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Differential fungicidal sensitivity of *Pythium* species associated with yellowing and wilting of black pepper (*Piper nigrum* L.)

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Abstract

Yellowing and wilting, which become prevalent with the advent of the post-monsoon season, are a serious threat to black pepper cultivation. Detailed investigations into the aetiology revealed an association of three *Pythium* species viz., *P. deliense, P. cucurbitacearum*, and *P. catenulatum,* with the syndrome. As a preliminary phase to develop a promising strategy to manage the yellowing and wilting, new generation fungicides, namely famoxadone-cymoxanil, cymoxanil-mancozeb, strobilurin fungicides [RIL-070/FI (72 FP) and kresoxim-methyl], propiconazole, propineb, chlorothalonil, iprovalicarb-propineb, and fenamidone-mancozeb were screened *in vitro* against the pathogens, and their efficacy was compared with commonly used copper oxychloride and metalaxyl-mancozeb at recommended dosages. Initially, metalaxyl-mancozeb (1250 ppm), propiconazole (2000 ppm), fenamidone-mancozeb (500 ppm), and RIL-070/FI (400 ppm) were found to be promising; however, the pathogens exhibited resurgence of growth, except for *P. cucurbitacearum*. *P. cucurbitacearum* did not show resurgence within 10 days, although delayed resurgence was observed after 12 days.The LD_{50} and LD_{90} values, as well as mycelial degradation, which occurred upon exposure to the fungicides, were also determined. In the Alamar Blue dye assay, a change of colour from blue to pink indicated resistance of the pathogens to the tested fungicides. The *in planta* assay showed typical wilting and root rot with propiconazole, while no visible symptoms were observed with metalaxyl-mancozeb and fenamidone-mancozeb. The results of the present study indicated the multiple fungicide tolerance of *Pythium* spp. associated with the yellowing and wilting of black pepper, which warrants further evaluation of the fungicides under field conditions in endemic regions.

Key words: Black pepper, fungicides, *Pythium deliense*, resurgence, wilting, yellowing

Introduction

Black pepper is one of the major globally traded crops in India, and its export contributes a significant share to the Indian economy. To increase production, it is highly imperative to expand the area under cultivation and also to protect the crop from major pests and diseases (Faisal *et al.*, 2023). Black pepper is susceptible to several diseases, among which foot rot caused by *Phytophthora capsici* and *P. tropicalis* (Suseela Bhai *et al*., 2022), as well as slow decline incited by nematodes in association with *Phytophthora,* are the major diseases (Anandaraj and Sarma, 1995). Post-monsoon yellowing and wilting due to *Pythium deliense* is found to be a dreaded problem in several potential black pepper cultivating tracts of Kerala and Karnataka (Subila and Suseela Bhai, 2020a, 2020b). The affected vines exhibit declining symptoms like yellowing, followed by defoliation, spike shedding and complete wilting during the post-monsoon season, subsequently leading to severe crop loss.

Moreover, the pathogens may also exhibit varied responses to fungicides with different modes of action (Kato *et al.*, 1990; Broders *et al.*, 2007). Chemical toxicity and persistence in plant or soil have adverse impacts on human beings and the biosphere *per se* (Aboutorabi, 2018). It is reported that applying fungicides with a similar mode of action in large quantities over an extended period leads to the development of fungicide resistance in the field population of the pathogens (Ali-Shtayeh *et al.,* 2003; Chung *et al.,* 2009; Moorman and Kim, 2004)

Extensive surveys during the post-monsoon season in black pepper cultivating tracts and further laboratory investigations resulted in the frequent isolation of *Pythium* spp., and *P. deliense* was found to be the most predominant (Subila and Suseela Bhai, 2020b; Subila *et al.,* 2025). The pathogens were characterized by the capability to survive under high soil temperature conditions, while the other major pathogens, like *Phytophthora* and nematodes, are inactivated due to the lack of soil moisture and high temperature. Before the aetiology of yellowing was understood, various fungicide-based and integrated management strategies were being adopted, but, with little success. As the association of *Pythium* spp. with yellowing of black pepper is a new constraint, and the pathogens showed a resurgence of growth after a few days of incubation under *In vitro* conditions, it is imperative to reconsider the sensitivity of the pathogens to fungicides. Hence, the current study examined the efficacy of various widely used as well as new generation fungicides against the pathogens through *In vitro* screening and *in planta* evaluation.

