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RESEARCH ARTICLE

Plant growth promoting microbial consortia against late blight disease of tomato under natural epiphytotic conditions

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Abstract

An indigenous microbial consortium was developed in order to combat late blight disease of tomato. For obtaining better insight in the antagonistic potential of native bioagents, 33 isolates of bioagents were isolated and evaluated against *Phytophthora infestans* causing late blight of tomato. Upon in-vitro screening of the varied isolates, the highest growth inhibition of the pathogen was recorded in *Pseudomonas* isolates; Pf-2 (81.33%) and Pf-3 (73.33%) followed by *Trichoderma* isolates; T-11 (73.73%) and T-14 (66.67%). All potent native microbial isolates showed consistent ability to produce siderophore, ammonia, IAA, HCN, volatile metabolites and also able to release inorganic phosphorus from tri-calcium phosphate. The potential isolates were identified as *T. asperellum* and *P. fluorescens* based on the molecular characterization. In-vitro compatibility analysis of microbial consortia showed positive interaction. The potent biocontrol consortial sets of *Trichoderma* and *Pseudomonas* were tested in-vitro and highest inhibition of the pathogen was recorded in the combination of Pf-2 + Pf-3 + T-11 + T-14 (83.33%) followed by Pf-2 + Pf-3 + T-11 (78.38%). Liquid bio-formulations were prepared using the best two microbial consortia (MC) which were utilized for the management of late blight through seed treatment (1%), soil application (1%) and foliar spray (1%) under natural epiphytotic conditions. The highest reduction of late blight severity was recorded in chemical control treatment (91.92%) followed by MC-1 (84.38%) and MC-2 (77.20%). The MC-1 also significantly promoted the tomato plant height (101.20%), number of leaves per plant (116.48%), number of branches per plant (146.57%), number of fruits per plant (185.52%), fresh weight of fruit (42.59%), root length (67.28%) and marketable fruit yield (313.02%) over control treatment whereas chemical treatment showed non-significant with all above parameters. Among the tested microbial consortia, outstanding results were obtained in MC-1 indicating better plant growth promoting potential and disease reduction potential and thus exhibiting tremendous potential for its commercial exploitation.

Keywords Plant growth promotion · Tomato · Microbial consortia · *Phytophthora* · Liquid bio-formulation

Introduction

Tomato is an indispensable vegetable crop which is the major source of nutrients and medicinal values, hence known as 'Nutraceutical vegetable' (Singh et al. 2019). Tomato is highly adaptive to warm season and can be grown successfully in plains as well as in hills. Cultivation of tomato in rainy season is assuming a great importance in the north-eastern region of India in general and Nagaland in particular owing to its high prices of produce obtained from other parts of the country during this period (Babu 2006).

Though tomato crop occupies a very important place among the vegetable crops cultivated in India, the average yield of this crop on farmers' fields is reasonably poor. One of the constrain for poor yield is the devastating effect of certain diseases. Among the diseases, late blight of tomato caused by *Phytophthora infestans* is destructive and wide spread in nature (Son et al. 2008). Worldwide losses were estimated is about $170 billion annually and thus this pathogen was considered as a major threat for global food security (Latijnhouwers et al. 2004; Wu et al. 2012). Yield losses up to 79% from late blight damage in tomato have been recorded in India (Arora et al. 2014; Chowdappa et al. 2015).

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Although, several management components viz., cultural practices, biological agents, host resistance and fungicides are available, but fungicides hold promise in managing the late blight disease of tomato. Use of fungicides is costly, may lead to environmental pollution and less effective due to increasing resistance of the pathogen. Under such conditions, the most effective method is the biological control (Harish et al. 2008). In recent years, emerging strategy is integrated biological control as microbial consortia. Under field conditions microbial consortia are much more efficient than single strain of organisms with diverse metabolic capabilities (Yan et al. 2002).

Hence, looking into the aforesaid realities, the use of native biological agents as a consortium and also not much systematic research work has been carried out on late blight disease of tomato under Nagaland condition. Hence, in the present study attempts were made to explore native isolates of BCAs and developing an indigenous microbial consortium package for developing a biointensive management strategy against late blight disease of tomato along with yield enhancement.

Materials and methods

Identification of the pathogen

The causal agent of late blight disease of tomato was isolated by standard tissue isolation technique on rye-A agar medium (Hollomon 1965). The purified isolate was subjected to pathogenicity test. For this purpose, isolated pathogen was inoculated on 4 weeks old susceptible tomato cv. Pusa Ruby (Loliam et al. 2012).

Morphological characters of the pathogen was studied on host as well as in pure culture on rye-B agar medium. The isolated pathogen was identified on the basis of morphological characters as documented by Waterhouse (1963).

Isolation and identification of bioagents

A field survey was undertaken for the collection of rhizospheric soil samples from different cropping areas in Nagaland, India (Table 1). Soil samples were taken from the rhizosphere of healthy plants and kept in polyethylene bags. The individual sample was mixed thoroughly after air drying. Thirty three isolates were obtained from the collected samples by soil dilution plate technique (Waksman 1927).

Initially isolated microbes were identified as *Trichoderma* spp. and *Pseudomonas* spp. based on morphological characteristics by use of selective media viz., *Trichoderma* selective medium (TSM) (Elad and Chet 1983) and King's B medium (King et al. 1954) respectively. Further, the potential isolates were identified as *T. asperellum* (T-11; Acc. No MK928414 and T-14; Acc. No MK928417) and *P. fluorescens* (Pf-2; Acc. No MN783298 and Pf-3; Acc. No MN783297) based on the molecular characterization (Singh et al. 2020).

In-vitro antagonistic tests

The antagonistic effect of *Trichoderma* and *Pseudomonas* isolates were evaluated against *Phytophthora infestans* by dual culture plate technique as per Sivakumar et al. (2000) and Georgakopoulos et al. (2002) respectively. Linear mycelial growth of the pathogen was recorded in Petri plate after full growth of pathogen attained in control treatment. The per cent inhibition of the growth of pathogen by antagonists over control was calculated (Vincent 1927).

