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Field evaluation of endophytic entomopathogenic fungi against tomato early blight

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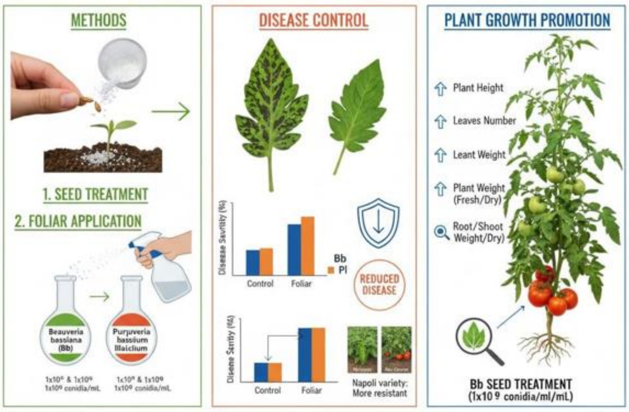
Graphical abstract



Abstract

The effect of seed treatment and foliar application with two endophytic entomopathogenic fungi (EPF), *Beauveria bassiana* and *Purpureocillium lilacinum*, was evaluated against tomato early blight under field conditions. Two conidial concentrations (1×10^8 and 1×10^9 conidia mL^{-1}) were tested under factorial design over two consecutive growing seasons. Disease severity, disease incidence and plant growth parameters were assessed. Both EPFs significantly reduced disease severity and incidence compared to untreated control. Seed treatment was more effective compared to foliar application particularly at 1×10^9 conidia mL^{-1} concentration. Among treatments, *B. bassiana* consistently showed the superior diseases suppression and growth promotion as compared to *P. lilacinum* of antifungal effect of endophytic EPFs against *A.* particularly at higher concentrations. Our results expend current understanding *solani* under field conditions for the sustainable management of tomato early blight.

Keywords: Endophytic entomopathogenic fungi, Early

1. Introduction

Entomopathogenic fungi (EPF) have long been considered for their potential as microbial control agents against insects (Vega *et al.* 2009). In addition to their entomopathogenic activity, many EPF are capable of colonize plants tissues endophytically (Elena *et al.* 2011; Gurulingappa *et al.* 2010; Jaber, 2015). This endophytic association contributes in plant growth promotion and antagonism against plant disease (Jaber and Enkerli, 2016; Jber and Ownley, 2017). These emerging but not fully understood ecological roles of entomopathogens provide opportunities for integrating EPF in sustainable disease management strategies. Among various application approaches, seed treatments provide practical and environmentally friendly alternative to chemicals pesticides (Gurulingappa *et al.* 2010; Anjum *et al.* 2019). Compared to foliar application, soil drenching or stem injection, seed treatments are simpler and require less field operations. Endophytic EPF have demonstrated antagonistic potential against several phytopathogens in addition to their efficacy against insects (Lacey *et al.* 2009; Ownley *et al.* 2004; Sanivada and Challa, 2014). Proposed mechanism of EPF include competition for food and space, mycoparasitism, antibiosis, production of secondary metabolites and induction of systemic resistance in host plant (Griffin, 2007; Ownley *et al.* 2010;

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Benhamou and Brodeur, 2000; Pieterse et al. 2014). *Beauveria bassiana* Vuillemin and *P. lilacinum* are well-known EPF that have been commercialized as mycopesticides (de Faria et al. 2007). These fungi are widely used against emerging insect pests in various cropping systems (Anastasiadis et al. 2008; Castillo-Lopez et al. 2014; Shah and Pell, 2003). Most recently, EPF has gain attention for their role as plant endophytes, plant growth promoters, antagonists of plant disease, and rhizosphere colonizers (Vega et al. 2009). Tomato (*Solanum lycopersicum L.*) is an economically important crop worldwide and a major source of vitamin A and C (Chohan et al. 2015). However, it productivity is substantially reduced by biotic and abiotic factors, particularly early blight caused by *Alternaria solani.* Early blight cause yield losses up to 50-91% in Pakistan under favorable conditions (Abdussamee et al. 2014; Saleem et al. 2015). Symptoms include concentric necrotic lesions on leaves and stems, and sunken, leathery lesions on fruit at the stem end (Chohan et al. 2015). Although synthetic chemicals are commonly applied to manage tomato early blight, but repeated applications lead to pathogen resistance and environmental pollution and ultimately potential risk to human health (Latha et al. 2009). Biological control agents are being used as an alternative for sustainable management of plant diseases (Khan et al. 2012; Ownley et al. 2010; McKinnon et al. 2017). While numerous studies demonstrated the entomopathogenic role of *B. bassiana*, comparatively fewer studies documented its role as plant disease antagonists under field conditions (Griffin et al. 2006; Jaber and Salem, 2014; Ownley et al. 2008; 2010). Therefore, present study was conducted to evaluate the antagonistic potential of *B. bassiana* and *P. lilacinum* against tomato early blight disease under field conditions using seed and foliar application approaches.

2. Materials and methods

2.1. Fungal cultures

Cultures of *Beauveria bassiana* and *Purpureocillium lilacinum* were obtained from Fungal Culture Bank, Department of Plant Pathology, University of Agriculture, Faisalabad, Pakistan. Cultured were maintained on Sabouraud Dextrose Agar (SDA) in dark conditions for four weeks to promote sporulation. Culture of *A. solani* (isolate 1) was maintained on Potato Dextrose Agar (PDA) at 25°C for 10 days for sufficient sporulation.