Materials and methods

Pathogens: Different species of *Pythium viz*., *P. deliense* IISR BPPy Tmsy1 (Accession No. MK416216), *P. cucurbitacearum* IISR BPPy Kdg5 (Accession No. MK416214), and *P. catenulatum* IISR BPPy Idk2 (Accession No. OK606004), isolated from the rhizosphere of yellowing affected black pepper were used.

Plants: The rooted plants of variety Panniyur 1, in which the yellowing was mostly observed under field conditions, were raised in plastic pots of size 14 cm diameter containing 500 g potting mixture (Soil: Sand: FYM) in the ratio 2:1:1. Three-month-old plants were used for pot evaluation studies.

Table 1. List of fungicides and their recommended doses

Fungicide	Trade name	Concentration (recommended dose (ppm)
Metalaxyl-mancozeb	Tata Master	1250
Copper oxychloride	Tata Blitox	2500
RIL	RIL-070/FI (72 FP)	400
Iprovalicarb-propineb	Melody Duo	1000
Cymoxanil-mancozeb	Curzate M8	2000
Propineb	Antracol	1000
Chlorothalonil	Chlorothalonil	1000
Propiconazole	Tilt	2000
Kresoxim methyl	Ergon	700
Famoxadone-cymoxanil	Equation Pro	1000
Fenamidone-mancozeb	Sectin	500

Chemicals: The fungicides and concentration used for initial screening are listed in Table 1.

***In vitro* screening of fungicides against *Pythium* spp.**: Eleven fungicides at their respective recommended doses were screened against the pathogens under *In vitro* conditions. Briefly, the fungicides at desired concentrations were amended in Potato Dextrose Agar (PDA) medium and dispensed into 90 mm Petri dishes. The pathogens were inoculated at the center as a mycelial plug (5 mm diameter) derived from the periphery of 72 h-old actively growing culture. The plates seeded with PDA without fungicides served as control and the plates were incubated for 24-240 h at room temperature (Borum and Sinclair, 1968). The per cent growth inhibition was calculated as, I= C-T/C x 100, where C is the growth in control and T is the growth in treatments (Hazarika and Das, 1998).

Determination of minimum lethal dose: For screening higher doses of efficacious fungicides, and to determine the minimum lethal dose, *i.e* ., LD_{50} and LD_{90}, metalaxyl-mancozeb, fenamidone-mancozeb, propiconazole and copper oxychloride, which were found promising to the pathogen, were used. The concentrations tested were 1250 ppm, 1500 ppm, 1750 ppm and 2000 ppm (metalaxyl-mancozeb), 500 ppm, 750 ppm and 1000 ppm (fenamidone-mancozeb), 2000 ppm, 2500 ppm and 3000 ppm (propiconazole) and 2500 ppm, 3000 ppm, 3500 ppm and 4000 ppm (copper oxychloride). The screening was done as described in previous section. LD_{50} and LD_{90} values were calculated based on Probit analysis (Finney, 1952).

Alamar Blue (AB) dye test for viability check: To perform the Alamar Blue (AB) Dye test, the mycelial plugs of 5 mm diameter scooped out from a 72 h-old culture were inoculated into a microtitre well plate (Nest Biotechnology Co. Ltd.) containing 2 ml of different concentrations of fungicide solution. Four replications were maintained for each concentration, along with a positive control with sterile distilled water in which mycelial plugs alone were placed. The plates were incubated for 10 days. The viability of the pathogen was assessed by adding 2 µl of AB dye (100 mg/ml) as an indicator of cellular respiration and incubating for 24 h with a negative control containing AB dye alone (Rampersad, 2011). The colour intensity was determined by measuring absorbance of the dye at 570 nm (λ1 red reflectance, blue light absorbance) and 600 nm (λ2 blue reflectance, red light absorbance). The correlation between radial growth and percentage reduction of AB dye was calculated by the equation ({[(εox λ2) (A λ1)] – [(εox λ1) (A λ2)]}/ {[(εred λ1)(A′ λ2)] – [(εred λ2)(A′ λ1)]}) × 100 where, εox is 117,216 (the molar extinction coefficient of oxidized (blue), and εred is 155,677 (the molar extinction coefficient of reduced (pink); A is the absorbance of test wells, A′ is the absorbance of negative control wells, λ1 is 570 nm, and λ2 is 600 nm (Cox *et al.,* 2009; Pettit *et al.,* 2005). *Pythium* species with percent reduction values >75% were classified as resistant, 25 to 75% as shifted (intermediate between resistant and sensitive) and <25% were considered as sensitive. Considering the predominance and virulent nature among the three species, *P. deliense* was used for further studies.

