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Evaluation of Endophytes and Green Nanoparticles for the Management of Pomegranate Wilt Complex

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ABSTRACT: The endophytes, *Trichoderma harzianum*-LF30, *T. asperellum*-SF33, Bacillus vallismortis-SB3 and green nanoparticle, *Pseudomonas fluorescens-based* zinc nanoparticles (Pf-ZnNPs) were first tested for their plant growth promotion activity with biocontrol agents *T. viride* (Multiplex Nisarga) and *B. amyloliquifaciens* in sorghum seeds. These endophytes and nanoparticles were tested for compatibility with each other and with chemical fungicides, mancozeb @ 0.2 percent, tebuconazole, propiconazole @ 0.1 percent, carbendazim 12% + mancozeb 63% and captan 70% + hexaconazole 5% @0.2%. Among endophytes and Pf-ZnNPs higher seed germination per cent was recorded in Pf-ZnNPs and seedling vigour was recorded in *B. vallismortis* -SB3.Based on compatibility test, consortia of *T. viride*-Multiplex Nisarga + *T. harzianum*-LF30 + *T. asperellum*- SF33 and *B. vallismortis*- SB3 + *B. amyloliquifaciens* were developed and included in management of pomegranate wilt study (pot culture). Among twelve treatments, these consortia and tebuconazole, propiconazole at 0.1% showed cent per cent disease reduction after 90 days of inoculation.

Keywords: Endophyte, management**,** nanoparticle, pomegranate wilt.

INTRODUCTION

Pomegranate wilt complex caused by *Ceratocystis fimbriata* and *Fusarium oxysporum* is becoming a major problem in northern Karnataka (Saranya *et al*., 2023). In the recent era, the exploitation of endophytes and green nanoparticles for the management of plant diseases is becoming more popular, as it is eco-friendly and sustainable in nature with cost effectiveness and efficiency. Endophytes live in plants as asymptomatic, may be mutualistic, and have been proven to be extremely beneficial to plants by promoting plant growth potential and acting as biological control agents against fungi, bacteria, viruses, and nematodes (Compant *et al*., 2005; Basamma and Naik 2018; Sunilkumar and Yashoda, 2018; Nakkeeran *et al*., 2021). In our study, we isolated 101 endophytes from pomegranate plants (Saranya and Yashoda 2019) and selected the three best endophytes based on their *in vitro* antagonist tests against *C. fimbriata* and *F. oxysporum*. They were *Trichoderma harzianum*-LF30, *T. asperellum*-SF33 and *Bacillusvallismortis*-SB3.

The use of nanoparticles in agricultural fields is a booming technology, as they are more effective at the nanoscale. Green nanoparticles are capturing attention as they offer an eco-friendly way to synthesise nanoparticles and are promoted as an alternative to chemical and physical methods. It is one of the cutting-edge technologies to manage plant diseases. Metallic nanoparticles with different forms, sizes, compositions, and physicochemical properties can be produced through biological processes, and their application has grown in recent years (Abdul *et al*., 2014; Nargund *et al*., 2016). In our study, we synthesised four different nanoparticles, *viz.*, pomegranate aril-based sulphur nanoparticles (PA-SNPS), pomegranate peel-based silver nanoparticles (PP-AgNPs), *Pseudomonas fluorescens-based* zinc nanoparticles (Pf-ZnNPs), and chitosan-based zinc nanoparticles (Ch-ZnNPs) (Unpublished). Among four different nanoparticles, Pf-ZnNPs were selected based on the *in vitro* studies.

This study was planned to evaluate best performing endophytes and green nanoparticles with selected biocontrol agents and fungicides under pot culture conditions. Before going to pot study, plant growth promotion activity and compatibility tests were carried out. Then the selected endophytes and green nanoparticles were evaluated *in vivo* with the selected chemical fungicides: mancozeb @ 0.2 percent, tebuconazole, propiconazole @ 0.1 percent, carbendazim 12% + mancozeb 63% and captan 70% + hexaconazole 5% @0.2% as well as biocontrol agents *T. viride* (Multiplex Nisarga) and *B. amyloliquifaciens*.