2.2. Harvesting of conidia

Conidia of *B. bassiana* and *P. lilacinum* were harvested from four-week-old cultures by using sterial camel hairbrush and suspended in sterile glass bottle containing 100mL distilled water supplemented with 0.1% Tween 20. Conidia of *A. solani* were harvested from 10-days-old PDA culture plates in a similar manner. Conidial viability was assessed by spreading 100µL each suspension on PDA media plates at 25^{o}C. Germination percentages were determined after 24h. Viability was 96% of both EPF isolates while 98% for *A. solani.*

2.3. Seed coating treatment

Tomato seeds of two varieties (Rio-Grande and Napoli) were obtained from local seed market of Bahawalpur, Punjab, Pakistan. Seeds of both tomato varieties were surface sterilized with 0.5% NaOCl solution for 2 min followed by three alternative rinses with distilled water. To confirm sterilization, 100 µL from final rinse was plated on PDA plates and incubated at 25 ± 1°C (Jaber and Ownley, 2018).

Two conidial suspension 1×10^8 and 1×10^9 conidia mL^{-1} were prepared and verified by hemocytometer count. Seeds were coated with 2 mL of 2% methyl cellulose containing 20µL Tween-20 as an adhesive agent. Seeds were soaked for 2h in conidial suspensions, air dried under sterile conditions and sown in plastic pots containing autoclaved loamy soil.

Successful colonization by EPF isolates was confirmed prior to field transplantation. After four weeks, seedlings were transferred to the field (Loamy soil, pH 7.7). Plant-to-plant and bed-to-bed spacing were 30 cm and 70 cm respectively.

Artificial disease pressure was established by spraying conidial suspension of *A. solani* (1×10^5 conidia mL^{-1}) with plastic hand sprayer. Disease severity and percent disease incidence were recorded after 7, 14, 21 and 28 days after *A. solani* inoculation. At the end of the experiment, Plant height, Plant weight, number of leaves, fresh and dry root shoot weight, data was recorded.

The entire experiment was repeated for two consecutive growing seasons. There were fifteen plants in a row as one replication. Each treatment has three replications.

2.4. Foliar application

For foliar application experiment, surface sterilized seeds were sown in nursery tray and transplanted to the field after one month. Concentrations of EPF, field spacing and replications were identical to seed treatment experiment. EPF were applied 7 days before the application of pathogen as protective treatment. Data on disease severity and percent disease incidence was recorded after 7, 14, 21 and 28 days of inoculation. Plant height, plant weight, number of leaves, fresh and dry root shoot weight were recorded at experiment completion.

2.5. Disease assessment

Disease severity was assessed by using modified disease rating scale (Chohan *et al.* 2015); where 0= No symptoms on leaves; 1=0-5% leaf area infected; 2=6-20% leaf area infected; 3= 21-40% leaf area infected; 4=41-70% leaf area infected, and 5= >71% leaf area infected.

Percent disease incidence was determined by using following formula (Wheeler, 1969).

$$\text{Disease Incidence} = \frac{T1}{T2} \times 100$$

where T1 represents number of leaves infected and T2 represents total number of leaves assessed.

2.6. Statistical analysis

The experiment was arranged in factorial complete block design (RCBD) with tomato varieties, EPF species, concentrations, application methods as fixed factors. Data was subjected to analysis of variance (ANOVA) using Statistix 8.1 software. Homogeneity of variance and normality were verified before analysis. Tuckey HSD test was used for treatments mean separation at P ≤ 0.05.

3. Results

3.1. Disease Severity

All the EPF treatments significantly reduced disease severity as compared to untreated control across both season (**Tables 1 and 2**). Among tested treatments, *B. bassiana* showed better reduction in disease severity compared to *P. lilacinum* particularly at higher concentrations 1×10^9 conidia mL^{-1}. In 2021, seed treatment with *B. bassiana* at 1×10^9 conidia mL^{-1} resulted in the lowest disease severity after 28 days of inoculation (DAI) with value of 2.46 in Napoli and 2.63 in Rio Grande. In contrast control treatment showed maximum disease severity 4.93 in Rio Grande and 4.34 in Napoli. A similar trend of disease severity reduction was observed in *P. lilacinum* at 1×10^9 conidia mL^{-1}, although diseases suppression was lower than *B. bassiana*.

Foliar application was less effective than seed treatment under field condition. *B. bassiana* at 1×10^9 conidia mL^{-1} showed 3.37 severity in Napoli while 3.54 in Rio Grande under foliar application at 28 DAI. While in case of untreated control the value reached 5.00 and 4.96 respectively. In case of *P. lilacinum* at 1×10^9 conidia mL^{-1} disease severity was 3.6 in Napoli and 3.92 in Rio Grande at 28 DAI (**Table 1**). Significant difference varietal response was observed, with Rio Grande gave maximum diseases severity than Nepoli in all observed intervals for both years (**Supplementary Tables 1&2**). In year 2022, similar pattern was observed, Seed treatment consistently performed better than foliar application. Higher concentration conidial concentration (1×10^9 conidia mL^{-1}) provided better disease suppression than 1×10^8 conidia mL^{-1}. Across the treatments and time intervals, Nepoli significantly performed better than Rio Grande against disease severity (**Table 1**). Treatment effects were highly significant (P ≤ 0.01) at all evaluation dates and intervals observed after analysis of variance.

Table 1. Effect of seed treatment and foliar application of *Beauveria bassiana* and *Purpureocillium lilacinum* at two conidial concentrations (1×10^8 and 1×10^9 conidia mL^{-1}) on the disease severity of tomato varieties Rio Grande and Napoli during 2021 and 2022 at 28 days after inoculation.