Investigation on the biocontrol mechanisms of antagonists

The effects of volatile metabolites and mycoparasitism activity of isolated BCAs were assessed against *P. infestans* by adopting the technique given by Dennis and Webster (1971) and Rodrigues (2010), respectively. The production of Ammonia, IAA and HCN by *Trichoderma* and *Pseudomonas* isolates were also determined in the qualitative assay technique given by Cappuccino and Sherman (1992), Gordon and Weber (1951) and Miller and Higgins (1970), respectively. Phosphate solubilization and siderophore production test was also conducted qualitatively by inoculation of *Trichoderma* and *Pseudomonas* isolates on National Botanical Research Institute's phosphate (NBRIP) agar medium (Nautiyal 1999) and chrome azurol sulfonate (CAS) agar medium (Milagres et al. 1999), respectively.

Selection of potential isolates and their compatibility study in-vitro

Based on in-vitro antagonistic capabilities of *Trichoderma* and *Pseudomonas* isolates against *P. infestans* and elucidation of their various biocontrol mechanisms, the potent isolates were selected for further studies. In-vitro compatibility test amongst microbial consortia of potent isolates of *Trichoderma* and *Pseudomonas* were evaluated by dual culture plate method (Siddiqui and Shaukat 2003) in order to determine the compatibility among different combination of consortia.

Antagonistic efficacy of microbial consortia against *P. infestans*

The in-vitro bioassay technique was used for the testing of microbial consortia against *P. infestans.* The mycelial disc

Table 1 Native biocontrol agents (BCAs) and their collection locations

Isolate code	Isolation from	Location
Pseudomonas isolates		
Pf-1	Tomato rhizosphere	Polyhouse-I, CIH, Medziphema, Dimapur, Nagaland
Pf-2	Tomato rhizosphere	Tomato field-I, Horticulture farm, SASRD, Medziphema, Dimapur
Pf-3	Tomato rhizosphere	Farmers' field-I, Merema, Kohima, Nagaland
Pf-4	Tomato rhizosphere	Farmers' field-I, Tsiesema, Kohima, Nagaland
Pf-5	Tomato rhizosphere	Polyhouse-II, CIH, Medziphema, Dimapur, Nagaland
Pf-6	Tomato rhizosphere	Tomato field-II, Horticulture farm, SASRD, Medziphema, Dimapur
Pf-7	Tomato rhizosphere	Farmers' field-II, Merema, Kohima, Nagaland
Pf-8	Tomato rhizosphere	Farmers' field-II, Tsiesema, Kohima, Nagaland
Trichoderma isolates		
T-1	Virgin forest soils	Dziilakie forest, Dimapur, Nagaland
T-2	Virgin forest soils	Dziilakie forest, Dimapur, Nagaland
T-3	Virgin forest soils	Dziilakie forest, Dimapur, Nagaland
T-4	Tomato rhizosphere	Polyhouse-I, CIH, Medziphema, Dimapur, Nagaland
T-5	Tomato rhizosphere	Tomato field-I, Horticulture farm, SASRD, Medziphema, Dimapur
T-6	Tomato rhizosphere	Farmers' field-I, Merema, Kohima, Nagaland
T-7	Tomato rhizosphere	Farmers' field-I, Tsiesema, Kohima, Nagaland
T-8	Tomato rhizosphere	Polyhouse-II, CIH, Medziphema, Dimapur, Nagaland
T-9	Tomato rhizosphere	Tomato field-II, Horticulture farm, SASRD, Medziphema, Dimapur
T-10	Tomato rhizosphere	Farmers' field-II, Merema, Kohima, Nagaland
T-11	Tomato rhizosphere	Farmers' field-II, Tsiesema, Kohima, Nagaland
T-12	Rice rhizosphere	Rice field-I, Agronomy farm, SASRD, Medziphema, Dimapur
T-13	Rice rhizosphere	Rice field-II, Agronomy farm, SASRD, Medziphema, Dimapur
T-14	Rice rhizosphere	Rice field-III, Agronomy farm, SASRD, Medziphema, Dimapur
T-15	Rice rhizosphere	Rice field-IV, Agronomy farm, SASRD, Medziphema, Dimapur
T-16	Rice rhizosphere	Rice field-V, Agronomy farm, SASRD, Medziphema, Dimapur
T-17	Soils	Fallow land, Agronomy farm, SASRD, Medziphema, Dimapur
T-18	Soils	Rice field-I, Agronomy farm, SASRD, Medziphema, Dimapur
T-19	Black gram rhizosphere	Agronomy farm, SASRD, Medziphema, Dimapur
T-20	Cauliflower rhizosphere	Horticulture farm, SASRD, Medziphema, Dimapur
T-21	Soils	Fallow land, Horticulture farm, SASRD, Medziphema, Dimapur
T-22	Soils	Rice field-II, Agronomy farm, SASRD, Medziphema, Dimapur
T-23	Soils	Rice field-III, Agronomy farm, SASRD, Medziphema, Dimapur
T-24	Soils	Rice field-IV, Agronomy farm, SASRD, Medziphema, Dimapur
T-25	Soybean rhizosphere	Agronomy farm, SASRD, Medziphema, Dimapur

(10 mm diameter) of the pathogen (9 days old) was placed at centre of Petri plate containing rye-B agar medium (20 ml). Simultaneously, 10 mm diameter disc of potent *Trichoderma* (T-11 and T-14) isolates (9 days old) and 20 µl of an overnight culture of potent *Pseudomonas* (Pf-2 and Pf-3) isolates were poured in wells (5 mm diameter) at different corner of Petri plate. Linear mycelial growth of the pathogen was recorded in Petri plate when mycelium of test pathogen touched any antagonists in any treatment. The per cent inhibition of the growth of pathogen by antagonists over control was calculated (Vincent 1927).

