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Integrated management of post-monsoon yellowing of black pepper (*Piper nigrum* L.) incited by *Pythium deliense*

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Abstract

Post-monsoon yellowing, incited by *Pythium deliense*, has recently emerged as a major threat to black pepper cultivation tracts in Kerala and Karnataka. To manage the disease, a field trial based on an integrated management approach was conducted from 2018 to 2020. Initial *in vitro* and greenhouse evaluation studies revealed that fenamidone-mancozeb inhibited the pathogen and was compatible with the bioagents *viz.*, *Trichoderma asperellum* and *Streptomyces albulus*. Combined application of *T. asperellum* as soil drench and fenamidone-mancozeb as foliar spray (1500 ppm) reduced the severity of yellowing from 78.8% to 21.67%, with a reduction of 72.33% and reduced the pathogen load in the soil from 32 to 2 disease potential index (DPI). The present study reports the efficacy of the combined application of *T. asperellum* and fenamidone-mancozeb as an integrated management strategy against post-monsoon yellowing incited by *P. deliense* in black pepper.

Key words: *Piper nigrum* L. (black pepper), fenamidone-mancozeb, *Pythium deliense, Streptomyces albulus*, *Trichoderma asperellum,* yellowing

Introduction

Climatic variability, characterised by erratic increases in atmospheric temperature and subsequent depletion of soil moisture, poses a major threat to agriculture, particularly in tropical regions. The combined effect of both biotic and abiotic factors has a profound adverse impact on black pepper cultivation in certain areas, where the vines exhibit declining symptoms, such as yellowing, defoliation, and wilting, during the post-monsoon season, resulting in severe crop loss. In general, the synergistic association of nematodes and *Phytophthora* spp. implicated to cause yellowing and slow decline disease of black pepper (Anandaraj and Sarma, 1995). Yellowing due to *Fusarium* spp. has also been documented (Shahnazi *et al.*, 2012). However, recent studies have unravelled the association of *Pythium deliense* with post-monsoon yellowing of black pepper, and the symptoms differ from those of slow decline disease (Subila and Suseela, 2020a, 2020b). Pathogenicity studies have indicated that *P. deliense* infects the roots, subsequently damaging the entire root system, which leads to the yellowing and wilting of the vines. Under field conditions, the affected vines exhibit declining symptoms, including yellowing of the foliage, defoliation, spike shedding, and wilting, which are most prominent during the post-monsoon season. To mitigate the problem, farmers adopted conventional management strategies, including commonly used fungicides at recommended concentrations and bioagents, but with limited success.

A strong understanding of the sensitivity of the pathogen to fungicides and bioagents is critical in formulating an effective, economical and eco-friendly integrated disease management strategy. Synthetic chemicals alone will not be feasible since the non-judicious application of fungicides may lead to the development of fungicide resistance in pathogenic microbes (Al-Sadi *et al.*, 2015). Hence, developing an integrated management approach that combines fungicides and bioagents with recommended agronomic practices is essential for managing diseases. In formulating a suitable integrated management strategy, assessing the compatibility of the fungicides and bioagents in an appropriate combination is also indispensable.

Chemical toxicity and persistence in plants, water, and soil have far-reaching adverse consequences for mankind and the biosphere (Aboutorabi, 2018). Biocontrol agents, such as *Trichoderma* and actinomycetes, are environment-friendly and safe alternatives for crop protection (Muthukumar *et al.*, 2011). Globally, extensive research is being undertaken to maintain and improve environmental health and combat fungicide-resistant pathogen populations. A proper combination of management measures would result in improved, broad-spectrum pathogen control with long-term efficacy concurrent with reduced pesticide usage. Many potential pathogens may coexist in the same field, and thus, different combinations of disease management practices may have additive or synergistic effects (Cohen *et al.*, 2000).