MATERIALS AND METHODS

A. Plant growth promoting activity of selected endophytes and green nanoparticles

Pomegranate is a perineal crop and is propagated through cuttings. So, the evaluation of plant growth promotion activity in pomegranate is difficult. Therefore, it was evaluated in sorghum seeds by the standard roll towel method (ISTA, 1993). The endophytic suspension of *T. harzianum*-LF30, *T.*

asperellum-SF33@(1×10^6 cfu/ml), *B. vallismortis*-SB3 (1 × 10^8cfu/ml), and green nanoparticle suspension of Pf-ZnNPs @ 0.15 % were prepared. Sorghum seeds were surface sterilized by submerging them in 4 per cent sodium hypochlorite for 3 minutes and then in 70 per cent ethanol for 2 minutes. The seeds were then rinsed in sterile water three times. Later, seeds were immersed in the prepared suspension overnight. The endophytes were compared with biocontrol agents *T. viride*-Multiplex Nisarga and *B. amyloliquifaciens.* Sterile distilled water was taken as a control treatment.

For this, 300 seeds were placed on paper towels in three sets of 100 seeds each and these 100 seeds were placed on presoaked germination paper. The seeds were then placed 2 cm below the edge of the sheet in a row using forceps. A strip of paper was wetted and placed over the seeds to keep them moist and prevent them from being displaced while rolling. The polythene sheet along with the seeds was then rolled up by using a rubber band to prevent unfurling of the roll and incubated in a growth chamber at 25°C. Three replications were kept for each treatment. Germination percentage was determined by counting the normal seedlings on the seventh day of incubation according to International Seed Testing Association protocols (ISTA, 1993) and shoot length, root length and seedling vigor index were also recorded. The percentage of seed germination was calculated using the formula given by Labouriau (1983).

$$\text{Percent seed germination} = \left(\frac{N}{A}\right) \times 100$$

Where,

N = Number of germinated seeds

A = Total number of seeds tested

The seedling vigor index was calculated using the formula given by Abdul- Baki and Anderson (1973).

Seedling vigor index = Germination percentage × (Mean root length + Mean shoot length)

Compatibility test

Effect of fungicides and nano particles on biocontrol agents/endophytes

The sterilized and cooled PDA was incorporated with following fungicides/nanoparticles.

Fungicides/Nanoparticles	Concentration (%)
PF-ZnNPs	0.1 %, 0.15%
Mancozeb 75%WP	0.2 %
Captan 70% + Hexaconazole 5% WP	0.1 %, 0.2%
Carbendazium 12 % + Mancozeb 63 % WP	0.1 %, 0.2%
Propiconazole 25 % EC	0.1 %, 0.2%
Tebuconazole 25 % EC	0.1 %, 0.2%

This medium was poured into sterile Petri plates and allowed to solidify. Actively growing biocontrol agent/endophyte was inoculated in the centre of Petri plate under aseptic condition. Each treatment was replicated thrice. The plates were incubated at 25 ±1ºC till it reaches the periphery of the plate.

Mutual compatibility of endophytes and biocontrol agents. The endophytes/biocontrol agents were subjected to *in vitro* compatibility experiment using dual culture technique. In case of fungi three mm mycelial discs of seven-day old cultures of two fungi were placed at opposite ends in Petri dishes with PDA (Potato Dextrose Agar). In between fungal and bacterial evaluation mycelial discs of fungal antagonist was inoculated at one side and bacterial antagonist was streaked at other side of the plate. The Plates were triplicated and kept at 25± 1°C for five days to observe the compatibility reaction. The compatibility between bacterial antagonists were tested by cross streak method. Two different bacterial isolates were streaked vertically with 4 cm gap on NA (Nutrient Agar) mediated plates. The plates were incubated for 42 h at 25± 1°C and observed for its compatibility. The following are the combinations.