Treatment	Concentration	2021				2022			
		Seed Treatment		Foliar Application		Seed Treatment		Foliar Application	
		Rio Grande	Napoli	Rio Grande	Napoli	Rio Grande	Napoli	Rio Grande	Napoli
B. bassiana	1×10^8 conidia mL^{-1}	3.87^b	3.56^b	4.03^b	3.87^b	3.78^b	3.32^{bc}	3.97^b	3.84^b
	1×10^9 conidia mL^{-1}	3.12^c	2.8^d	3.54^c	3.37^c	3.03^c	2.60^d	3.44^c	3.32^d
P. lilacinum	1×10^8 conidia mL^{-1}	4.08^b	3.74^b	4.13^b	3.98^b	3.95^b	3.54^b	4.11^b	3.94^b
	1×10^9 conidia mL^{-1}	3.38^c	3.16^c	3.92^b	3.60^c	3.33^c	3.15^c	3.82^b	3.56^c
Control		5.00^a	4.94^a	5.00^a	4.96^a	5.00^a	4.95^a	5.00^a	4.95^a
F-value		75.39	49.06	67.40	125.05	128.74	259.51	89.14	298.75
P		≤ 0.05	≤ 0.05	≤ 0.05	≤ 0.05	≤ 0.05	≤ 0.05	≤ 0.05	≤ 0.05

Means following the same letters are not significantly different from each other at P ≤ 0.05 level using Tukey HSD test

Table 2. Effect of seed treatment and foliar application of *Beauveria bassiana* and *Purpureocillium lilacinum* at two conidial concentrations (1×10^8 and 1×10^9 conidia mL^{-1}) on the disease incidence (%) of tomato varieties Rio Grande and Napoli during 2021 and 2022 at 28 days after inoculation.

Treatment	Concentration	2021				2022			
		Seed Treatment		Foliar Application		Seed Treatment		Foliar Application	
		Rio Grande	Napoli	Rio Grande	Napoli	Rio Grande	Napoli	Rio Grande	Napoli
B. bassiana	1×10^8 conidia mL^{-1}	78.93^b	3.56^b	4.03^b	3.87^b	3.78^b	3.32^{bc}	3.97^b	3.84^b
	1×10^9 conidia mL^{-1}	58.20^c	2.8^d	3.54^c	3.37^c	3.03^c	2.60^d	3.44^c	3.32^d
P. lilacinum	1×10^8 conidia mL^{-1}	82.77^b	3.74^b	4.13^b	3.98^b	3.95^b	3.54^b	4.11^b	3.94^b
	1×10^9 conidia mL^{-1}	62.07^c	3.16^c	3.92^b	3.60^c	3.33^c	3.15^c	3.82^b	3.56^c
Control		5.00^a	100a	5.00^a	4.96^a	5.00^a	4.95^a	5.00^a	4.95^a
F-value		75.39	135.35	67.40	125.05	128.74	259.51	89.14	298.75
P		≤ 0.05	≤0.05	≤ 0.05	≤ 0.05	≤ 0.05	≤ 0.05	≤ 0.05	≤ 0.05

3.2. Disease Incidence (%)

Entomopathogenic fungi (EPF) significantly reduced disease incidence (%) in both growing seasons compared to untreated control (**Table 2**). In 2021, Napoli (48.90%) demonstrated minimum disease incidence compared to Rio Grande (59.56%) after seed treatment at 1×10^9 conidia mL^{-1} after 28 DAI. At lower concentration (1×10^8 conidia mL^{-1}) disease incidence value exceeded 70% for both varieties which represent completely ineffectiveness. Highest disease incidence 98.66% in Napoli and 100% in Rio Grande was observed in untreated control. Across all treatments, Napoli performed better against disease with lower disease incidence than Rio Grande with higher disease incidence. Similar to disease severity, seed treatment was more effective against disease incidence than foliar application. Foliar application of *B. bassiana* at 1×10^9 conidia mL^{-1} reduce disease incidence to 54.30% in Napoli in 2021 whereas untreated control exhibited 97% disease incidence at 28 DAI (**Table 2**). Similar pattern was observed in 2022, seed treatment reduced disease incidence to 48.10% in Napoli and 55.83% in Rio Grande compared to untreated control with 98.33% in Napoli and 100% in Rio Grande. Overall, under all treatment conditions, *B. bassiana* was more effective than *P. lilacinum* at higher concentration as it provided superior disease suppression. Seed treatment was more effective than foliar application. Napoli performed better against early blight incidence and severity by demonstrating greater resistance than Rio Grande

3.2.1. Plant Growth Parameters

3.2.1.1 Vegetative growth traits

a. Plant Height

Both EPF, *B. bassiana* and *P. lilacinum,* significantly enhanced the plant height of both tomato varieties, Napoli and Rio-Grande compared to untreated control (**Figure 1**). Overall, *B. bassiana* improved plant height greater than *P. lilacinum* particularly at higher concentration 1×10^9 conidia mL^{-1}. In 2021, Seeds treated with *B. bassiana* at 1×10^9 conidia mL^{-1} resulted in maximum plant height in Napoli (75.6cm) while followed by Rio Grande (60cm). While minimum plant height was observed in untreated control (50.6 cm). Foliar application also enhanced the plant height but lower than seed treatment which consistently produced superior results. Similar trend was observed in 2022 with Seed treatment at higher concentration of *B. bassiana* exhibited greater height in Napoli followed by Rio Grande (**Figure 1**).

b. Number of Leaves

Significant increase in total number of leaves in both tomato varieties was observed when inoculated with EPF as compared to control. In 2021, Napoli produced maximum number of leaves (77.6) while control treatment produced 60 leaves in seed treatment with *B. bassiana* at 1×10^9 conidia mL^{-9} while in Rio Grande, maximum leaves 65.3 were observed compared to untreated control 49.6 leaves. In foliar application, Napoli produces maximum leaves 77.3 while Rio Grande produced 61.6 leaves (**Figure 1**). Same trend was observed in 2022, higher concentration of *B. bassiana* at 1×10^9 conidia mL^{-1} produced maximum number of leaves followed by *P. lilacinum*.