Effect of fungicides on mycelial degradation and virulence of *P. deliense*: The mycelial plugs of 5 mm size derived from 72-h old culture of *P. deliense* were inoculated into a microtitre well plate containing 2 ml of different concentrations of fungicides and observed for growth and sporangial formation. The mycelial plugs in sterile distilled water served as a control. For the virulence assay, fungicide-treated mycelial plugs from the microtitre well plate were placed on detached black pepper leaves (variety: Panniyur 1) and observed for lesion development (Holliday, 1963).

***In planta* evaluation**: The promising fungicides, *viz*., propiconazole (3000 ppm), metalaxyl-mancozeb (2000 ppm), fenamidone-mancozeb (1000 ppm), and copper oxychloride (4000 ppm), were evaluated *in planta* on the variety Panniyur 1. Pathogen control (*P. deliense*) and absolute control (plants without any fungicide treatment) were also included as treatments with an experimental design CRD and six replications per treatment. After three months of establishment, the plants were inoculated with the pathogen. The pathogen was cultured in potato dextrose agar medium for 72-h, macerated with sterile distilled water and 100 ml of the inoculum was drenched at the root zone of all the treatments except in the absolute control (Bhai *et al.,* 2007). The fungicides were applied as soil drench before and after challenge inoculation. The plants were uprooted after the appearance of disease symptoms and observations were recorded on plant/root infection. The disease potential index (DPI) was analyzed to estimate the pathogen load in the soil. The experiments were repeated thrice for confirmation.

Statistical analysis: The data were statistically analyzed using SAS version 9.2 (www.sas.com/en_us/software). The significance of treatment effects was determined by Analysis of Variance and the means were ranked by Duncan's Multiple Range Test at P=0.05.

Results

***In vitro* screening of fungicides against *Pythium* spp.**: Under *In vitro* screening, at recommended doses, several fungicides failed to suppress growth, while selected fungicides (metalaxyl-mancozeb, RIL-070/FI, cymoxanil-mancozeb, famoxadone-cymoxanil, fenamidone-mancozeb, and propiconazole) showed initial inhibition followed by resurgence. However, after 5 days of incubation with these fungicides, *P. deliense* and *P. catenulatum* regained the viability and overgrew the growth medium in 10 days (Fig. 1 and Table 2), indicating resurgence of growth at the tested concentrations. Whereas, *P. cucurbitacearum* showed resurgence only after 12 days and was inhibited by famoxadone-cymoxanil and propiconazole. There was only <25% inhibition

Table 2. Sensitivity of *Pythium* spp. towards recommended fungicides

Fungicide	Concentration (ppm)	*P. deliense*			*P. cucurbitacearum*			*P. catenulatum*		
		Radial growth in cm (5^{th} day)	Radial growth in cm (10^{th} day)	% inhibition (10^{th} day)	Radial growth in cm (5^{th} day)	Radial growth in cm (10^{th} day)	% inhibition (10^{th} day)	Radial growth in cm (5^{th} day)	Radial growth in mm (10^{th} day)	% inhibition (10^{th} day)
Metalaxyl-mancozeb	1250	0.00	3.50	22.2	0.000	0.000	100	0.000	3.000	33.33
RIL-070/FI	400	0.00	4.00	11.1	2.000	2.000	56.56	0.167	3.000	33.33
Iprovalicarb-propineb	1000	2.53	4.50	0	1.900	4.333	0	2.300	4.500	0
Copper oxychloride	2500	2.67	4.50	0	2.600	4.500	0	2.000	4.500	0
Cymoxanil-mancozeb	2000	0.43	4.00	11.1	0.100	3.000	33.33	0.567	3.500	22.22
Propineb	1000	3.50	4.50	0	3.000	3.967	11.11	3.333	4.500	0
Chlorothalonil	1000	3.37	4.50	0	1.500	4.000	11.11	2.100	4.500	0
Propiconazole	2000	0.10	4.00	11.1	0.000	0.000	100	0.267	3.000	33.33
Kresoxim methyl	700	3.50	4.50	0	3.000	4.500	0	3.500	4.500	0
Famoxadone-cymoxanil	1000	0.23	4.50	0	0.000	0.000	100	0.233	3.500	22.22
Fenamidone-mancozeb	500	4.50	3.70	17.7	0.000	0.000	100	0.000	1.233	72.67
Control		4.50	4.50	0	4.333	4.500		4.333	4.500	
CV (%)		4.293	1.850		5.741	3.311		8.837	0.452	
CD at 5%		0.126	0.116		0.149	0.143		0.233	0.028	

