26.72 (31.12)	29.07 (32.62)	32.92 (35.01)	29.57 (32.94)
4	Zineb (75WP)	74.73 (59.82)	84.31 (66.66)	100 (90.00)	86.34 (68.30)
5	Copper Oxy Chloride (50WP)	28.51 (32.27)	38.52 (38.36)	42.09 (40.44)	36.37 (37.09)
6	Chlorothalonil (75WP)	75.94 (60.62)	76.10 (60.73)	83.87 (66.32)	78.63 (62.46)
7	Propineb (70WP)	70.51 (57.10)	75.01 (60.00)	86.88 (68.76)	77.46 (61.65)
Mean		66.03 (54.34)	70.87 (57.33)	77.96 (62.00)	71.62 (57.80)
			S. Em±		**C.D. at 1%**
	Fungicides (F)		**0.22**		**0.82**
	Concentration (C)		**0.13**		**0.50**
	F × C		**0.38**		**1.42**

*Mean of three replications

**Values in the parentheses are arc sine transformed

Fig.1 Efficacy of non systemic fungicides against *P. aphanidermatum*

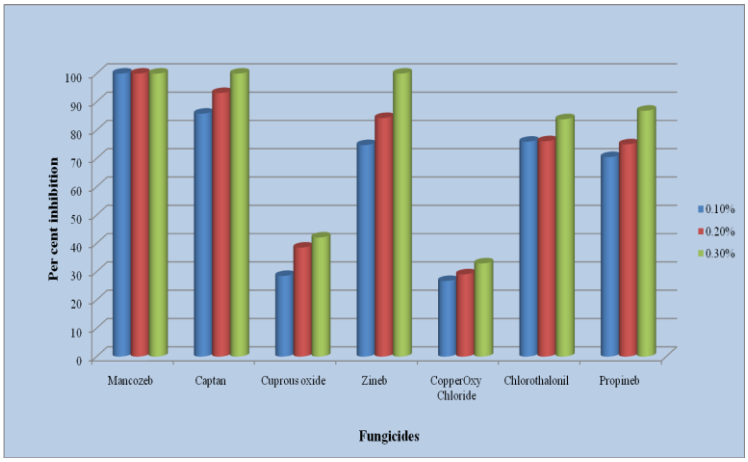


Table.2 Efficacy of systemic fungicides against *P. aphanidermatum*

Sl. No.	Fungicides	Per cent inhibition			
		Concentration (%)			
		0.05	0.1	0.15	Mean
1	Fosetyl-Al (80 WP)	34.20* (35.79)**	40.76 (39.67)	51.47 (45.84)	42.14 (40.44)
2	Carbendazim (50 WP)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
3	Tridemorph (75EC)	59.41 (50.42)	64.11 (53.20)	68.24 (55.70)	63.92 (53.11)
4	Azoxystrobin (23SC)	61.83 (51.85)	67.61 (55.31)	69.81 (56.67)	66.42 (54.61)
5	Tebuconazole (25EC)	68.61 (55.93)	69.78 (56.65)	72.22 (58.19)	70.20 (56.92)
6	Triadimefon (20EC)	76.59 (61.06)	82.67 (65.41)	86.21 (68.21)	81.82 (64.89)
7	Hexaconazole (5EC)	63.35 (52.74)	72.24 (58.20)	74.44 (59.63)	70.01 (56.86)
8	Carboxin (75WP)	77.79 (61.88)	87.70 (69.47)	100.00 (90.00)	88.50 (59.91)
Mean		67.72 (55.37)	73.10 (58.75)	77.78 (61.87)	72.86 (58.60)
			S. Em±	**C.D. at 1%**	
	Fungicides (F)		0.140	0.54	
	Concentration (C)		0.180	0.70	
	F × C		0.312	1.21	