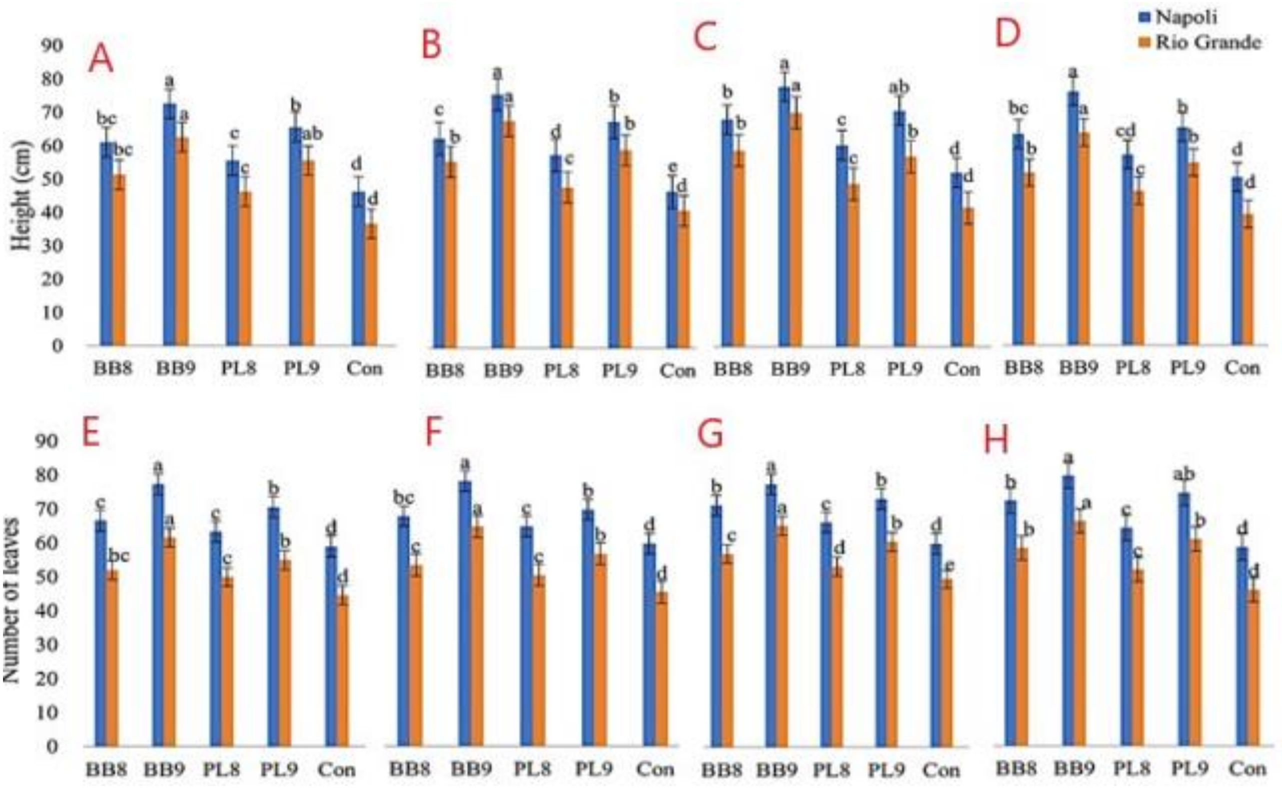

Figure 1. Vegetative growth trait of tomato varieties (Napoli and Rio Grande).