Sr. No.	Combinations
1.	*Trichoderma viride*-Multiplex Nisarga + *Bacillus amyloliquifaciens*
2.	*T. harzianum*-LF30 + *B. amyloliquifaciens*
3.	*T. asperellum*-SF33 + *B. amyloliquifaciens*
4.	*T. viride*-Multiplex Nisarga + *B. vallismortis*-SB3
5.	*T. harzianum*-LF30 + *B. vallismortis*-SB3
6.	*T. asperellum*-SF33 + *B. vallismortis*-SB3
7.	*B. amyloliquifaciens* + *B. vallismortis*-SB3
8.	*T. harzianum*-LF30 + *T. viride*-Multiplex Nisarga
9.	*T. harzianum*-LF30 + *T. asperellum*-SF33

Efficacy of effective treatments against wilt complex under in vivo condition. This study was conducted in a glasshouse. There, the cleaned soil mixture (Soil, sand & FYM in 3:1:1 ratio) was transferred to a cement floor and four per cent formalin was added for sterilization. The soil bed was prepared and made airtight by covering it with a polythene sheet. Then the polythene sheet was removed after 10 days of fumigation and then the soil was spread to facilitate the escape of toxic fumes, if any. This soil was filled in the pot measuring 36 × 34 × 17 cm (upper calibre × height × bottom diameter). Then the one-month-old pomegranate seedlings (Bhagva variety) were transferred to pots and maintained till they were 6 months old. The following treatments were assigned to each pot randomly with three replications (two pots per replication).

The endophytes and biocontrol agents were applied to the soil @ 50ml/plant having concentrations of 1 × 10^6cfu/ml for fungi and 1 × 10 ^{8}cfu/ml for bacteria. While in combination treatments, the amount of inoculum was equally divided and distributed after make up to 50ml/plant. Challenge inoculation of the pathogen was done by application of four per cent giant culture of pathogens at 7 days after inoculation of endophytes and biocontrol agents. Fungicides and nanoparticles were applied after 3 days of pathogen inoculation by drenching method. Wilt incidence was recorded at 60, 75 and 90 days after inoculation. Per cent disease incidence (PDI) and plant height was recorded. Per cent wilt incidence was calculated using the following formula.

Per cent wilt incidence = (No. of plants wilted/Total no. of plants observed) × 100

Treatments	Treatment details
T_1	Tebuconazole 25 % EC @ 0.1 % @ 3 DAI of pathogens
T_2	Probiconazole 25 % EC @ 0.1 % @ 3 DAI of pathogens
T_3	Mancozeb 75 % WP @ 0.2 % @ 3 DAI of pathogens
T_4	Taqat 75 % WP @ 0.2 % @ 3 DAI of pathogens
T_5	SAAF 75 % WP @ 0.2 % @ 3 DAI of pathogens
T_6	Pf-ZnNPs @ 0.15 % @ 3 DAI of pathogens
T_7	*T. harzianum*-LF30 @ 7 DBI of pathogens
T_8	*T. harzianum*-LF30 @ 7 DBI of pathogens + *T. viride*-Multiplex Nisarga @ 7 DBI of pathogens + Pf-ZnNPs 0.15 % @ 3 DAI of pathogens
T_9	*T. harzianum*-LF30 @ 7 DBI of pathogens + *T. viride*-Multiplex Nisarga@ 7 DBI of pathogens + Mancozeb 0.2 % @ 3 DAI of pathogens
T_{10}	*T. viride*-Multiplex Nisarga @ 7 DBI of pathogens + *T. harzianum*-LF30 @ 7 DBI of pathogens + *T. asperellum*-SF 33 @ 7 DBI of pathogens
T_{11}	*B. vallismortis*-SB 3 @ 7 DBI of pathogens + *B. amyloliquifaciens*@ 7 DBI of pathogens
T_{12}	Control