Subila and Suseela (2021) assessed the sensitivity of *P. deliense* to various fungicides and bioagents at different concentrations *in vitro* and *in planta*. The *in vitro* studies revealed that the fungicides, metalaxyl-mancozeb (2000 ppm), propiconazole (3000 ppm) and fenamidone-mancozeb (1000 ppm) were inhibitory to the pathogen whereas, *in planta* studies with propiconazole showed wilting symptoms followed by root rot (15%) and metalaxyl-mancozeb and fenamidone-mancozeb showed 11.1% and 16.7% root rot, respectively without manifestation of any aerial symptoms (data unpublished). Another trial with bioagents showed that *Trichoderma harzianum* and *Streptomyces albulus* were completely inhibitory to the pathogen both *in vitro* and *in vivo* (Subila and Suseela, 2021). Based on the results from the previous experiments, a field trial was conducted using these promising fungicides and bioagents to manage the pre-monsoon yellowing of black pepper caused by *P. deliense*.

Materials and methods

The experiments were conducted in three phases: *in vitro*, in planta, and under field conditions. In the first phase, compatibility of the fungicides and bioagents was assessed under *in vitro* conditions. The second phase involved *in planta* evaluation, and in the third phase, the promising combinations were evaluated under field conditions.

Pathogen: *Pythium deliense* (IISR BPPY Mp2; Acc. No. MH017856) isolated from the rhizosphere of yellowing and wilt-affected black pepper (Kozhikode, Kerala, India; 76.0878° E, 11.4438° N) was used for *in vitro* screening studies. The pathogen was cultured on potato dextrose agar (PDA) medium for 72 hours, macerated in sterile distilled water and used for challenge inoculation of the plants under pot culture conditions.

Biocontrol agents, including *Trichoderma asperellum* NAIMCC 0049, Streptomyces albulus (Acc. No. KM361516), and *Pseudomonas fluorescens* (IISR BPP2), were obtained from the repository of beneficial isolates at the ICAR-Indian Institute of Spices Research, Kozhikode, Kerala, India. The spores of *T. asperellum* were harvested from the fungal colony cultured on PDA and applied to the pots as a spore suspension containing 10^8 spores/mL. *S. albulus* was mass multiplied in malt extract broth and applied @ 10^8 CFU/mL.

Fungicides: The commercial products of metalaxyl-mancozeb and fenamidone-mancozeb were used in the study.

Plants: Black pepper rooted plants of 3-4 leaf stage of the variety Panniyur 1 transplanted in plastic pots containing 500 g solarized potting mixture (Soil: Sand: FYM at 2:1:1 ratio) were used and the treatments were imposed after the establishment of the plants.

***In vitro* assay**: A dual-plate assay was employed to assess the direct inhibition potential of the bioagents against *P. deliense*. *Streptomyces albulus* and *Pseudomonas fluorescens* were streaked on PDA plates as two lines, equidistant from the edge of the plate. A mycelial plug derived from a 72-hour culture of *P. deliense* was placed at the centre and incubated at 30°C for 72 hours. Mycelial plugs of *T. asperellum* and *P. deliense* were placed in a single PDA plate equidistant from the edge of the plate on the opposite side and incubated at 30°C for 72 hours. The fungicide sensitivity of *P. deliense* was tested by culturing on media amended with fungicides (metalaxyl-mancozeb at 2250 ppm, fenamidone-mancozeb at 1500 ppm, and copper oxychloride at 3500 ppm). *P. deliense* on the PDA plate served as the control. Six replications were maintained for each treatment. The per cent inhibition of mycelial growth of the pathogen was calculated using the formula:

$I = (C-T)/C \times 100$ (Vincent 1947)

Where, I - % inhibition, C - radial growth in control plate, T - radial growth of the pathogen in bioagent/fungicide inoculated plate

Compatibility of fungicides and bioagents: The fungicides (metalaxyl-mancozeb and fenamidone-mancozeb) were incorporated into the PDA medium at the previously mentioned concentrations, mixed well, and poured into 90 mm Petri dishes using the poisoned food technique. A mycelial disc of 5 mm excised from the growing margin of a 5-day-old culture of *T. asperellum* was inoculated at the center of the Petri dish. Similarly, *S. albulus* was streak cultured on PDA amended with the fungicides. The PDA plates, without fungicide but inoculated with bioagents, served as the control. All plates were incubated at 26±2°C for 5 days, and growth was recorded (Waghe *et al.*, 2015).