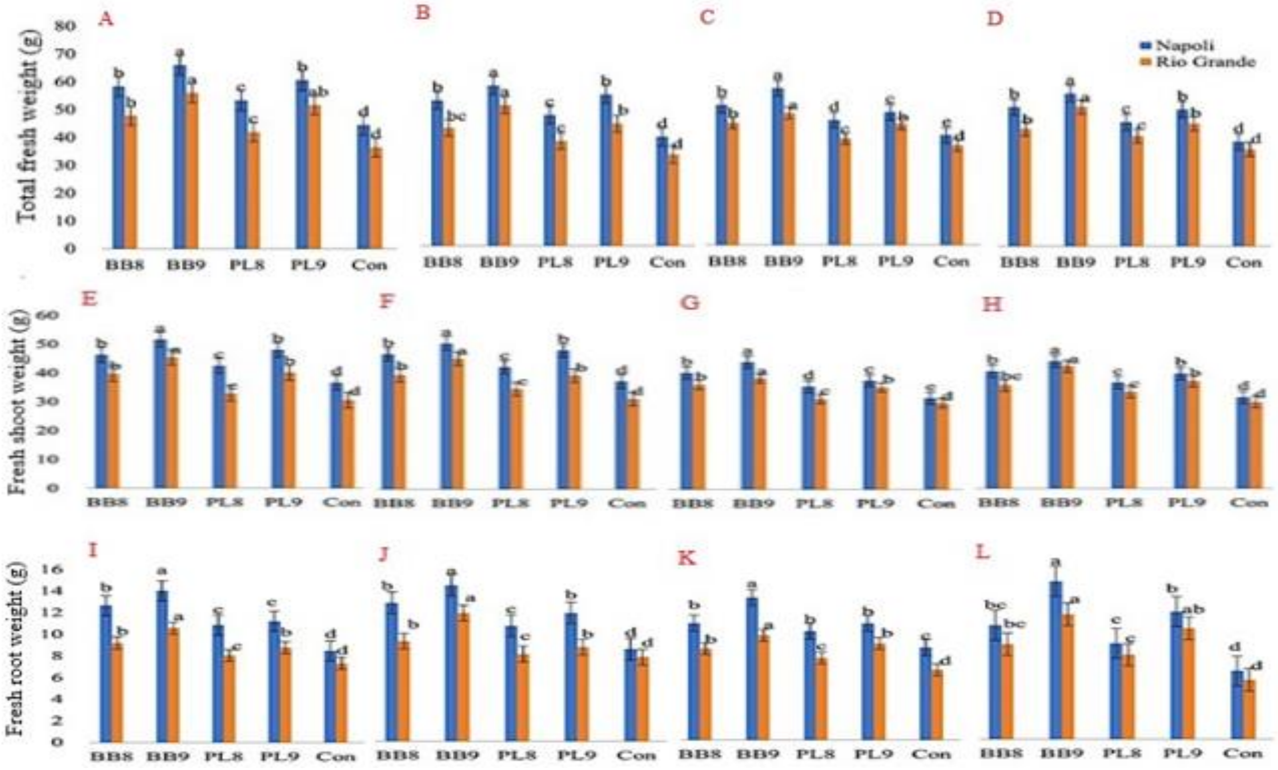

Figure 2. Fresh Biomass accumulation of tomato varieties (Napoli and Rio Grande).

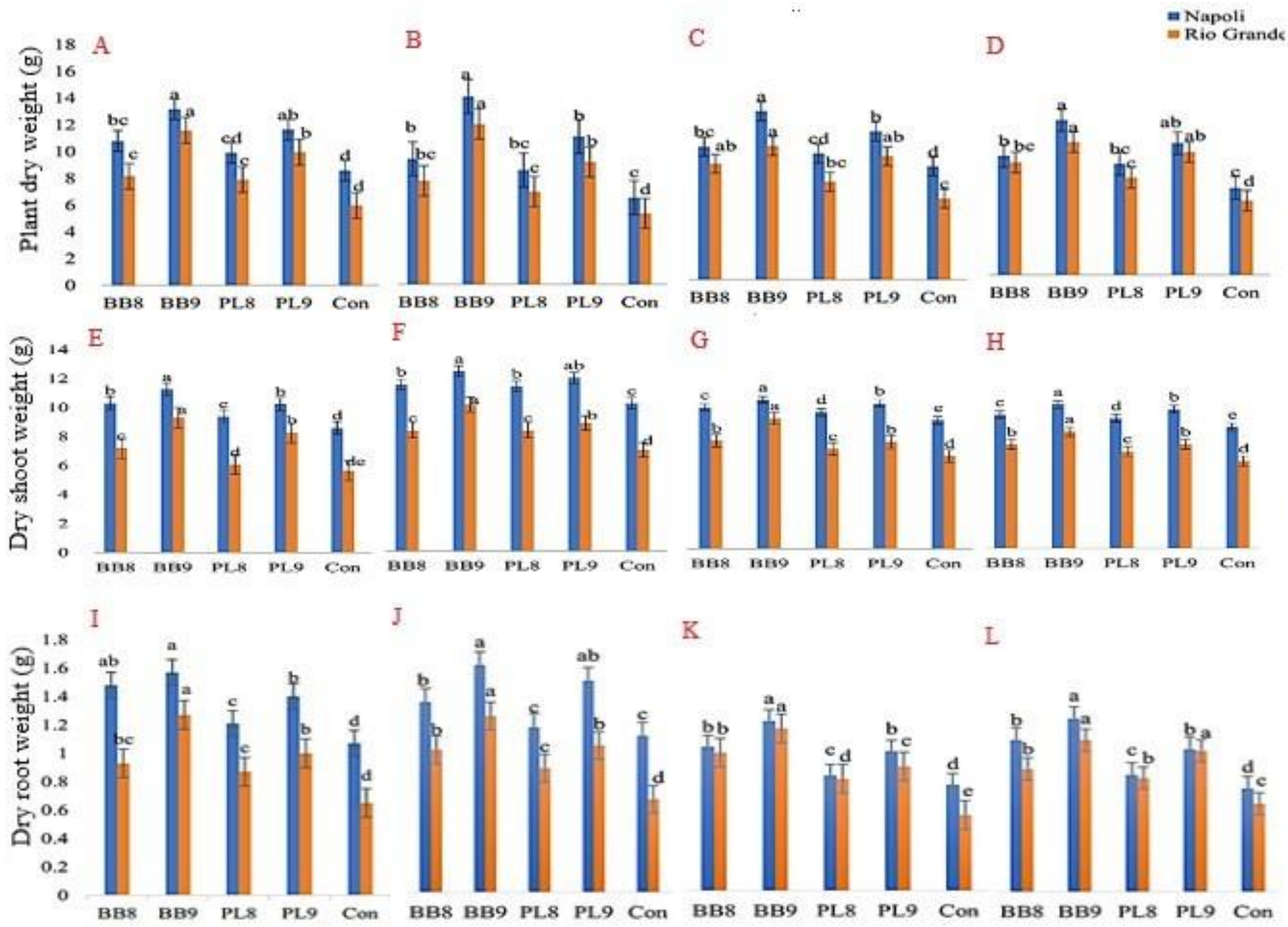

Figure 3. Dry biomass accumulation of tomato varieties (Napoli and Rio Grande).