Preparation of liquid based bio-formulation of microbial consortia

The conidial suspension of each selected isolates of *T. asperellum* (T-11 and T-14) was prepared from 9 days old PDA plates. The plates were rinsed with sterile distilled water and the mycelia were carefully scraped off with a bent glass rod. This suspension was filtered through filter paper (Whatman No.1) to separate the spores from the mycelia. The spore concentration was adjusted to 3.7×10^8 spores/ml (Dubos 1987) with the help of haemocytometer. Similarly, selected *P. fluorescens* isolates (Pf-2 and Pf-3) cell

suspension was prepared by inoculating into King's B broth followed by shaking for 48 h (150 rpm) at 28 °C. The bacterial suspension was adjusted optically at 1×10^9 cfu/ml (Mulya et al. 1996). Liquid based bio-formulations of consortia were prepared by mixing equal volume of each selected isolate just before use for field experiment (Srinivasan and Mathivanan 2009).

Field evaluation of liquid bio-formulation of microbial consortia against late blight of tomato under natural epiphytotic conditions

The field trials were conducted during the tomato growing seasons (Sept.–Jan.) of 2017–2018 and 2018–2019. The research field site is located in the foothills of Nagaland (India) and situated at 25° 45′ 45″ North latitude and 93° 51′ 45″ East longitudes at an elevation of 310 m above mean sea level.

The bio-formulation of microbial consortia (MC) and chemical treated seeds (400 seeds/treatment) of tomato were sown in nursery beds (8 × 1 cm at 1 cm depth) after 15 days of formalin (2%) treated soil. The tomato cv. Pusa Ruby was used in the field experiment, which is known to be highly susceptible to *P. infestans* in India (Singh et al. 2019). The 28 days old seedlings were transplanted (60 × 45 cm) in main field during second week of October in raised plot (1.8 m × 1.8 m). The each plot was framed at 50 cm distance apart. All the recommended standard cultural operations were followed.

The field experiment was laid out in a randomized block design (RBD) with six replications (72 plants per treatment). A total of four treatments viz., T_1 (MC-1; seed treatment (1%) + soil application (1%) + foliar sprays (1%) at 15, 30 and 45 DAT), T_2 (MC-2; seed treatment (1%) + soil application (1%) + foliar sprays (1%) at 15, 30 and 45 DAT), T_3 (Chemical control; seed dressing with 0.3% Captan 50% WP + soil application of Mancozeb 75% WP (0.2%) + foliar sprays of Ridomil MZ 72% WP (0.25%) at 15, 30 and 45 DAT) and T_4 (Control, sterile distilled water) were used.

Application methods of liquid microbial consortia

Seed treatment

The surface sterilized (1.0% sodium hypochlorite for 2 min) seeds were soaked in conidial suspension of microbial consortia at 1%, chemical control treatment (0.3% of captan 50% WP) and control treatment (soaked in sterile distilled water). All the treated seeds were dried by keeping under aseptic condition in laminar air flow for 5 h (Srinivasan and Mathivanan 2009).

Soil application

The soil application treatment was done with 1% of MC inoculated in FYM, Mancozeb 75% WP at 0.2% and sterile distilled water for control treatment at 10 days before transplanting (Srinivasan and Mathivanan 2011).

Foliar spray

Three foliar sprays were done with 1% of MC, Mancozeb + Metalaxyl-72% WP at 0.25% and sterile distilled water for control treatment at 15, 30 and 45 DAT. The total spray solution of 150 ml was used in each plot (12 plants) (Srinivasan et al. 2009).

Observations

The late blight disease severity was assessed visually on leaves, stems and fruits of all plants in each replication following rating scale as per Irzhansky and Cohen (2006), when all plants in control treatment infested with late blight disease under natural epiphytotic conditions. The severity grades were converted into percentage disease index (PDI) for analysis as per the formula given by Wheeler (1969).

Plant growth promoting attributes like plant height, number of leaves, number of branches, number of fruits per plant, fresh weight of fruit, marketable fruit yield and root length were recorded.

Statistical analysis

The data were analyzed using WASP 2.0 software developed by the Central Coastal Agricultural Research Institute, Goa (India).

Results

Identification of the pathogen

The pure culture was obtained from the diseased specimens were identified as *P. infestans* based on macroscopic and microscopic characters. The phenotypic characteristics of isolate were observed fluffy cottony mycelium and slow growth rate on the rye-B agar medium. Microscopic observation revealed that the fungal hyphae were hyaline, moderately thick hyphae, coenocytic and profusely branched. Sporangiosphores were sympodial with a small swelling at the base of each branch. Sporangia were terminal or lateral, ellipsoid, ovoid or limoniform, semipapillate, deciduous and pedicelless and they comparatively more frequently

observed on the tomato plants than in pure culture. Chlamydospores of the pathogen were also recorded in diseased specimens (Fig. 1).