***In planta* evaluation**: The *in planta* evaluation was conducted on the black pepper variety Panniyur 1, with treatments including *T. asperellum, S. albulus*, metalaxyl-mancozeb (2, 250 ppm), and fenamidone-mancozeb (1, 500 ppm), compared with an absolute control without any application (Bhai *et al.*, 2007), with six replications. Fungicides and bioagents were applied in their respective concentrations as a soil drench before and after challenge inoculation with the pathogen. The plants were inoculated with 100 mL of a macerated culture of the pathogen at 10^5 CFU/mL in the root zone of all treatments except the absolute control. Plants were uprooted after the appearance of disease symptoms, and observations were recorded on the plant and root infection. The pathogen load in the soil was estimated for the disease potential index (DPI) of the soil. The experiment was repeated twice for confirmation.

Field evaluation: The field evaluation was conducted from 2018 to 2020 in a farmer's plot located at Ambalavayal (76.18138° E, 11.59805°N), Wayanad, Kerala, India, where yellowing was found to be severe and a recurring problem. The experiment was conducted in a uniformly infected plot (more than 75% yellowing) with 5-year-old black pepper vines yielding black pepper, intercropped with areca nut, coffee, and banana.

The treatments were selected based on the compatibility of fungicides and bioagents (based on the results of *in vitro* studies), which included (1) *T. asperellum* alone, (2) *S. albulus* (IISR BP Act 1) alone, (3) *T. asperellum* + fenamidone-mancozeb, (4) *S. albulus* + fenamidone-mancozeb, (5) fenamidone-mancozeb alone and (6) absolute control without any treatments. Five replications were maintained for each treatment, with each replication consisting of six plants. Bioagents at one litre of each culture/vine (10^8 CFU/mL) were applied at the basin as soil drench along with 250 g well-decomposed cow dung. Fenamidone-mancozeb (1500 ppm) was applied as a foliar spray at monthly intervals for three months, followed by bimonthly intervals. In treatment five, fenamidone-mancozeb was applied alone as both a soil drench and a foliar spray.

The intensity of yellowing and the disease potential index were recorded at monthly intervals, in addition to recording the yield per plant. The rhizospheric soil samples from each treatment were collected at a depth of 20 cm from the root zone of the vines pooled together and three replications from each treatment were collected for further studies. The soil samples for microbial analysis were stored at 4°C. Each vine was indexed for the severity of yellowing using a 0-4 scale (0 = no infection, 1 = 25%, 2 = 50%, 3 = 75%, and 4 = 100% infection) (Eapen *et al.*, 2009). The disease potential index of the soil was determined using the soil dilution endpoint method (Anandaraj and Sharma, 1990), with *Bauhinia variegata* leaflets as bait (Anoop, 2011).

Statistical analysis: The software SAS version 9.2 was used to scrutinize the significance of the treatments. The data were analysed using the analysis of variance (ANOVA) test, and the values were ranked by Duncan's multiple range test (DMRT) at a significance level of 5%.

Results

In vitro **assay**: The *in vitro* assay using bioagents and fungicides showed that all treatments were effective in inhibiting the mycelial growth of *P. deliense*. All the bioagents tested showed more than 75% inhibition (Table 1), wherein *T. asperellum* and *S. albulus* were found to be superior (99.67% inhibition) compared with *P. fluorescens* (78.67% inhibition). *T. asperellum* and *S. albulus* overgrew the pathogen completely after 5 days of incubation (Fig. 1). Hence, *T. asperellum* and *S. albulus* were shortlisted for subsequent studies. Among the fungicides, fenamidone-mancozeb showed 98% inhibition, followed by metalaxyl-mancozeb (96.67%), whereas copper oxychloride exhibited 45.33% inhibition only. The results from *in vitro* studies with fungicides indicated that copper oxychloride was ineffective against *P. deliense*; therefore, it was excluded from *in planta* and field evaluations.