Seed treatment and foliar application of *Beauveria bassiana* and *Purpureocillium lilacinum* response at 1×10^8 and 1×10^9 conidia mL^{-1} across two growing seasons. A&B, E&F = Seed treatment year 1& 2, C&D, G&H= Foliar application year 1&2

3.2.2. Biomass Accumulation

A. Fresh Biomass

The EPF treatments significantly increased the fresh biomass accumulation in plant ($P \leq 0.05$). Plants treated with higher concentration 1×10^9 conidia mL^{-1} of *B. bassiana* demonstrated higher total fresh weight, fresh shoot weight and fresh root weight followed by lower concentration (1×10^8 conidia mL^{-1}) and *P. lilacinum* treatments. Lowest fresh biomass accumulation was observed in untreated control (**Figure 2**). Result of seed treatment was encouraging with higher fresh biomass accumulation followed by foliar application. Napoli consistently performed better against early blight with higher fresh biomass accumulation than Rio Grande under corresponding treatments. In both growing season untreated control resulted in lowest fresh biomass accumulation. This enhancement in fresh biomass accumulation corresponds to improved vegetative vigor and nutrient absorption in EPF treated plants (**Figure 2**).

Seed treatment and foliar application of *Beauveria bassiana* and *Purpureocillium lilacinum* response at 1×10^8 and 1×10^9 conidia mL^{-1} across two growing seasons. Total fresh weight = A&B (year 1), C&D = year 2: Fresh shoot weight E&F (year 1), G&H (year 2): Fresh root weight I&J (year 1), K&L (year 2).

B. Dry Biomass

Dry biomass accumulation followed similar trends of fresh biomass. B. bassiana (1×10^9 conidia mL^{-1}) treated plant significantly resulted in higher total dry weight, dry shoot weight and dry root weight ($P \leq 0.05$) (**Figure 3**). Following the pattern, seed treatment significantly outperformed foliar application in dry matter accumulation. Across the studied years, Napoli produced higher dry biomass compared to Rio Grande. Untreated control plants, highly affected by early blight diseases, resulted in lowest dry biomass values. The enhanced dry biomass refers to enhanced physiological and structural development in EPF treated plants. These results reflect the sustained plant growth rather than temporarily water related increase in fresh weight (**Figure 3**).

Seed treatment and foliar application of *Beauveria bassiana* and *Purpureocillium lilacinum* response at 1×10^8 and 1×10^9 conidia mL^{-1} across two growing seasons. Total dry weight = A&B (year 1), C&D = year 2: Dry shoot weight E&F (year 1), G&H (year 2): Dry root weight I&J (year 1), K&L (year 2).

3.3. Overall summary of growth response

For vegetative and biomass parameters, *B. bassiana* was more favorable compared with *P. lilacinum*, especially that the concentration of 1 × 10^9 conidia mL^{-1} was significantly better than other concentrations. The seed treatment was superior to the foliar application, and Napoli was more responsive than Rio Grande. The stability of the trends for two years validates the observed growth promoting effects.

4. Discussion

The present study evaluated the endophytic potential of *B. bassiana* and *P. lilacinum* applied through seed treatment and foliar application against tomato early blight and their influence on plant growth parameters under field conditions. The results demonstrated that both fungi significantly reduced disease severity and incidence compared to the untreated control. The endophytic fungi also enhanced the vegetative growth and biomass accumulation. To our knowledge, limited field-based studies have evaluated the endophytic potential of *B. bassiana* against the plant pathogens, particularly under open-field tomato cultivation systems. This study provides additional field evidence supporting the antifungal potential of endophytic *B. bassiana* and *P. lilacinum* against sustainable management of tomato early blight. Present results also demonstrated that seed treatment was more effective than foliar application. Previous research evaluated the endophytic potential of *B. bassiana* against seedling blight of cotton (Griffin *et al.* 2006), zucchini yellow mosaic virus in squash (Jaber and Salem, 2014), and damping off of tomato (Ownley *et al.* 2004) through seed treatments. Similarly, downy mildew of grapes was managed through foliar application (Jabber, 2015). These studies were completely aligned with the present study, where both treatments were effective, with seed treatment being the most effective. Effectiveness of seed treatment may be attributed to early and stable colonization, which facilitates better interaction between plant tissue and the endophyte. The underlying mechanism of EPF for disease suppression is most likely multifactorial (Ownley *et al.* 2010; Vega *et al.* 2009). Direct mechanisms involve the production of antifungal secondary metabolites, i.e., oosporein and beauvericin (Ownley *et al.* 2010), competition for resources and space, and mycoparasitism. However, under field conditions direct involvement of antifungal secondary metabolites in disease suppression is still unclear. On the other hand, the indirect mode of action of biocontrol includes induction of defense enzymes and plant growth promotion, which may also be triggered by *B. bassiana* that suppressed the plant disease (Ownley *et al.* 2010). Proteomic analysis of date palm leaves colonized with *B. bassiana* showed the induction of a specific protein that is directly related to defense mechanisms (Gómez-Vidal *et al.* 2009). Production of PR protein by plant defense genes may also be involved in the induction of resistance (Shehata and El-Borollosy, 2013). On the other hand, our study also confirmed the additional role of endophytic EPF that promoted the tomato plant growth under field conditions. An increase in plant height, leaf number, and fresh and dry biomass was observed. These findings are inconsistent with previous reports that endophytic EPF positively enhanced the plant growth (Jaber and Ownley, 2018; Sasan and Bidochka, 2013; Sun *et al.* 2018). Among application methods, seed treatment efficiently increased plant growth as compared to foliar application. It is supposed that multiple mechanisms were involved in growth promotion, such as increased soil nutrient uptake (Behie and Bidochka, 2014) in inoculated plants and up-movement of specific proteins that are involved in metabolism, photosynthesis (Gomez-Vidal *et al.* 2009), and phytohormones (McKinnon *et al.* 2006). This dual role of EPF in growth promotion and disease suppression increases the possibility of inclusion of EPF in sustainable disease management programs. Varietal difference regarding performance against early blight was also observed. Napoli performed better against disease compared to Rio Grande. This difference suggests that genotypic differences also influence the interaction with endophytes, as previously described by Schulz and Boyle (2005), who found that a plant-endophyte interaction ranges from mutualistic to parasitic, which may depend on plant genotype-endophyte genotype interaction. Present research work is one of the few studies that investigated the role of endophytic EPF against plant pathogens. Consistent results of disease suppression across both growing seasons strengthen the reliability of these findings. These findings also support the practical possibility of including EPF in integrated plant disease management strategies at the national level. The EPF integration (*B. bassiana and P. lilacinum*) into tomato disease management program appears economically feasible. Compared to foliar spray, seed treatment being most effective application method, provide distinct economic advantages i.e. lower inoculum requirement, single pre-sowing application, and reduced labor and equipment requirements. Furthermore, the overall input costs is reduced by reducing the need of multiple fungicides sprays due to early colonization through seed treatment. Lower fungicides used minimize environmental and health risk associated with synthetic chemical fungicides. Commercial formulations of *B. bassiana* are already available in many agricultural markets, indicating existing infrastructure for mass production and distribution. Production of EPF through solid-state or liquid fermentation is well established, and formulation technologies such as wettable powders and oil-based suspensions enhance shelf life and field stability. The dual benefit observed in this study disease suppression combined with plant growth promotion further improves cost benefit potential by increasing yield alongside reducing disease pressure (Jaber and Ownley, 2018; Sasan and Bidochka, 2013; Sun *et al.* 2018). However, large-scale adoption will require cost benefit ratio analyses under different agroecological zones, testing of compatibility with existing agronomic practices, and most important of all persistence under farmer field conditions. Nonetheless, given the increasing restrictions on chemical fungicides and the demand for sustainable crop protection strategies, EPF based seed treatment represents a promising, economically viable component of integrated disease management programs at regional and national levels.