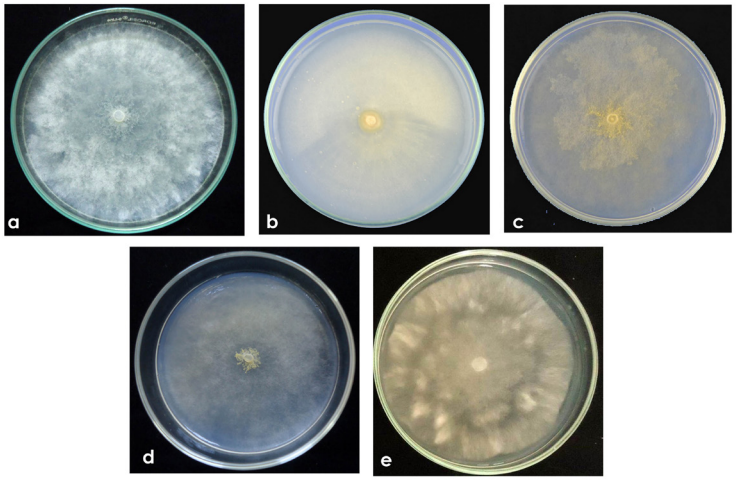

Fig. 1. Resurgence of *P. deliense* on prolonged incubation (10^{th} day) a. Cymoxanil-mancozeb, b. Metalaxyl-mancozeb, c. Fenamidone-mancozeb, d. Propiconazole and e. RIL070/FI

with recommended doses of metalaxyl-mancozeb, RIL-070/FI, cymoxanil-mancozeb, famoxadone-cymoxanil, fenamidone-mancozeb, and propiconazole, indicating the need for still higher concentrations of these fungicides for complete inhibition. Based on the sensitivity, metalaxyl-mancozeb, fenamidone-mancozeb, and propiconazole were shortlisted for further studies and compared with copper oxychloride, the commonly recommended fungicide against oomycetes.

Determination of minimum lethal dose: Higher concentrations of metalaxyl-mancozeb, fenamidone-mancozeb, propiconazole and copper oxychloride were tested *In vitro* to determine the minimum lethal dose. The results showed that metalaxyl-mancozeb is completely inhibitory at 1750 ppm while propiconazole, fenamidone-mancozeb and copper oxychloride showed 100% inhibition at 3000 ppm, 1000 ppm and 4000 ppm, respectively. The LD_{90} values were found to be, 1496.86 ppm, 604.99 ppm, 2497 ppm, and 3804 ppm, for metalaxyl-mancozeb, fenamidone-mancozeb, propiconazole and copper oxychloride, respectively (Table 3).