RESULTS AND DISCUSSION

Effect of endophytes and Pf-ZnNPs on plant growth promotion activity. Among all treatment tested (Table 1) for their growth promotion activities, the higher per cent seed germination was recorded in Pf-ZnNPs @ 0.15 % (86.67 %) which was on par with *B. amyloliquifaciens* (84.33 %), *T. harzianum*-LF30 (83.33 %) and *B. vallismortis* -SB3 (83.33 %) and were significantly superior to the untreated control. Lower germination was recorded in untreated control (75.33 %). The maximum shoot length and root length was recorded in SB3 (12.18 and 20.11 cm), which was on par with shoot length of *T. harzianum*-LF30 (11.50 cm). The minimum shoot length and root length was recorded in untreated control (9.59 and 15.60 cm). The higher seedling vigor index was observed in *B.vallismortis*-SB3 (2127.86) which were on par with Pf- ZnNPs @ 0.15 % (1990.52) and *T. harzianum*-LF30 (1984.62). The lower seedling vigor index was recorded in untreated control (1516.57) followed by *T. asperellum*-SF33 (1786.80) (Fig. 1).

T_1–*T. viride*-Multiplex Nisarga; T_2–*B. amyloliquifaciens;* T_3-Pf- ZnNPs @0.15%; T_4- *T. asperellum*-SF33; T_5 - *T. harzianum*-LF30; T_6 - *B. vallismortis*-SB3; C - Control (Water)

Fig. 1. Effect of endophytes, biocontrol agent and nanoparticle on seedling vigour of sorghum.

Table 1: Effect of green nanoparticles, endophytes and bioagent on seedling vigour of sorghum.

Sr. No.	Treatment	Per cent germination	Shoot length (cm)	Root length (cm)	Seedling vigour index
1.	*T. viride*-Multiplex Nisarga	81.33 (64.40) *	10.36	17.57	1798.54
2.	*B. amyloliquifaciens*	84.33 (66.68)	10.20	17.54	1849.35
3.	Pf- ZnNPs @ 0.15 %	86.67 (68.58)	10.62	18.40	1990.52
4.	*T. asperellum*-SF33	82.00 (64.90)	10.23	17.30	1786.80
5.	*T. harzianum* -LF30	83.33 (65.91)	11.50	18.61	1984.62
6.	*B. vallismortis*-SB3	83.33 (65.91)	12.18	20.11	2127.86
7.	Control (Water)	75.33 (60.22)	9.59	15.60	1516.57
	S.Em. ±	0.86	0.14	0.25	36.11
	C.D. @ 1 %	3.65	0.59	1.05	152.01
	C.V.	2.30	2.27	2.43	3.35

*Arc sine transformed values

Endophytic microorganisms can influence the plant growth, which differs among species and strains and there may be many mechanisms through which plant growth is promoted. Conceptually, researchers have speculated that plant growth promoting endophytes may influence plant growth either directly or indirectly. Direct promotion of plant growth occurs when either (i) the plant growth promoting endophytes enable the attaining of resources from the environment including potassium, nitrogen, phosphorus and iron; (ii) modify plant growth by providing or regulating various plant hormones including cytokinin, auxin or ethylene. Indirect promotion of plant growth by endophytes through production of metabolites, HCN and antibiotics against pathogenic bacteria and fungi (Rekha *et al.*, 2017). Similar to our results, Adhikari *et al.* (2016) and

Estrada-Urbina *et al.* (2018) reported that, Zinc oxide nanoparticles enhanced the germination and growth of maize.

Compatibility test

Effect of fungicides and PF- ZnNPs on growth of endophytes and bioagents. To develop an effective disease management strategy, the compatibility of potential bio agents and endophytes with fungicides and green nano-particle, is essential. The result presented in the Table 2 depicts that among different treatments Pf-ZnNPs at 0.1 per cent was compatible for *T. harzianum*-LF30 and *T. viride* (Multiplex Nisarga) and their mycelial growth was 8.15 cm and 8.4 cm respectively. In Pf-ZnNPs at 0.2 per cent concentration the mycelial growth was recorded as 8.05cm and 8.2 cm respectively. The next best treatment mancozeb at 0.2 per cent did not affect the mycelial growth (8.5) of *T. harzianum* (Multiplex Nisarga) but sporulation was less as compared with control treatment. The endophyte *T. harzianum* -LF30 was partially compatible (4.2cm) in mancozeb 0.2 per cent concentration. The fungicides *viz.*, taqat, SAAF, propiconazole and tebuconazole at 0.1 and 0.2 per cent concentration did not allow the growth of fungal antagonists. In case of bacterial antagonists, both *B. amyloliquifaciens* and *B. vallismortis*-SB3 showed incompatible reaction for all tested fungicides and Pf-ZnNPs. This study clearly indicates that the endophyte *T. harzianum*-LF30 can be used along with the fungicide mancozeb and Pf-ZnNPs. Similar to our study, Wedajo (2015) tested *T. harzianum* (AUT1) and *T. viride* (AUT2) for their compatibility in mancozeb and curzate at 100, 200, 400, 600, 800 and 1000 ppm concentrations. Maximum compatibility was found at lowest concentrations, while at 400 and 600 ppm the antagonists showed tolerance. However, beyond 1000 ppm concentrations the growth was completely inhibited.