5. Conclusions

The current study demonstrates that endophytic EPF, *B. bassiana* and *P. lilacinum* decrease tomato early blight disease and promote the plant growth and biomass under field conditions. Seed treatment with higher concentration (1×10^9 conidia mL^{-1}) of *B. bassiana* was more effective compared to other treatments. Napoli variety showed minimum diseases level as compared to Rio-Grande under field conditions. These finding supports the inclusion of B. bassiana as seed treatment particularly at higher concentration as promising alternative to chemical fungicides for tomato disease management program. Further studies regarding cost benefit ratio under large field environment would strengthen the practical possibilities.

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Supplementary Tables:

Table 1. Effect of seed treatment and foliar application with *B. bassiana* and *P. lilacinum* at two conidial concentrations (1×10^8 and 1×10^9 conidia mL-1) on the disease severity on two varieties Rio Grande and Napoli during 2021.

Seed treatment									
Treatment	**Concentration**	**7th day**		**14th day**		**21st day**		**28th day**	
		Rio-Grande	**Napoli**	**Rio-Grande**	**Napoli**	**Rio-Grande**	**Napoli**	**Rio-Grande**	**Napoli**
B. bassiana	1×10^8 conidia mL-1	2.42bc	2.2bc	3bc	2.55c	3.4b	2.98bc	3.87b	3.56b
	1×10^9 conidia mL-1	1.74d	1.57d	2.3d	2.38c	2.63d	2.46c	3.12c	2.8d
P. lilacinum	1×10^8 conidia mL-1	2.53b	2.33b	3.11b	2.8b	3.47b	3.06b	4.08b	3.74b
	1×10^9 conidia mL-1	2.19c	2.08c	2.74c	2.42c	2.99c	2.83bc	3.38c	3.16c
Control		3.76a	3.4a	4.11a	3.95a	4.93a	4.34a	5±0.01a	4.94a
F-value		133.31	194.48	99.58	319.99	219.69	42.97	75.39	49.06
P		≤0.001	≤0.001	≤0.001	≤0.001	≤0.001	≤0.001	≤0.001	≤0.001
Foliar application									
Treatment	**Concentration**	**7th day**		**14th day**		**21st day**		**28th day**	
		Rio-Grande	**Napoli**	**Rio-Grande**	**Napoli**	**Rio-Grande**	**Napoli**	**Rio-Grande**	**Napoli**
B. bassiana	1×10^8 conidia mL-1	2.72b	2.39bc	3.08b	3.04bc	3.47b	3.42b	4.03b	3.87b
	1×10^9 conidia mL-1	2.27d	2.26c	2.69c	2.65d	3.08c	3.05c	3.54c	3.37c
P. lilacinum	1×10^8 conidia mL-1	2.8b	2.69b	3.15b	3.3b	3.58b	3.52b	4.13b	3.98b
	1×10^9 conidia mL-1	2.4c	2.38bc	2.98b	2.92cd	3.32b	3.35b	3.92b	3.6c
Control		3.98a	3.4a	4.28a	4.02a	4.83a	4.36a	5±00a	4.96a
F-value		750.61	30.64S	41.57	60.12	86.62	76.27	67.40	125.05
P		≤0.001	≤0.001	≤0.001	≤0.001	≤0.001	≤0.001	≤0.001	≤0.001

Means following the same letters are not significantly different from each other according to Tukey HSD test at P≤0.05.