*Mean of three replications

**Values in the parentheses are arc sine transformed

Fig.2 Efficacy of systemic fungicides against *P. aphanidermatum*

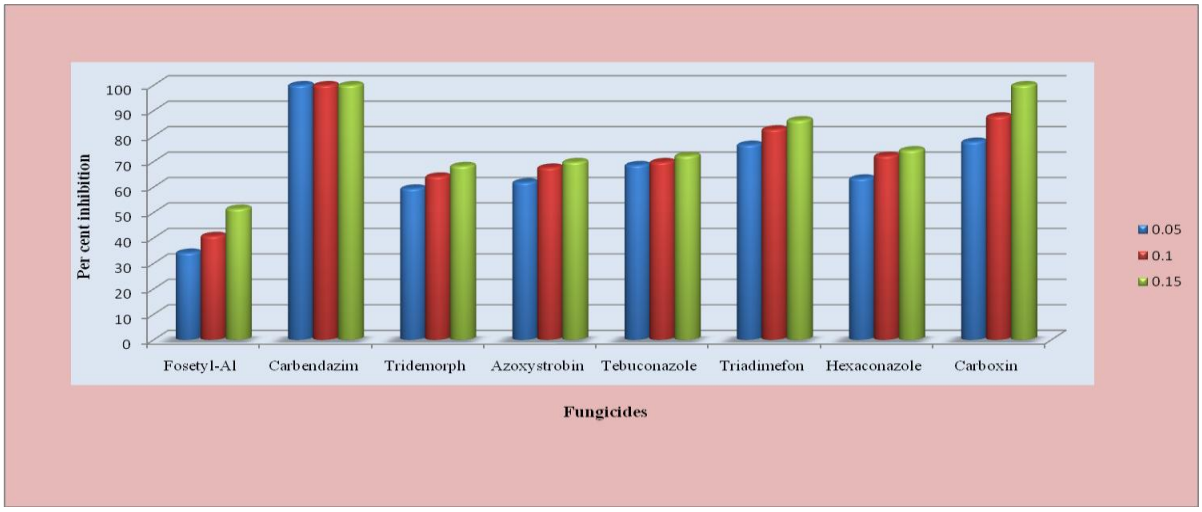


Table.3 Efficacy of combi fungicides against *P. aphanidermatum*

Sl. No.	Combi fungicides	Per cent inhibition			
		Concentration (%)			
		0.1	0.2	0.3	Mean
1	Captan 70% + Hexaxonazole 5% WP	51.30* (45.74)**	82.96 (65.63)	97.04 (80.13)	77.10 (63.83)
2	Carbendazim 12% + Mancozeb 63% WP	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
3	Iprodione 25%+ Carbendazim 25% WP	65.00 (53.73)	70.56 (57.14)	100.00 (90.00)	78.52 (66.69)
4	Metalaxyl 8%+ Mancozeb 64% WG	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
5	Zineb 68% + Hexaconazole 4% WP	58.44 (49.86)	73.89 (59.27)	78.70 (62.52)	70.34 (57.22)
6	Cymoxanil 8% + Mancozeb 64% WP	69.10 (55.01)	70.55 (57.14)	76.85 (61.24)	72.17 (57.80)
Mean		73.97 (59.32)	82.99 (65.64)	92.09 (73.66)	83.01 (65.65)
			S. Em±		**C.D. at 1%**
	Fungicides (F)		**0.18**		**0.68**
	Concentration (C)		**0.11**		**0.42**
	F × C		**0.31**		**1.19**