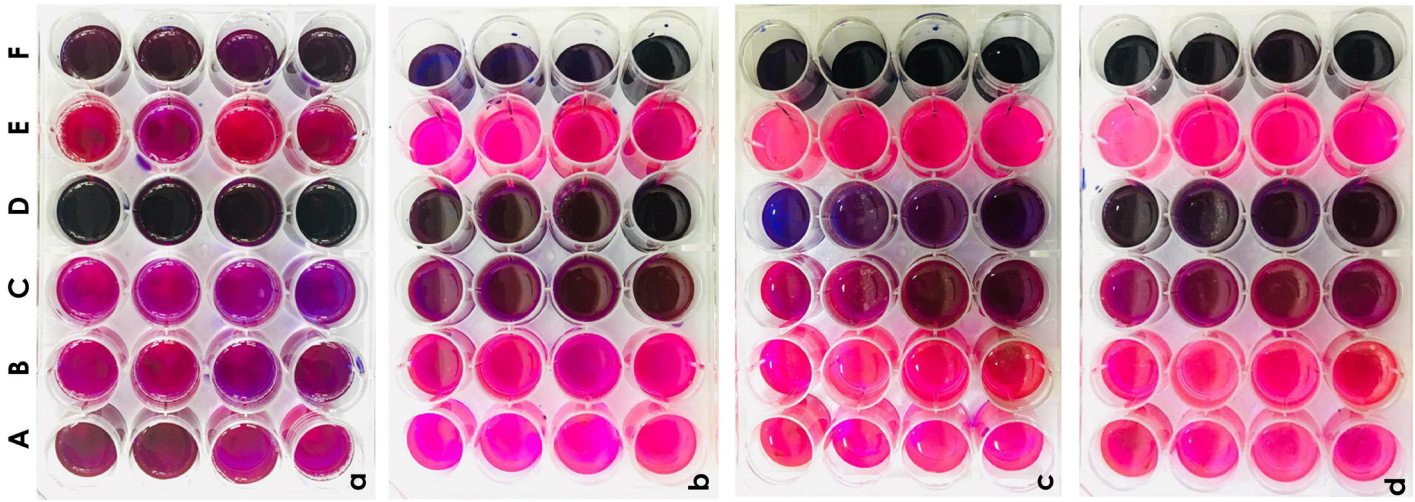

Fig. 2. Alamar Blue (AB) Dye assay: a. Reduction of AB dye by Metalaxyl-mancozeb – A. 1250 ppm, B. 1500 ppm, C. 1750 ppm, D. 2000 ppm, E. positive control and F. negative control (AB dye alone); b. Reduction of AB dye by propiconazole- A. 1500 ppm, B. 2000 ppm, C. 2500 ppm, D. 3000 ppm, E. positive control and F. negative control; c. Reduction of AB dye by fenamidone-mancozeb- A. 250 ppm, B. 500 ppm, C. 750 ppm, D. 1000 ppm, E. positive control and F. negative control and d. Reduction of AB dye by copper oxychloride- A. 2500 ppm, B. 3000 ppm, C. 3500 ppm, D. 4000 ppm, E. positive control and F. negative control

Table 3. Minimum lethal dose of fungicides against *Pythium* species

Pythium species	Metalaxyl-mancozeb		Fenamidone-mancozeb		Propiconazole		Copper oxychloride	
	LD_{50} (ppm)	LD_{90} (ppm)	LD_{50} (ppm)	LD_{90} (ppm)	LD_{50} (ppm)	LD_{90} (ppm)	LD_{50} (ppm)	LD_{90} (ppm)
P. deliense	1337.76	1496.86	186.82	604.99	1678.00	2497.00	2378.00	3804.00
P. cucurbitacearum	1086.64	1186.23	217.43	749.53	704.91	1369.00	1696.00	4376.00
P. catenulatum	1259.83	1277.27	358.64	752.38	1688.00	2555.00	1729.00	4323.00

Alamar Blue dye test for viability check: In the AB dye test, wells with the dye that changed colour from blue to pink indicated that cellular respiration occurred at that concentration of fungicide, indicating cellular viability and resistance of the pathogen. Wells with blue colour indicated that the cells lost their respiratory power due to fungicide sensitivity. In the assay, pink colour was observed up to 1500 ppm of metalaxyl-mancozeb, 2500 ppm of propiconazole, 750 ppm of fenamidone-mancozeb, and 3500 ppm of copper oxychloride, indicating resistance towards the fungicides at the concentrations tested. The percentage reduction of AB dye varied with the pathogen's sensitivity towards fungicides (Fig. 2 & Table 4). It was found that the fungicides showed highest per cent reduction at lower concentrations. In the present study, *P. deliense* was found sensitive to 2000 ppm of metalaxyl-mancozeb, 1000 ppm of fenamidone-mancozeb, 3000 ppm of propiconazole and copper oxychloride at 4000 ppm. Correlation analysis showed that there is a positive correlation between radial growth and per cent reduction of fungicide concentration (metalaxyl-mancozeb-0.66; fenamidone-mancozeb-0.96, propiconazole-0.99 and copper oxychloride-0.98).