Table 2. Effect of seed treatment and foliar application with *B. bassiana* and *P. lilacinum* at two conidial concentrations (1×108 and 1×109 conidia mL-1) on the disease severity on two varieties Rio Grande and Napoli during 2022.

Seed treatment									
Treatment	**Concentration**	**7th day**		**14th day**		**21st day**		**28th day**	
		Rio-Grande	**Napoli**	**Rio-Grande**	**Napoli**	**Rio-Grande**	**Napoli**	**Rio-Grande**	**Napoli**
B. bassiana	1×10^8 conidia mL-1	2.4b	2.11c	2.92bc	2.54c	3.35b	2.87b	3.78b	3.32bc
	1×10^9 conidia mL-1	1.74c	1.5d	2.19d	2.35d	2.58c	2.42d	3.03c	2.6d
P. lilacinum	1×10^8 conidia mL-1	2.43b	2.33b	3.04b	2.72b	3.42b	3.02b	3.95b	3.54b
	1×10^9 conidia mL-1	2.12bc	2.02c	2.66c	2.4d	2.9c	2.7c	3.33c	3.15c
Control		3.74a	3.42a	4.08a	3.84a	4.88a	4.37a	5a	4.95a
F-value		46.62	341.96	107.74	594.03	167.40	498.64	128.74	259.51
P		≤0.001	≤0.001	≤0.001	≤0.001	≤0.001	≤0.001	≤0.001	≤0.001
Foliar application									
Treatment	Concentration	7th day		14th day		21st day		28th day	
		Rio-Grande	Napoli	Rio-Grande	Napoli	Rio-Grande	Napoli	Rio-Grande	Napoli
B. bassiana	1×10^8 conidia mL-1	2.62b	2.42bc	3b	2.92c	3.33bc	3.27bc	3.97b	3.84b

	1×10⁹ conidia mL-1	2.22c	2.22c	2.65c	2.59d	3.05c	3c	3.44c	3.32d
P. lilacinum	1×10⁸ conidia mL-1	2.72b	2.64b	3.12b	3.19b	3.54b	3.4b	4.11b	3.94b
	1×10⁹ conidia mL-1	2.37c	2.28c	2.92b	2.82cd	3.52b	3.25bc	3.82b	3.56c
Control		3.95a	3.35a	4.27a	3.96a	4.78a	4.32a	5a	4.95a
F-value		418.44	45.98	32.34	109.84	75.39	49.06	89.14	298.75
P		≤0.001	≤0.001	≤0.001	≤0.001	≤0.001	≤0.001	≤0.001	≤0.001

Means in each column designated by the same latter are not significantly different at (P≤0.05) level using Tukey HSD test.

Table 3. Effect of seed treatment and foliar application with *B. bassiana* and *P. lilacinum* at two conidial concentrations (1×10^8 and 1×10^9 conidia mL-1) on the disease incidence (%) on two varieties Rio Grande and Napoli during 2021.

Seed treatment									
Treatment	**Concentration**	**7th day**		**14th day**		**21st day**		**28th day**	
		Rio-Grande	**Napoli**	**Rio-Grande**	**Napoli**	**Rio-Grande**	**Napoli**	**Rio-Grande**	**Napoli**
B. bassiana	1×10⁸ conidia mL-1	44bc	38.1bc	51.2c	44.56b	69.66b	54.4b	78.93b	70.96b
	1×10⁹ conidia mL-1	31.06d	31.1c	38.5d	31.9d	44.16d	38c	58.2c	48.9d
P. lilacinum	1×10⁸ conidia mL-1	50.7b	44.96b	59.96b	46.93b	75.36b	55.63b	82.77b	71.83b
	1×10⁹ conidia mL-1	38.6cd	33.86c	44.13d	36.43c	53.8c	47.9b	62.07c	59.56c
Control		67a	58.16a	71.6a	68.53a	95.4a	85.23a	100a	98.66a
F-value		55.66	30.80	239.80	113.33	108.28	107.07	135.35	288.84
P		≤0.001	≤0.001	≤0.001	≤0.001	≤0.001	≤0.001	≤0.001	≤0.001
Foliar application									
Treatment	**Concentration**	**7th day**		**14th day**		**21st day**		**28th day**	
		Rio-Grande	**Napoli**	**Rio-Grande**	**Napoli**	**Rio-Grande**	**Napoli**	**Rio-Grande**	**Napoli**
B. bassiana	1×10⁸ conidia mL-1	47.4b	38.7bc	60.16b	48.83b	69.6bc	55.83b	80.33b	78.33b
	1×10⁹ conidia mL-1	35.6c	32.43c	43.83c	38.13c	45.66d	41.36c	57.4d	54.3d
P. lilacinum	1×10⁸ conidia mL-1	53.3b	44.86b	66.56ab	50.13b	77.1b	56b	83.67b	74.66b
	1×10⁹ conidia mL-1	40.13c	34.33c	47.13c	40.9c	57.96c	54b	67.33c	64.33c
Control		64.8a	59.36a	72.33a	69.33a	91.16a	83.4a	100a	97.33a
F-value		85.43	52.54	25.95	113.61	52.38	73.91	223.85	83.38
P		≤0.001	≤0.001	≤0.001	≤0.001	≤0.0001	≤0.0001	≤0.001	≤0.001

Means in each column designated by the same latter are not significantly different at (P≤0.05) level using Tukey HSD test.