Table 2: Compatibility of endophytes/biocontrol agent with nanoparticle/fungicides.

Treatment	Mycelial growth (cm)			Colony growth	
	***T. asperellum*-SF33**	***T. harzianum*-LF30**	***T. viride*-Multiplex Nisarga**	***B. amyloliquifaciens***	***B. vallismortis*-SB3**
PF-ZnNPs @ 0.1 %	**0 (-) *	8.15	8.4	-	-
PF-ZnNPs @ 0.15 %	0 (-)	8.05	8.2	-	-
Mancozeb 75 %WP @ 0.2 %	0 (-)	4.2	8.5	-	-
Captan 70 % + Hexaconazole 5 % WP @ 0.1 %	0 (-)	0 (-)	0 (-)	-	-
Captan 70 % + Hexaconazole 5 % WP @ 0.2 %	0 (-)	0 (-)	0 (-)	-	-
Carbendazium 12 % + Mancozeb 63 % WP @ 0.1 %	0 (-)	0 (-)	0 (-)	-	-
Carbendazium 12 % + Mancozeb 63 % WP @ 0.1 %	0 (-)	0 (-)	0 (-)	-	-
Propiconazole 25 % EC @ 0.1 %	0 (-)	0 (-)	0 (-)	-	-
Propiconazole 25 % EC @ 0.2 %	0 (-)	0 (-)	0 (-)	-	-
Tebuconazole 25 % EC @ 0.1 %	0 (-)	0 (-)	0 (-)	-	-
Tebuconazole 25 % EC @ 0.2 %	0 (-)	0 (-)	0 (-)	-	-
Control	8.5	8.5	8.5	+	+

* - Incompatible, ** Diameter, - no growth, + growth was observed

To test the mutual compatibility of endophytes and biocontrol agents. There were totally of ten combinations among the fungi and bacterial antagonists. The different interactions observed were intermingling of hyphae, presence of thick mycelial band, clear demarcation at the meeting point, heavy sporulation at the meeting point and yellow pigmented band at the interaction site. Incompatible combinations showed over growth, lysis of colony and reduction in size of colonies.

Combinations of antagonist for plant diseases management include mixtures of fungi and mixtures of fungi and bacteria. Most reports on bioconsortia showed that, combinations of antagonists resulted in improved biocontrol activity. However, they are also resulted in suppression of disease compared with their individual bioagent. Incompatibility of the co-inoculated antagonists may arise because biocontrol agents inhibit each other as well as the target pathogen. Therefore, the effective endophytes and bioagents were subjected to mutual compatibility test for the development of efficient microbial consortia.

Among ten combinations of fungal and bacterial antagonists, five combinations gave compatible result (Table 3). They were *T. asperellum*-SF33 + *B. vallismortis*-SB3, *T. harzianum*-LF30 + *T. viride*-Multiplex Nisarga, *T. harzianum*-LF30 + *T. asperellum*-SF33, *T. viride*-Multiplex Nisarga + *T.*

asperellum-SF33 and *B. amyloliquifaciens* + *B. vallismortis*-SB3. In remaining combination either fungal antagonist overgrown on bacterial antagonist or lysis of fungal mycelium by bacterium was observed. Similar kind of study was done by James and Mathew (2017).