Table 1. Mycelial growth inhibition of *P. deliense* by bioagents and fungicides

Bioagents/Fungicides	Inhibition (%)
Trichoderma asperellum	99.667[a]
Streptomyces albulus	99.667[a]
Pseudomonas fluorescens	78.667[c]
Fenamidone-mancozeb (1500 ppm)	98.000[b]
Metalaxyl-mancozeb (2250 ppm)	96.667[b]
Copper oxychloride (3500 ppm)	45.333[d]
CV (%)	0.905
CD (5%)	1.391

Compatibility assay: In the compatibility assay, *T. asperellum* exhibited considerable growth on the medium amended with metalaxyl-mancozeb (2250 ppm) and fenamidone-mancozeb (1500 ppm), however, some morphological changes were noticed (Fig. 2). Conversely, *S. albulus* displayed reduced growth and notable morphological deformations, indicating sensitivity to these fungicides. The study revealed that *T. asperellum* is compatible with the fungicides, while *S. albulus* is not.

***In planta* evaluation**: In the *in planta* evaluation, plants treated with *T. asperellum*, *S. albulus* and fenamidone-mancozeb showed 100 % root rot inhibition with a reduction in DPI compared to control. Although there were no external symptoms due to infection in the case of metalaxyl-mancozeb, root rot was observed at a rate of 12.67%. The disease potential index (pathogen load) in the rhizosphere was reduced from DPI 32 to 2 in the case of biocontrol agents followed by fenamidone-mancozeb (from 32 to 3.33) (Fig. 3 and Table 2). In contrast, for metalaxyl-mancozeb, the DPI was reduced to 6.67 only. Hence, based on *in planta* evaluation, *T. asperellum*, *S. albulus* and fenamidone-mancozeb were selected for the subsequent assessment under field conditions. Due to the incompatible nature of *S. albulus* with the fungicides, fenamidone-mancozeb, being a systemic cum contact fungicide, was applied as a foliar spray along with basal application of bioagents.

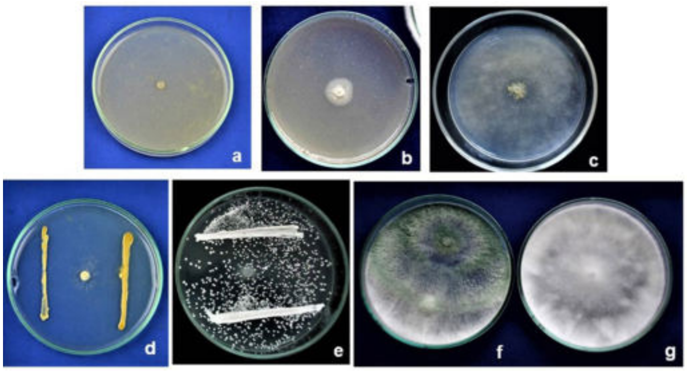

Fig. 1. *In vitro* evaluation of bioagents and fungicides against *P. deliense* (a) fenamidone-mancozeb (b) metalaxyl-mancozeb (c) copper oxychloride (d) *Pseudomonas fluorescens* (e) *Streptomyces albulus* (f) *T. asperellum* (g) control (*P. deliense* alone)

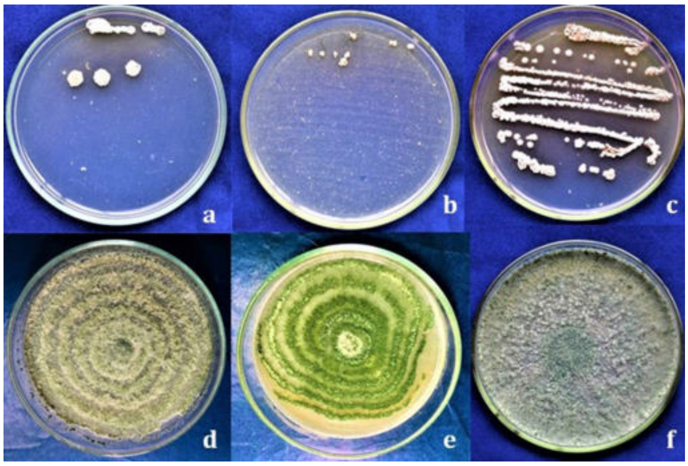

Fig. 2. Compatibility assay of bioagents with fungicides (a) *S. albulus* in fenamidone-mancozeb (b) *S. albulus* in metalaxyl-mancozeb (c) *S. albulus* on PDA (d) *T. harzianum* in fenamidone-mancozeb (e) *S. albulus* in metalaxyl-mancozeb (f) *T. harzianum* on PDA