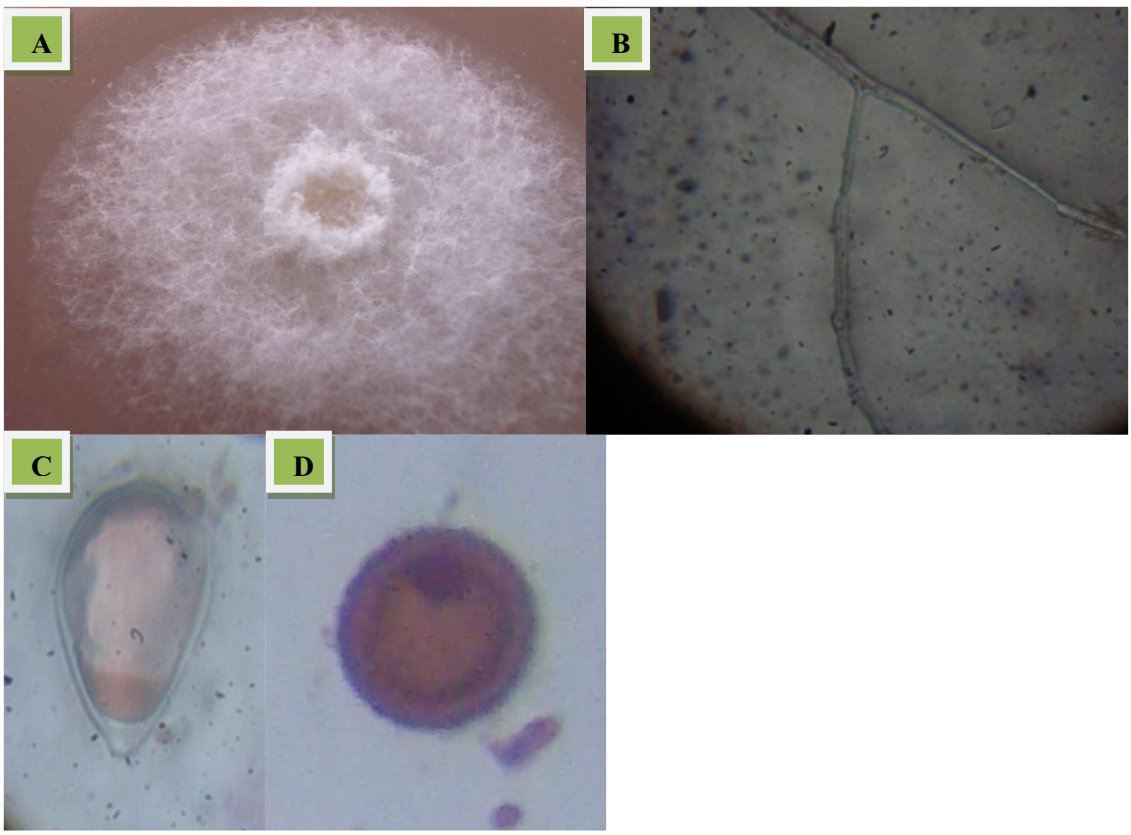
In-vitro antagonistic tests

Altogether 25 isolates of *Trichoderma* were screened for their inhibitory action on the radial growth of *P. infestans.* It was found that the growth of the pathogen in dual culture plates progressed until they come in contact with the leading edges of the antagonist. The per cent inhibition over control was calculated and it was observed that T-11 was the most promising isolate against *P. infestans* with 73.73 per cent inhibition. Next best isolate was T-14 (66.67%) followed by T-5 (64.93%), T-25 (64.00%) and the least antagonistic effect was observed in T-17 (51.07%) at 8 days after incubation at 18 ± 1 °C (Table 2).

The antagonistic effects of *Pseudomonas* isolates were evaluated against *P. infestans* which significantly inhibited the growth of the pathogen as compared to control treatment. Among the *Pseudomonas* isolates, maximum per cent inhibition was observed in Pf-2 (81.33%) which is significantly superior to all other treatments followed by Pf-3 (73.33%), Pf-1 (69.33%), Pf-7 (66.67%) and Pf-4 (65.33%) at 8 days after incubation at 18 ± 1 °C (Table 2). The clear zone of inhibition was also observed in the dual culture plate of Pf-2 and Pf-3.

Investigation on the biocontrol mechanisms of antagonists

The effects of volatile metabolites of *Trichoderma* and *Pseudomonas* isolates were assessed against *P. infestans*. Among the tested isolates, the per cent inhibition over control was calculated and it was recorded that *Trichoderma* (T-11) and *Pseudomonas* (Pf-3) was found to be most promising in production of volatile compounds against *P. infestans* with 45.55 and 53.67 per cent inhibition (Table 2).



(A) Fluffy cottony mycelium
(B) Sympodial with nodal swellings of sporangiophore under 45 x
(C) Microscopic view of a hyaline, limoniform, an apical papilla on sporangium under100 x
(D) Microscopic view of chlamydospore under 45 x

Fig. 1 Characterization of the pathogen (*P. infestans*)

Table 2 In-vitro screening of native biocontrol agents (BCAs) against *P. infestans* and their mechanisms

BCAs	Per cent inhibition of pathogen growth in dual culture	Biocontrol mechanisms						
		Per cent inhibition of pathogen growth by volatile metabolites	Ammonia production	IAA production	Phosphate solubility	Siderophore production	HCN production	Mycoparasitism
Pseudomonas isolates								
Pf-1	69.33	44.78	+++	−	++	++	−	*
Pf-2	81.33	53.67	+++	+++	+++	++	++	*
Pf-3	73.33	48.11	++++	+++	+++	+++	++	*
Pf-4	65.33	25.22	++++	−	−	++	−	*
Pf-5	60.44	29.22	+++	−	−	+	−	*
Pf-6	60.00	35.89	+++	−	−	++	−	*
Pf-7	66.67	24.78	++++	−	+	++	−	*
Pf-8	64.00	25.56	++++	+	+++	+++	++	*
Trichoderma isolates								
T-1	60.40	34.78	++++	−	−	+	*	+
T-2	54.27	26.33	++++	+	−	+	*	+
T-3	52.93	19.67	+++	−	+	+++	*	+
T-4	56.00	32.22	++	−	−	+++	*	+
T-5	64.93	14.11	++	−	+	+++	*	+
T-6	57.33	21.89	+++	+	−	++	*	+
T-7	52.00	14.44	+++	+	−	+++	*	+
T-8	61.33	18.11	++	+	−	+++	*	+
T-9	57.73	31.11	+++	+	−	+++	*	+
T-10	53.33	28.11	+++	−	+	+++	*	+
T-11	73.73	45.55	++++	+++	+++	+++	*	+
T-12	53.73	16.67	+	−	−	++	*	+
T-13	60.00	25.89	+	−	−	++	*	+
T-14	66.67	35.55	+++	+++	+++	+++	*	+
T-15	55.60	30.00	+++	+	−	+++	*	+
T-16	52.40	27.00	+	+	+	+	*	+
T-17	51.07	15.22	+	+	−	++	*	+
T-18	59.60	12.22	+++	−	−	+++	*	+
T-19	62.67	25.55	+++	+	+	++	*	+
T-20	60.93	23.67	+++	−	−	++	*	+
T-21	56.93	35.22	++	−	−	+++	*	+
T-22	58.67	25.89	++	−	−	+	*	+
T-23	56.00	13.00	+++	−	−	++	*	+
T-24	57.73	16.67	+++	−	−	+	*	+
T-25	64.00	32.55	++++	+	+	++	*	+

Whereas, − = negative, + = positive/low production, ++ = moderate production, +++ = strong production and * = not tested

The mycoparasitism activity of 25 isolates of *Trichoderma* were also assessed against *P. infestans* and they showed the presence of coiling as hyphal interactions between them (Table 2).