Table 3: Mutual compatibility between biocontrol agents and endophytes.

Sr. No.	Treatment	Interaction	Compatibility
1.	*T. viride*-Multiplex Nisarga+ *B. amyloliquifaciens*	Over growth of *T. viride*	-
2.	*T. harzianum*-LF30 + *B. amyloliquifaciens*	Lysis of *T. harzianum*	-
3.	*T. asperellum*-SF33 + *B. amyloliquifaciens*	Over growth of SF33, mycelial thickening and clear demarcation at meeting point	-
4.	*T. viride*-Multiplex Nisarga + *B. vallismortis*-SB3	Reduced growth of *T. viride*	-
5.	*T. harzianum*-LF30 + *B. vallismortis*-SB3	Reduced growth of LF30 and lysis of mycelia	-
6.	*T. asperellum*-SF33 + *B. vallismortis*-SB3	Over growth ofSF33	+
7.	*B. amyloliquifaciens* + *B. vallismortis*-SB3	Equal growth observed	+
8.	*T. harzianum*-LF30 + *T. viride*-Multiplex Nisarga	Mycelial thickening at the meeting point	+
9.	*T. harzianum*-LF30 + *T. asperellum*-SF33	Intermingling of hyphae	+
10.	*T. viride*-Multiplex Nisarga + *T. asperellum*-SF33	Intermingling of hyphae; and heavy sporulation of SF33 at meeting point	+

+ Compatible- Incompatible

To test the efficacy of effective treatments against wilt complex under in vivo condition. A pot culture experiment was conducted to test the efficacy of different treatments (Table 4) on wilt incidence at different intervals (60, 75 and 90 DAI) and plant growth was measured at 90 DAI. Among the twelve treatments tested, mancozeb showed 50 per cent wilt incidence at 60 days after inoculation. The remaining treatments are significantly superior over untreated control (66.67 %) and did not produce wilting symptoms up to 60 DAI. At 75 DAI drenching of individual treatments like T1 (Tebuconazole 25 % EC @ 0.1 %) and T2 (Probiconazole 25 %EC @ 0.1 %) did not show wilt incidence, where as in case of combinations T10 (*T. viride*-Multiplex Nisarga + *T. harzianum*-LF30 + *T. asperellum*-SF33) and T11 (*B. vallismortis*-SB3 + *B. amyloliquifaciens*) were recorded zero per cent disease incidence, and which accounted cent per cent disease reduction over control and it was followed by T6 (Pf-ZnNPs @ 0.15 %), T8 (*T. harzianum*-LF30 + *T. viride*-Multiplex Nisarga + Pf-ZnNPs @ 0.15 %) and T9 (*T. harzianum*-LF30+ *T. viride*-Multiplex Nisarga + mancozeb 75 % WP @ 0.2 %) by recording 83.33 per cent disease reduction over control. The least per cent disease reduction (16.67 %) was recorded in T3 (Mancozeb 75 % WP @ 0.2 %) and T7 (*T. harzianum*-LF30) as compared to control (Table 30). The observation at 90DAI recorded the maximum disease reduction over control in T1 (Tebuconazole 25 % EC @ 0.1 %), T2 (Probiconazole 25 %EC @ 0.1 %), T10 (Multiplex Nisarga + *T. harzianum*-LF30 + *T. asperellum*-SF33) and T11 (*B. vallismortis*-SB3 + *B. amyloliquifaciens*). These four treatments are significantly superior over all other treatments.

The effect of different treatments on the plant height of pomegranate revealed that the treatment T10 (*T. viride*-Multiplex Nisarga + *T. harzianum*-LF30 + *T. asperellum*-SF33) recorded maximum plant height of 105.33 cm, which was on par with T11 (*B. vallismortis*-SB3 + *B. amyloliquifaciens*) (100.33 cm) and the next best treatment was T9 (*T. harzianum*-LF30 + Multiplex Nisarga + mancozeb 75 % WP @ 0.2 %) (94.00 cm). The least plant height was recorded in T4 (Taqat 75 %WP @ 0.2 %) (70.33 cm) while compared to all treatments.