Table 2. *In planta* evaluation of bioagents and fungicides

Treatments	Symptoms	Plant infection (%)	Root rot (%)	DPI
Trichoderma asperellum	No infection	0	0.00	2.00
Streptomyces albulus	No infection	0	0.00	2.00
Metalaxyl-mancozeb	Root rot	0	12.67	6.67
Fenamidone-mancozeb	No infection	0	0.00	3.33
Inoculated control	Collar rot, wilt, defoliation	100	100.00	32.00
Uninoculated control	No infection	0	0.00	0.00
CV (%)		NS	4.53	12.79
CD (5%)			1.27	1.47

Field evaluation: Among the treatments, *T. asperellum,* along with fenamidone-mancozeb, was found to be the most promising treatment in reducing *the* yellowing of black pepper vines. A more than 70% reduction was achieved in the treatment, and the DPI was reduced from 32 to 2 (Table 3). Treatment with *T. asperellum* alone showed a decrease in the intensity of yellowing to 64.16%

Fig. 3. *In planta* evaluation of bioagents and fungicides (a) *T. harzianum* (b) *S. albulus* (c) pathogen inoculated (d) metalaxyl-mancozeb (e) fenamidone-mancozeb (f) uninoculated control

Fig. 4. Field evaluation of bioagents and fungicide (a) *S. albulus* (b) *T. asperellum* (c) fenamidone-mancozeb (d) *S. albulus* + fenamidone-mancozeb (e) *T. asperellum* + fenamidone-mancozeb (f) untreated control (g) before application (h) after application (*T. asperellum* + fenamidone-mancozeb)

Table 3. Field evaluation of fungicide

Treatments	Intensity of yellowing (%)		Intensity reduction (%)	DPI		Fresh yield (kg) per standard/vine	
	2018*	2020		2018*	2020	2019	2020
T. asperellum	80.0	28.67	64.16	16	2.67	12.00	17.33
S. albulus	80.0	31.67	60.41	16	2.67	11.67	17.00
T. asperellum + fenamidone-mancozeb	78.33	21.67	72.33	16	2.00	11.67	23.33
S. albulus + fenamidone-mancozeb	78.33	36.67	53.18	16	4.00	12.00	20.67
Fenamidone-mancozeb	78.33	41.67	46.80	16	8.00	11.33	15.33
Untreated control	80.0	73.33	8.33	16	21.33	11.67	12.67
CV (%)	2.57	7.07		NS	16.25	5.32	5.59
CD (5%)	3.06	4.57			5.69	0.94	1.49

* before application

followed by *S. albulus* alone (60.41%) whereas, combined application of *S. albulus* with fenamidone-mancozeb showed a 53.18% reduction only (Fig. 4). Application of bioagents alone also recorded a low level of DPI (2.67). Hence, based on these results, combined application of *T. asperellum* and fenamidone-mancozeb (1500 ppm) was found to be suitable for managing post-monsoon yellowing induced by *P. deliense.* Furthermore, a 50% yield increase was also recorded in these treatments.

Discussion

A diverse array of biotic and abiotic factors threatens the black pepper industry, adversely affecting the production potential, even with promising high-yielding varieties and landraces in traditional black pepper cultivation tracts. Pan globe, black pepper production suffers setbacks due to several plant pathogens with the potential to inflict damage to both aerial and subterranean plant parts, leading to severe economic losses (Anandaraj, 2000). Footrot incited by *Phytophthora capsici* and *P. tropicalis*), stunt disease caused by cucumber mosaic and *Piper* yellow mottle viruses, slow decline/yellows induced by *Meloidogyne* spp. and *Radopholus similis* and anthracnose (different species of *Colletotrichum*) are considered as the most cosmopolitan and economically important diseases in black pepper (Biju *et al.*, 2021).