The production of Ammonia by *Trichoderma* and *Pseudomonas* isolates were also determined in the qualitative assay. Among the tested isolates, *Pseudomonas* isolates (Pf-3, Pf-4, Pf-7 and Pf-8) and *Trichoderma* isolates (T-1, T-2, T-11, T-14 and T-25) exhibited strong ammonia production by turning initial peptone water broth from yellow to dark brown colour (Table 2). The results of qualitative assay of IAA production by different native BCAs revealed that *Pseudomonas* isolates (Pf-2 and Pf-3) and *Trichoderma* isolates (T-11 and T-14) exhibited strong IAA production. The moderate production of HCN was observed in *Pseudomonas* isolates (Pf-2, Pf-3 and Pf-8) (Table 2).

The results of qualitative assay of phosphate solubilization by different native BCAs revealed that *Pseudomonas*

isolates (Pf-2, Pf-3 and Pf-8) and *Trichoderma* isolates (T-11 and T-14) elucidated strong phosphate solubility activity (Table 2). The siderophore production test was also conducted qualitatively by inoculation of *Trichoderma* and *Pseudomonas* isolates on chrome azurol sulfonate (CAS) agar medium. All 33 isolates showed positive results for siderophore production. Among the tested isolates, *Pseudomonas* isolates (Pf-3 and Pf-8) and *Trichoderma* isolates (T-3, T-4, T-5, T-7, T-8, T-9, T-10, T-11, T-14, T-15, T-18 and T-21) exhibited strong siderophore production by pink and orange halo colour development (Table 2).

Selection of potential isolates and their compatibility study in-vitro

Based on in-vitro antagonistic capabilities of *Trichoderma* and *Pseudomonas* isolates against *P. infestans* and elucidation of their various biocontrol mechanisms, the potent isolates of *Pseudomonas* (Pf-2 and Pf-3) and *Trichoderma* (T-11 and T-14) were used for further studies. Selected native microbial isolates were showed consistent ability to produce siderophore, ammonia, IAA, volatile metabolites and also able to release inorganic phosphorus from tri-calcium phosphate (Table 2).

In-vitro experiment was carried out in all permutations and combination amongst the potent isolates of *Trichoderma* and *Pseudomonas*. Altogether 11 treatment combinations were tested and compared with growth of Pf-2 (Control-1), Pf-3 (Control-2), T-11 (Control-3) and T-14 (Control-4). The microorganisms showing positive compatibility among them was recorded, tabulated and selected for further study. The data showed compatibility among all the treatment combinations of the four bioactive microorganisms in-vitro. No clear inhibition zone was recorded between the tested microbial consortia. Absence of inhibition zone indicated that the potential isolates of *Trichoderma* and *Pseudomonas* were compatible with each other.

Antagonistic efficacy of microbial consortia against *P. infestans*

A total of 12 treatment combinations were compared. Eleven consortia produced varying inhibitions (%) in-vitro against *P. infestans* (Table 3 and Fig. 2). All consortia tested against *P. infestans* were significantly superior over control. Among the different consortial sets tested in-vitro the significant highest inhibition of pathogen was recorded in the combination of Pf-2 + Pf-3 + T-11 + T-14 (83.33%) followed by Pf-2 + Pf-3 + T-11 (78.38%), Pf-2 + Pf-3 (77.43%) and Pf-2 + T-14 (76.54%) respectively at 5 days after incubation at 18 ± 1 °C.