Effect of fungicides on mycelial degradation and virulence of *P. deliense*: In treatment with metalaxyl-mancozeb, mycelial degradation occurred at 1750 ppm, whereas with propiconazole at 2500 ppm and with fenamidone-mancozeb at 750 ppm. There was no mycelial degradation in treatment with copper oxychloride. At higher concentrations of these fungicides, sporangial formation was completely inhibited (Fig. 3A). The detached leaf assay showed that mycelial discs from 1750 ppm of metalaxyl-mancozeb did not infect the leaves indicating non-viability of the pathogen (Fig. 3B). In the AB dye test, 1750 ppm showed shifted character, but here it was near to sensitivity (64.5%) (Table 4).

Table 4. AB dye assay for evaluating fungicide sensitivity

Fungicide	Concentration (ppm)	*P. deliense*		*P. cucurbitacearum*		*P. catenulatum*	
		Reduction (%)	Sensitivity classification	Reduction (%)	Sensitivity classification	Reduction (%)	Sensitivity classification
Metalaxyl-mancozeb	1250	77.5	Resistant	28.9	Shifted	87.8	Resistant
	1500	75.3	Resistant	21.8	Sensitive	75.4	Resistant
	1750	64.5	Shifted	11.7	Sensitive	68.0	Shifted
	2000	21.5	Sensitive	9.3	Sensitive	23.3	Sensitive
Fenamidone-mancozeb	250	86.1	Resistant	42.8	Shifted	88.9	Resistant
	500	64.5	Shifted	23.3	Sensitive	77.5	Resistant
	750	43.0	Shifted	24.3	Sensitive	42.8	Shifted
	1000	10.8	Sensitive	10.7	Sensitive	15.7	Sensitive
Propiconazole	1500	75.5	Resistant	29.1	Shifted	79.6	Resistant
	2000	76.3	Resistant	27.2	Shifted	74.5	Resistant
	2500	48.7	Shifted	8.8	Sensitive	67.8	Shifted
	3000	11.1	Sensitive	2.3	Sensitive	9.5	Sensitive