The plant treated with chemical fungicide Propiconazole recorded minimum wilt incidence and this finding was similar with Somasekhara *et al.* (2009); Sonyal (2010). Saravanakumar *et al.* (2007); Kim *et al.* (2008) have proved that the use of biocontrol agents as consortia was more effective for management of plant diseases as compared to individual bioagent. Similarly, our combined use of endophytes and biocontrol agents (*T. viride*-Multiplex Nisarga+*T. harzianum*-LF30 + *T. asperellum* -SF33) and (*B. vallismortis* -SB3 + *B. amyloliquifaciens*) have given the best result as like chemical treatment. It has been established that *Trichoderma* spp. prevents the entry of pathogen through the mechanisms of mycoparasitism, antibiosis and competition (Anwar *et al.*, 2008).

Table 4: Evaluation of endophytes and nanoparticles against *Ceratocystis fimbriata* and *Fusarium oxysporum* in pot culture.

Tr. No.	Treatments	Plant height (cm)	Wilt incidence (%)			Disease reduction over control (%)
			60 DAI	75 DAI	90 DAI	
1	Tebuconazole 25% EC @ 0.1% @ 3 DAI of pathogens	72.00	0.00 (1.00)*	0.00 (1.00)	0.00 (1.00)	100.00
2	Probiconazole 25% EC @ 0.1% @ 3 DAI of pathogens	71.83	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	100.00
3	Mancozeb 75% WP @ 0.2% @ 3 DAI of pathogens	75.50	50.00 (7.14)	66.67 (8.23)	83.33 (9.18)	16.67
4	Taqat 75% WP @ 0.2% @ 3 DAI of pathogens	70.33	0.00 (1.00)	33.33 (5.86)	50.00 (7.14)	50.00
5	SAAF 75% WP @ 0.2% @ 3 DAI of pathogens	85.33	0.00 (1.00)	33.33 (5.86)	33.33 (5.86)	66.67
6	Pf-ZnNPs @ 0.15% @ 3 DAI of pathogens	81.83	0.00 (1.00)	16.67 (4.20)	16.67 (4.20)	83.33
7	*T. harzianum*-LF30 @ 7 DBI of pathogens	84.00	0.00 (1.00)	66.67 (8.23)	83.33 (9.18)	16.67
8	*T. harzianum*-LF30 @ 7 DBI of pathogens + *T. viride*-Multiplex Nisarga @ 7 DBI of pathogens + Pf-ZnNPs 0.15 % @ 3 DAI of pathogens	89.50	0.00 (1.00)	0.00 (1.00)	16.67 (4.20)	83.33
9	*T. harzianum*-LF30 @ 7 DBI of pathogens + *T. viride*-Multiplex Nisarga@ 7 DBI of pathogens + Mancozeb 0.2 % @ 3 DAI of pathogens	94.00	0.00 (1.00)	0.00 (1.00)	16.67 (4.20)	83.33
10	*T. viride*-Multiplex Nisarga @ 7 DBI of pathogens + *T. harzianum*-LF30 @ 7 DBI of pathogens + *T. asperellum*-SF 33 @ 7 DBI of pathogens	105.33	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	100.00
11	*B. vallismortis*-SB 3 @ 7 DBI of pathogens + *B. amyloliquifaciens*@ 7 DBI of pathogens	100.33	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	100.00
12	Control	59.67	66.67 (8.23)	100 (10.05)	100.00 (10.05)	-
	S.Em. ±	2.37	0.87	1.37	1.46	
	C.D. at 1 %	9.39	3.44	5.43	5.80	

* √ x+1 transformed values DAI: Days after inoculation of pathogen DBI: Days before inoculation of pathogen

Author contribution. Saranya R. and Yashoda R. H. designed and conceived the study. Material preparation and data collection were performed by Saranya R. The analysis was done by Sarsanya R. The original draft was prepared by Saranya R and it was reviewed and edited by Yashoda R. H.

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Conflict of Interest. None.

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