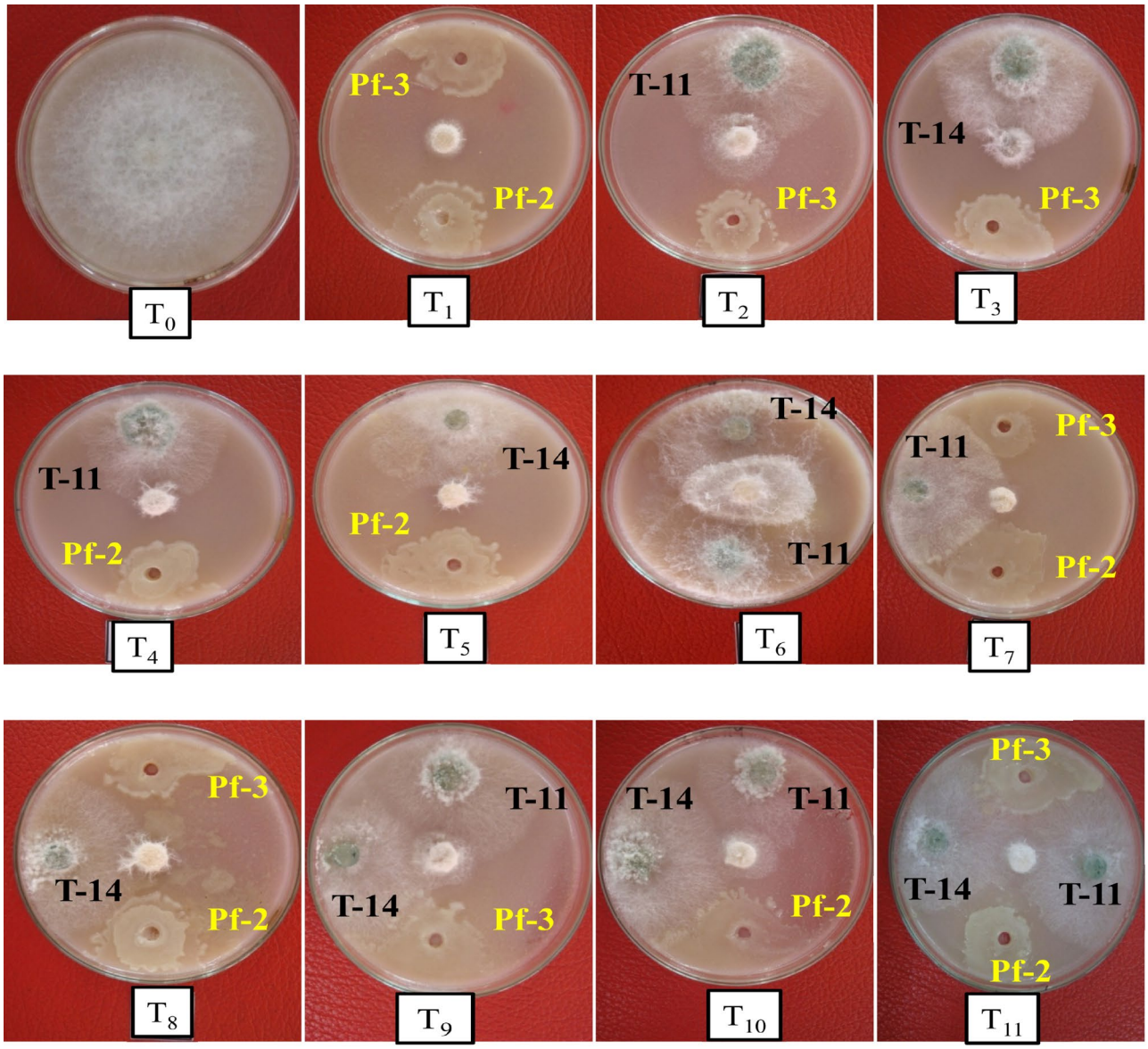
An in-vitro study was taken up to select the two best microbial consortia (MC) against the test pathogen. The MC-1 (*P. fluorescens* Pf-2 + *P. fluorescens* Pf-3 + *T. asperellum* T-11 + *T. asperellum* T-14) and MC-2 (*P. fluorescens* Pf-2 + *P. fluorescens* Pf-3 + *T. asperellum* T-11) inhibited the pathogen significantly and were found to be the most effective consortia. Hence, they were selected for field study.

Table 3 In-vitro antagonistic effect of microbial consortia on radial growth and per cent inhibition of *P. infestans*

Treatment	Treatment combination	Inhibition of *P. infestans* growth[a]			
		Radial growth (cm)	Radial growth (cm) inhibited	Individual's Inhibition (%)	Combines inhibition (%)
T_0	Control *P. infestans*	3.90	00.00	00.00	00.00 (4.05)
T_1	Pf-2 + Pf-3	0.83 + 0.93	3.07 + 2.97	78.72 + 76.15	77.43 (61.66)
T_2	Pf-3 + T-11	1.30 + 0.93	2.60 + 2.97	66.67 + 76.15	71.41 (57.67)
T_3	Pf-3 + T-14	0.90 + 1.00	3.00 + 2.90	76.92 + 74.36	75.64 (60.43)
T_4	Pf-2 + T-11	0.93 + 1.07	2.97 + 2.83	76.15 + 72.56	74.36 (59.58)
T_5	Pf-2 + T-14	0.87 + 0.96	3.03 + 2.94	77.69 + 75.38	76.54 (61.05)
T_6	T-11 + T-14	0.90 + 1.20	3.00 + 2.70	76.92 + 69.23	73.08 (58.76)
T_7	Pf-2 + Pf-3 + T-11	0.70 + 0.90 + 0.93	3.20 + 3.00 + 2.97	82.05 + 76.92 + 76.15	78.38 (62.29)
T_8	Pf-2 + Pf-3 + T-14	1.03 + 1.23 + 1.30	2.87 + 2.67 + 2.60	73.59 + 68.46 + 66.67	69.57 (56.33)
T_9	Pf-3 + T-11 + T-14	1.03 + 1.07 + 1.27	2.87 + 2.83 + 2.63	73.59 + 72.56 + 67.44	71.20 (57.56)
T_{10}	Pf-2 + T-11 + T-14	0.97 + 0.87 + 1.10	2.93 + 3.03 + 2.80	75.13 + 77.69 + 71.79	74.87 (59.91)
T_{11}	Pf-2 + Pf-3 + T-11 + T-14	0.63 + 0.67 + 0.60 + 0.70	3.27 + 3.23 + 3.30 + 3.20	83.85 + 82.82 + 84.61 + 82.05	83.33 (65.90)
SEm ±					0.91 (0.60)
C.V. (%)					2.29 (1.86)
CD ($p = 0.01$)					3.60 (2.36)

Values in parentheses are angular transformed values

[a]Means of three replications



Whereas; T_0 (Control, *P. infestans* alone)**;** T_1 (Pf-2 + Pf-3); T_2 (Pf-3 + T-11); T_3 (Pf-3 + T-14); T_4 (Pf-2 + T-11); T_5 (Pf-2 + T-14); T_6 (T-11 +T-14); T_7 (Pf-2 + Pf-3 + T-11); T_8 (Pf-2 + Pf-3 +T-14); T_9 (Pf-3+ T-11 +T-14); T_{10} (Pf-2 + T-11+T-14) and T_{11} (Pf-2 + Pf-3 + T-11 +T-14).