*Mean of three replications
**Values in the parentheses are arc sine transformed

Fig.3 Efficacy of combi fungicides against *P. aphanidermatum*

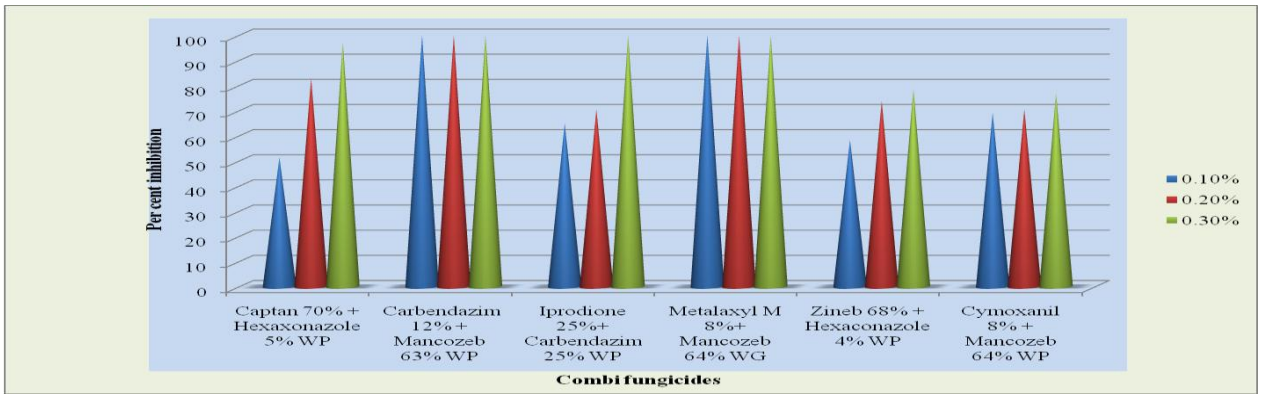
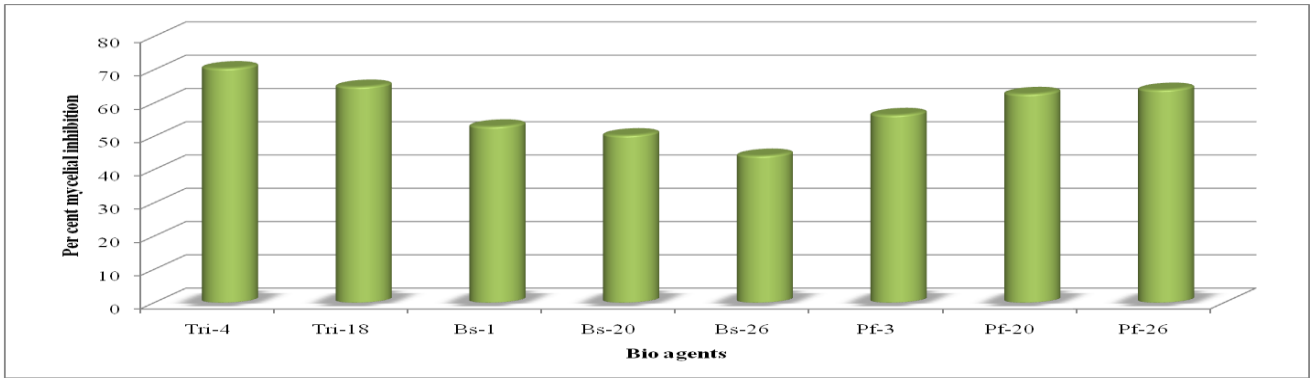


Table.4 Bioefficacy of bio agents against *P. aphanidermatum*

Sl. No.	Bio agent	Strain	Per cent mycelial inhibition
1	*T. harzianum*	Th-4	70.40* (57.03)**
2	*T. harzianum*	Th-18	64.85 (53.63)
3	*Bacillus subtillis*	Bs-1	52.90 (46.66)
4	*B. subtillis*	Bs-20	50.25 (45.14)
5	*B. subtillis*	Bs-26	44.11 (41.61)
6	*Pseudomonas fluorescencs*	Pf-3	56.36 (48.65)
7	*P. fluorescencs*	Pf-20	62.67 (52.34)
8	*P. fluorescencs*	Pf-26	63.94 (53.10)
Mean			**58.18**
S. Em ±			**0.44**
C.D. at 1%			**1.84**

*Mean of three replications
**Values in the parentheses are arc sine transformed

Fig.4 Bioefficacy of bio agents against *P. aphanidermatum*



Tri-4 - *T. harzianum* **Bs-26** - *B. subtillis* **Tri -18** - *T. harzianum*
Pf-3 - *P. fluorescencs* **Bs-1** - *B. subtillis* **Pf -20** – *P. fluorescencs*
Bs-20 - *B. subtillis* **Pf-26** - *P. fluorescencs*