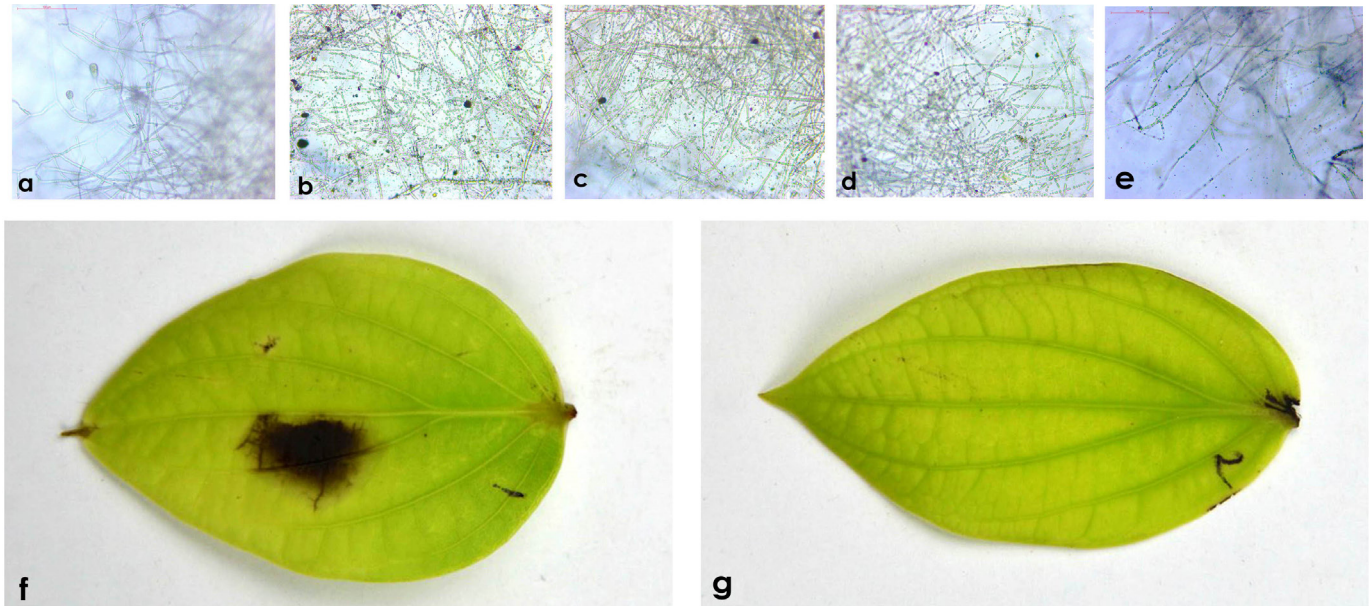

Fig. 3. Fungicide sensitivity in solution: A. mycelial degradation of *P. deliense* by - a. control b. Metalaxyl-mancozeb at 1750 ppm; c. Propiconazole at 2500 ppm; d. Fenamidone-mancozeb at 750 ppm and e. Copper oxychloride at 4000 ppm; B. Detached leaf assay- f. Metalaxyl-mancozeb at 1500 ppm and g. Metalaxyl-mancozeb at 1750ppm

Fig. 4. *In planta* evaluation of fungicides: a- Propiconazole, b- Metalaxyl-mancozeb, c- Copper oxychloride, d- Fenamidone-mancozeb, e- Pathogen control and f- Absolute control

Table 5. Pot evaluation under greenhouse conditions

Treatment	Visible/aerial infections	Plant infection (%)	Root rot (%)	DPI
Propiconazole	Collar rot and wilt	33.3	15	16
Metalaxyl-mancozeb	Root rot	0	11.1	8
Copper oxy chloride	Collar rot, wilt and defoliation	100	86.7	24
Fenamidone-mancozeb	Root rot	0	16.7	8
Pathogen control	Collar rot, wilt and defoliation	100	100.0	32
Absolute control	No infection	0	0.00	0
CV (%)			3.6	
LSD at 5%			1.05	

***In planta* evaluation**: *In planta* evaluation of the higher effective dose of fungicides showed no visible symptoms like yellowing or wilting with metalaxyl-mancozeb (2000 ppm) and fenamidone-mancozeb (1000 ppm), indicating that these chemicals suppressed the pathogen. However, treatment with propiconazole showed visible symptoms of wilting and root rot (15%). Even though there were no visible symptoms with metalaxyl-mancozeb and fenamidone-mancozeb, root rot was observed in both cases at 11.1% and 16.7%, respectively. But the DPI of the soil was reduced four times (DPI 8) compared to pathogen-alone treated plants (DPI 32) (Table 5 & Fig. 4). The plants treated with copper oxychloride showed defoliation, wilting and severe root rot (86.7%), indicating that the prevailing recommended dose is not effective against *P. deliense* induced yellowing.

Discussion

Pythium deliense is a new report in the black pepper disease catalogue (Subila and Suseela Bhai, 2020a) and hence, appropriate management strategies are not available. Hence, in the present study, recommended doses of already certified fungicides against oomycete pathogens *viz*., famoxadone-cymoxanil, cymoxanil-mancozeb, RIL-070/FI, propiconazole, propineb, chlorothalonil, kresoxim-methyl, iprovalicarb-propineb and fenamidone-mancozeb, along with commonly used fungicides in black pepper, such as copper oxychloride and metalaxyl-mancozeb, at recommended dosages, were screened for sensitivity against *Pythium* spp. both *In vitro* and *in planta*. The results showed that the recommended dose of these fungicides was not effective against *P. deliense*. In the present investigation, the recommended dosage of the fungicides (for black pepper) was initially inhibitory but resulted in the resurgence of the pathogen after a prolonged incubation period. So higher doses of these fungicides were subsequently assessed for their inhibitory effect and the LD_{50} and LD_{90} values were estimated. Mycelial degradation was also recorded at higher concentrations. The colourimetric assay with AB dye was used to confirm the viability of the pathogen at different concentrations of the fungicides (Cox *et al.,* 2009). The change in colour from blue to pink showed viability of the cell and the percentage reduction of dye classified the pathogens from resistant to sensitive. *In planta* evaluation revealed the need for higher concentrations of fungicides for disease suppression, as all the fungicides at lower or recommended doses did not suppress root infection on black pepper, though the DPI was considerably reduced to four-fold under greenhouse evaluation.