Fig. 2 In-vitro antagonistic effect of microbial consortia (MC) on radial growth of *P. infestans*

Field evaluation of liquid bio-formulation of microbial consortia against late blight of tomato under natural epiphytotic conditions

Among different treatments, liquid microbial consortia (MC)-1 significantly decreased the late blight severity (12.08 PDI) compared to all other treatments. This was comparable with the chemical treatment (6.25 PDI). In case of untreated control, high severity of 77.36 PDI was recorded at 45 DAT (Table 4). These results revealed that the chemical control significantly decreased late blight severity (91.92%) over control treatment. Next in order of merit was MC-1 (84.38%) and MC-2 (77.20%). The mortality per cent significantly decreased in all the treatment (100%) over control treatment (Table 4). Simultaneously, liquid bio-formulation of MC-1 significantly increased the tomato plant height (101.20% and Fig. 3), number of leaves per plant (116.48%), number of branches per plant (146.57%), number of fruits per plant (185.52%), fresh weight of fruit (42.59%), root length (67.28%) and

Table 4 In-vivo effects of microbial consortia (MC) on per cent decrease of late blight severity and mortality per cent and per cent increase of marketable tomato fruit yield over control

Treatment	Disease severity (PDI)[a] at 45 DAT				[c]Mortality per cent at 77 DAT				Marketable fruit yield (g plant^{-1})				Calculated marketable fruit yield (t ha^{-1})			
	2017–2018	2018–2019	Pooled	% decrease over control[b]	2017–2018	2018–2019	Pooled	% decrease over control	2017–2018	2018–2019	Pooled	% increase over control	2017–2018	2018–2019	Pooled	% increase over control
T_1 (MC-1)	12.50 (20.58)	11.67 (19.89)	12.08 (20.28)	84.38	00.00 (4.05)[a]	00.00 (4.05)	00.00 (4.05)	100	1185.70	1147.87	1166.78	313.02	43.91	42.51	43.21	313.10
T_2 (MC-2)	19.44 (26.09)	15.83 (23.32)	17.64 (24.80)	77.20	00.00 (4.05)	00.00 (4.05)	00.00 (4.05)	100	718.00	752.90	735.45	160.34	26.59	27.88	27.23	160.32
T_3 (chemical control)	06.94 (15.16)	05.55 (13.50)	06.25 (14.39)	91.92	00.00 (4.05)	00.00 (4.05)	00.00 (4.05)	100	467.83	482.80	475.31	68.25	17.32	17.88	17.60	68.26
T_4 (control)	77.78 (61.97)	76.94 (61.35)	77.36 (61.62)	–	23.61 (28.51)	25.00 (29.79)	24.30 (29.36)	–	243.67	321.33	282.50	–	09.02	11.90	10.46	–
SEm ±	1.18 (0.91)	1.34 (1.08)	0.75 (0.36)	–	2.26 (1.58)	1.52 (1.02)	1.46 (0.98)	–	92.79	61.16	72.92	–	3.47	2.26	2.70	–
C.V. (%)	9.95 (7.17)	11.98 (8.97)	6.54 (4.94)	–	19.82 (7.85)	11.95 (4.69)	15.69 (6.36)	–	16.85	17.10	16.86	–	17.56	14.46	16.86	–
CD ($p=0.05$)	3.57 (2.73)	4.05 (3.26)	2.28 (1.84)	–	6.81 (4.77)	4.58 (3.07)	4.42 (2.87)	–	279.63	184.33	219.76	–	10.46	6.83	8.14	–

Values in parentheses are angular transformed values

[a]PDI = (Sum of numerical rating/no. of plant scored × maximum score in scale) × 100

[b]The per cent reduction over control treatment: Disease reduction (%) $= \frac{\text{PDI in control} - \text{PDI in treatment}}{\text{PDI in control}} \times 100$

[c]Mortality per cent = (Number of diseased dead plants/total number of plants) × 100

Fig. 3 Effect of microbial consortia (MC) on tomato plant height, number of leaves, fruits and branches; T_1 (MC-1), T_2 (MC-2), T_3 (Chemical control) and T_4 (Control)

marketable fruit yield (313.02%) over untreated control (Tables 5 and 6).

Discussions

The pathogen was identified as *P. infestans* based on the nature of disease observed, morphological and cultural characters seen under the microscope. These characters were further compared with the characters reported by Waterhouse (1963) and description given by Agrios (1997) and Zentmyer (1983). The present observations corroborates with the descriptions and findings of earlier workers.

The development of native bio-formulation is more efficient antagonistic player in plant disease management and growth promotion. Antagonistic effect of *Trichoderma* and *Pseudomonas* isolates against *P. infestans* has already been reported by several research workers (Kabir et al. 2013; Kumar et al. 2015; Lamsal et al. 2013; Patel and Mukadam 2011; Zegeye et al. 2011). In this study, the probable reasons of high inhibitory activity of the antagonists observed on *P. infestans* in dual cultures may be due to production of antifungal metabolites such as mycoparasitic activities, volatile gases, cell-wall degrading enzymes, HCN, siderophores, pyoluteorin, pyrrolnitrin and 2-4 diacetyl phloroglucinol.

Many secondary metabolites have been recorded to be involved in microbial interactions (Dennis and Webster 1971; Kapri and Tewari 2010; Vespermann et al. 2007). These reports are commensurate with result of the present investigation, which suggests that the production of secondary metabolites by both *Trichoderma* and *Pseudomonas* isolates have definite influence on the high degree of inhibition of *P. infestans.*

The mycoparasitic potential of *Trichoderma* spp. against *P. infestans* is well documented in previous findings (Ezziyyani et al. 2007; Pugeg and Ian 2006; Zegeye et al. 2011). However, in the present investigation, working with the native isolates, the mycoparasitic potential was further manifested by characteristic envelopment and coiling around the hyphae by all isolates of *Trichoderma* spp. The hyphae of *Trichoderma* spp. was also observed to grow in close proximity to the hyphae of *P. infestans* before coagulation and disintegration occurred.