The next best fungicides found were Azoxystrobin (90.70 %) and Thiophanate methyl (90.35 %). Prabhakaran Nair (2013) concluded that treating the seed rhizomes for 30 min with Mancozeb (0.3%) or carbendazim (0.3%) in the case of soft rot prior

Carbendazim have a low toxicity and widely used in agriculture. The carbendazim show a striking resemblance to the secondary plant metabolite colchicine, which disrupts mitosis and meiosis in animal and plant cells by inactivating the spindle. These compounds interfere with mycelial growth and affect conidia formation and also inhibit spore germination.

In vitro evaluation of combifungicides

The different combinations of fungicides *viz.*, Captan 70% + Hexaxonazole 5% WP, Carbendazim 12% + Mancozeb 63% WP, Iprodione 25% + Carbendazim 25% WP, Metalaxyl M 8%+ Mancozeb 64% WG, Zineb 68% + Hexaconazole 4% WP, Cymoxanil 8% + Mancozeb 64% WP were evaluated for both *P. aphanidermatum* and *F. oxysporum* f. sp. *zingiberi* at 0.1, 0.2 and 0.3 % concentrations.

Among the six combi fungicides tested against *P. aphanidermatum* Both Carbendazim 12% + Mancozeb 63% WP and Metalaxyl M 8%+ Mancozeb 64% WG showed the maximum inhibition (100 %, 100 % and 100 %) of the test fungus at all the three concentrations (0.1, 0.2 and 0.3 %) respectively. It was followed by Iprodione 25%+ Carbendazim 25% WP (65.00 %, 70.56 % and 100 %) at 0.1, 0.2 and 0.3 per cent concentrations respectively. The least inhibition was observed in with Zineb 68% + Hexaconazole 4% WP with 70.34 per cent inhibition (Fig. 3). The results are depicted in Table 3.

Carbendazim 12% + Mancozeb 63% WP inhibit spore germination and mycelial growth of fungus. Metalaxyl-M is a systemic fungicide which is rapidly taken up by the green plant part (within 30 min.) transported upwards in the sap stream and is distributed thus provides control of fungi from within the plant. Mancozeb provides a protective film over plant surfaces hence inhibits germination of the spores.

The results of the *in vitro* evaluation of combi fungicides were contrast with the findings of Singh *et al.,* (2004). Who carried out *in vivo* field study for control of rhizome rot diseases of ginger using fungicides. The effect of Dithane 2 %, Ridomil 0.3 %, Bavistin 0.1 %, Saaf 0.2 %, Shield 0.2 %, Blitox 0.3 % and Dithane 0.25% + Bavistin 0.05% was studied against rhizome rot pathogen *P. aphanidermatum* from Bihar.

In vitro evaluation of bioagents

The effect of biocontrol agents on *F. oxysporum* f. sp. *zingiberi* and *P. aphanidermatum* was studied *in vitro* by using dual culture technique in the form of interaction. Among different bio agents tested, against *P. aphanidermatum, T. harzianum* (Tri-4) inhibited maximum mycelia growth of the test fungus (70.40 %) followed by *T. harzianum* (Tri-18) of 64.85 per cent inhibition. *P. fluorescens* (Pf-26) was the next best treatment which inhibited 63.94 per cent mycelia growth of the test fungus which is on par with *P. fluorescens* (Pf-20) with 62.67 per cent inhibition. *B. subtilis* (Bs-1) strain showed 52.90 per cent inhibition and the least inhibition of 44.11 per cent was recorded by *B. subtilis* (Bs-26). The results are depicted in Table 4 and Fig. 4.

Dohroo *et al.,* (2012) found that application of *T. harzianum* bioformulation and seed application with onion and garlic extract were useful in inhibiting the soft rot of ginger and improving the production and yield parameters. Karima *et al.,* (2012) observed the antagonistic effect against *P. aphanidermatum* pathogen reducing the mycelial growth by *T. harizianum i.e.,* followed by *T. viride, B. subtilis* and *P. fluorescens*.

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