In the present study, the application of *T. asperellum* to the soil and spraying fenamidone-mancozeb at 1500 ppm showed control over the intensity of yellowing (72.33% reduction) in black pepper after two years of trial. Fenamidone-mancozeb was also reported as promising in controlling *Phytophthora capsici* infection in black pepper (Rini and Remya, 2020). For the control of *P. capsici* infection in black pepper under field conditions, among the new-generation fungicides and biopesticides, fenamidone-mancozeb (2000 ppm) as a soil drench and spray was the most effective, followed by copper hydroxide (2000 ppm). Many researchers have proven the efficacy of fenamidone-mancozeb against *Pythium* spp. (Yadav and Joshi, 2012; Ravichandra *et al.*, 2023; Dam and Sreedhar, 2023). According to Dam and Sreedhar (2023), fenamidone-mancozeb was the most effective as it inhibited the growth of *P. aphanidermatum* even at 100 ppm. According to our previous findings, the soil application of *T. harzianum* (later confirmed as *T. asperellum* by DNA fingerprinting) and *S. albulus* during the post-monsoon season proved to be an effective management method for reducing the severity of black pepper yellowing (Subila and Suseela, 2021). Under pot culture conditions, Küçükyumuk *et al.* (2014) found that a blend of zinc and *Glomus intraradices* could regulate *P. deliense*-induced seedling rot in cucumbers. According to Feng *et al.* (2020), *P. deliense* associated with soybean was found to be sensitive to mefanoxam (13.33 ppm) and metalaxyl (30 ppm) *in vitro*. Abdelzaher *et al.* (2000) conducted a study on the disease management of maize caused by *P. deliense* using antagonistic soil fungi and stated that coating maize grains and roots with *Aspergillus terreus*, *Chaetomium globosum* and *Myrothecium verrucaria* protected maize seedlings from root-rot and pre- and post-emergence damping-off.

T. asperellum is reported to be antagonistic against soil-borne pathogens such as *Phytophthora ramorum* (Widmer, 2014), *P. capsici* (Segarra *et al.*, 2013), *Pythium myriotylum* (Mbarga *et al.*, 2012), *Macrophomina phaseolina* and *Fusarium solani* (Pastrana *et al.*, 2016), *Verticillium dahliae* (Carrero-Carron *et al.*, 2016), *F. oxysporum* f. sp. *lycopersici* (El Komy *et al.*, 2015), *F. oxysporum* f. sp. *cubense* (Chaves *et al.*, 2016) and *Rhizoctonia solani* (Santos de França *et al.*, 2015) and aerial pathogens such as *Colletotrichum gloeosporioides* (de los Santos-Villalobos *et al.*, 2013) and *Ramularia areola* (da Silva *et al.*, 2017). *T. asperellum* isolates have also been observed to enhance plant growth (Nieto-Jacobo *et al.*, 2017). Integrated pest or disease management (IPM or IDM) involves combining conventional pest or disease management measures with pesticides to reduce pesticide applications.

Three isolates were selected to investigate the survival of conidia in soil after different fungicides were applied. No fungicide killed the conidia, but certain fungicides affected their survival. According to Ashwani *et al.* (2012), *Trichoderma* was found to be highly sensitive to Ridomil (metalaxyl-mancozeb). The integration of compatible bioagents with pesticides may enhance disease control effectiveness and improve soil-borne disease management (Papavizas and Lewis, 1981). The combination of biological control agents with fungicides would provide disease suppression similar to that achieved with higher fungicide use (Monte, 2001).

Hence, based on the present investigation, the combined application of *T. asperellum* and fenamidone-mancozeb (1500 ppm) was found to be promising for managing post-monsoon yellowing induced by *P. deliense*, as it reduced both the intensity of yellowing and the pathogen load in the soil. The systemic nature of fenamidone-mancozeb protects the roots from infection. *T. asperellum* in the rhizosphere antagonises the pathogen, preventing it from entering the roots and thereby protecting the plants. Additionally, it imparts resistance to plants through the exudation of antimicrobial compounds (Guzmán-Guzmán *et al.*, 2023). The results of the present investigation clearly indicated that the integrated management strategy, including the soil application of *T. asperellum* along with FYM and aerial spraying with fenamidone-mancozeb, will improve soil quality, suppress the pathogen, and thereby increase yield.

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