Dixit et al. (2015) evaluated 11 isolates of fluorescent *Pseudomonas* for ammonia production. All isolates showed positive result for ammonia production. Lalngaihawmi and Bhattacharyya (2019) also evaluated *Trichoderma* spp. for ammonia production and results revealed that all the *Trichoderma* spp. showed positive result. These reports are in agreement with the result of

Table 5 In-vivo effects of microbial consortia (MC) on tomato plant height, number of leaves and branches per plant at 94 DAT

Treatment	Plant height (cm)				No. of leaves per plant				No. of branches per plant			
	2017–2018	2018–2019	Pooled	% increase over control[a]	2017–2018	2018–2019	Pooled	% increase over control[a]	2017–2018	2018–2019	Pooled	% increase over control[a]
T_1 (MC-1)	43.40	47.27	45.33	101.20	27.57	30.23	28.90	116.48	08.20	08.33	08.26	146.57
T_2 (MC-2)	34.10	37.20	35.65	58.23	20.87	22.93	21.90	64.04	06.17	05.50	05.83	77.01
T_3 (chemical control)	29.33	22.83	26.08	15.76	14.00	16.00	15.00	12.36	04.20	04.17	04.18	24.78
T_4 (control)	24.43	20.63	22.53	–	13.03	13.67	13.35	–	03.23	03.47	03.25	–
SEm ±	1.52	1.74	1.17	–	1.32	1.61	1.22	–	0.43	0.41	0.38	–
C.V. (%)	11.38	13.31	8.89	–	17.20	19.02	15.19	–	19.18	18.76	17.15	–
CD ($p=0.05$)	4.59	5.24	3.54	–	3.99	4.85	3.70	–	1.29	1.24	1.14	–

[a]The per cent increase over control treatment: per cent increase (%) $=\frac{\text{Treatment value}-\text{Control value}}{\text{Control value}} \times 100$

Table 6 In-vivo effects of microbial consortia (MC) on number of tomato fruit per plant, fresh weight of fruit and root length

Treatment	No. of fruit per plant at 94 DAT				Fresh weight of fruit (g) at 98 DAT				Root length (cm) at 100 DAT			
	2017–2018	2018–2019	Pooled	% increase over control[a]	2017–2018	2018–2019	Pooled	% increase over control[a]	2017–2018	2018–2019	Pooled	% increase over control[a]
T_1 (MC-1)	35.65	35.73	35.69	185.52	33.30	32.73	33.01	42.59	44.93	44.33	44.63	67.28
T_2 (MC-2)	24.60	25.37	24.98	99.84	29.73	31.54	30.63	32.31	37.07	35.80	36.43	36.54
T_3 (Chemical control)	19.53	18.50	19.01	52.08	24.33	26.31	25.32	09.37	30.20	28.70	29.45	10.38
T_4 (Control)	11.07	13.93	12.50	–	23.07	23.24	23.15	–	27.30	26.07	26.68	–
SEm ±	2.11	2.06	1.98	–	1.41	1.55	1.22	–	1.23	1.59	1.04	–
C.V. (%)	17.90	19.21	18.02		14.24	15.51	13.80		8.61	11.54	7.47	
CD ($p=0.05$)	6.36	6.20	5.96	–	4.25	4.68	4.69	–	5.11	4.79	3.15	–

[a]The per cent increase over control treatment: per cent increase (%) $=\frac{\text{Treatment value}-\text{Control value}}{\text{Control value}} \times 100$

the present investigation, which suggests that the production of ammonia by both *Trichoderma* and *Pseudomonas* isolates have positive impact on the plant growth.

Dixit et al. (2015) further evaluated 20 isolates of *Trichoderma* for IAA production. All *Trichoderma* spp. isolates elucidated positive results for IAA production. Prasad et al. (2017) also evaluated 24 isolates of *Trichoderma* spp. and 12 isolates of *B. subtilis* and *P. fluorescens* for IAA production. In the present investigation, 3 isolate of *Pseudomonas* and 12 isolates of *Trichoderma* were observed to produce IAA at varying intensity. This occurrence may be ascribed to the heterogeneous nature of the source and the strains of the antagonists.

Corbett (1974) described that HCN inhibits proper functioning of enzymes and natural receptors reversible mechanism of inhibition in the pathogens. This report is in agreement with the result of the present investigation, which suggests that the production of HCN by *Pseudomonas* isolates have absolute influence on the high degree of inhibition of *P. infestans.*

It has been observed by many investigators (Bhakthavatchalu et al. 2013; Gangwar et al. 2012; Kapri and Tewari 2010; Prasad et al. 2017; Rai 2017) that a high proportion of phosphate solubilizing microorganisms (PSMs) reside in the rhizosphere of plants and play an important role in solubilization of bound phosphates, making them available to the plants. This report is in agreement with the result of the present investigation, which suggests that the phosphate solubilization by both *Trichoderma* and *Pseudomonas* isolates have obvious influence on the plant growth.

In this present study, the strong and positive siderophore production exhibited by *Pseudomonas* isolates (Pf-3 and Pf-8) and *Trichoderma* isolates (T-3, T-4, T-5, T-7, T-8, T-9, T-10, T-11, T-14, T-15, T-18 and T-21) explicate the corresponding inhibited radial growth and high per cent inhibition of *P. infestans*.

Microbial consortia are known to enhance plant growth, which can result in development of various plant parts and higher growth leads to significant enhancement of vegetative growth attributes through plant growth promotion, whereas growth promotion was absent in chemical control in addition to disease suppression. Presence of consortia in the rhizoshphere increases the availability of nutrients through solubilization of insoluble sparingly soluble minerals have better nutrient uptake thereby enhancing plant growth (Biam and Majumder 2019; Harish et al. 2008; Idris et al. 2007; Raupach and Kloepper 1998; Yan et al. 2002).

Based on activities of biological control mechanism and plant growth promotion studies, the best microbial consortium was identified as MC-1. This promising indigenous liquid consortium has promoted the tomato plant growth and reduced the losses due to late blight disease in an eco-friendly manner exhibiting tremendous potential for its commercial exploitation.

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