In *Pythium* and *Phytophthora*, metalaxyl and mefenoxam inhibit ribosomal RNA synthesis (Cohen and Coffey, 1986). However, a mutation in the *RNA pol I* gene regulated by an incompletely dominant single gene renders *Pythium* and *Phytophthora* insensitive to metalaxyl (Bhat *et al.,* 1993; Gisi and Cohen, 1996; Randall *et al.,* 2014). Earlier, researchers have proved the inhibitory effect of metalaxyl on Pythiaceae due to retarded respiration and interfering with spindle formation during mitosis, leading to distorted germtubes and hyphae (Abdel-Fattah and Baka, 2000). Kato *et al.* (1990) evaluated the sensitivity of several *Pythium* spp. and other oomycetes to hymexazol and metalaxyl and found that different species of *Pythium* had a range of sensitivities to both metalaxyl and hymexazol. Broders *et al.* (2007) reported the fungicide sensitivity of *Pythium* spp. infecting corn and soybeans. Ferry and Farrar (2009) reported the effect of azoxystrobin or higher rates of mefenoxam in reducing the incidence of cavity spot in carrot caused by *Pythium* spp. Flores and Garzon (2009) observed reduced mycelial growth of *P. aphanidermatum* with mefenoxam.

Resistance to mefenoxam is reported in *P. aphanidermatum* infecting poinsettia and geranium under protected cultivation (Moorman *et al.,* 2002; Moorman and Kim, 2004). The cucurbit and pepper isolates of *Phytophthora capsici,* as well as *P. erythroseptica* and *Pythium ultimum* infecting potato, exhibited sensitivity towards mefenoxam (Lamour and Hausbeck, 2003; Taylor *et al.,* 2006). *P. deliense* infecting soybean was found to be sensitive to mefenoxam (13.33 ppm) and metalaxyl (30 ppm) (Feng *et al.,* 2020). Hymexazol resistance in some *Fusarium* and *Pythium ultimum* has been reported (Ali-Shtayeh *et al.,* 2003; Chung *et al.,* 2009; Moorman and Kim, 2004). Chavan *et al.* (2017) reported that copper oxychloride is 100% inhibitory, whereas chlorothalonil exhibited only 81.4% inhibition at 2500 ppm against *P. aphanidermatum*, the rhizome rot pathogen of turmeric. While metalaxyl-mancozeb (2500 ppm), cymoxanil-mancozeb (2500 ppm) and propiconazole (1500 ppm) showed 60.96%, 64.4% and 82.17% inhibition, respectively. In the present study, copper oxychloride was not found to be inhibitory to *P. deliense* which is in accordance with earlier reports. Broders *et al.* (2007) studied the sensitivity of mefenoxam (5-100 ppm), azoxystrobin (10-100 ppm), trifloxystrobin (10-100 ppm) and captan (50-200 ppm) against 58 *Pythium* isolates and found that, the tested fungicides were not effective along with differential response of the isolates to the fungicides. All these reports support our study that both sensitivity and dose of the fungicides are species-dependent and should be evaluated for each species separately. Our study showed that the fungicides metalaxyl-mancozeb and fenamidone-mancozeb at higher doses *i.e* ., 2000 ppm and 1000 ppm, respectively prevented the disease development, but with slight root rot, suggesting the need for higher concentrations to manage the disease. But the application of the same fungicides in large quantities for a prolonged period

has been reported to the build-up of fungicide resistance in the field population of the pathogens (Chung *et al.,* 2009). Based on our study, metalaxyl-mancozeb and fenamidone-mancozeb were effective at higher doses and can be alternated for field-level management of the disease, as well as for preventing the development of resistance in the pathogen. It is concluded that the fungicides metalaxyl-mancozeb and fenamidone-mancozeb at 2000 ppm and 1000 ppm, respectively, suppress the disease based on *in planta* studies, which need to be further evaluated under field conditions in order to develop a sustainable management strategy.

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