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Effect of Consortia Bioformulation of Rhizobacteria on Induction of Systemic Resistance in Tuberose Against Peduncle Blight Disease

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Abstract

Bioformulations of mixtures of plant growth promoting bacterial (*Pseudomnas flourescences* Pf_1 *Bacillus subtilis* Bs_{10}) and fungal isolates (*Trichoderma viridae* Tv_1) were effective in reducing the incidence of peduncle blight under green-house conditions (85.50%). Peduncle blight, hitherto an unknown disease was found to be a major limiting factor to the cultivation of tuberose. Though *Lasiodiplodia theobromae* is an ubiquitous pathogen, its occurrence on tuberose is a new record in India. In addition to disease control, a significant increase in the yield (53.33%) was noticed in the bioprimed plants when compared to the control plants. Pathogenesis-related (PR) proteins, chitinase and b-1, 3-glucanase and defense-related proteins, peroxidase, polyphenol oxidase, phenylalanine ammonialyase and phenolic compounds were significantly activated in the consortia bioformulation treated plants, thus inducing resistance against *Lasiodiplodia theobromae*. Native gel electrophoresis also revealed the expression of more isoforms of pathogenesis-related proteins and other defense enzymes viz., polyphenol oxidase and peroxidase in plants treated with Pf_1+Bs_{10} strain mixture challenged with L. *theobromae*. Populations of bacteria also remained high and stable throughout the growing period. The present study reveals that, microbe inoculated bulblets showed sustained, timely induction and accumulation of these defense enzymes and PR-proteins which enhances the resistance in tuberose against peduncle blight disease and also improved the vegetative growth, physiological attributes, PR-proteins and phenol contents.

Keywords: Tuberose, resistance, peduncle blight, peroxidase, pathogenesis related proteins

1. Introduction

Tuberose (*Polianthes tuberosa* L.) is one of the most popular bulbous ornamental plants of tropical and sub-tropical areas. The flowers having excellent keeping quality and are widely used as cut flowers. (Patel et al., 2006) Diseases and pest were appear to be the major constraints to the production of tuberose. Among the diseases, the peduncle blight (*Lasiodiplodia theobromae*) is an emerging disease in the southern parts of India. Yield loss was reported up to 40–60% in India and in some cases it will extend up to 92%. Though *Lasiodiplodia theobromae* is an ubiquitous pathogen, its occurrence on tuberose is a new record (Durgadevi and Sankaralingam, 2012). The fungus induced confounding symptoms which included blossom blight, peduncle blight and leaf blight at tips as well. This fungus L. *theobromae* has a wide host range and is found throughout the tropics and subtropics (Punithalingam, 1980). The management of L. *theobromae* has been almost exclusively based on the application of chemical fungicides. Several effective fungicides have been recommended for use against the pathogen, but they are not considered to be long-term solutions, due to concerns of expense, exposure risks, residue persistence, elimination of natural enemies and other health and environmental hazards. Therefore, the need for alternative methods of control of this emerging disease has become vital. Unfortunately there is no ecofriendly viable practice currently available for this purpose.

Microbial inoculants, primarily bacteria, are used as propagule priming agents, both as in vitro co-cultures and on transplanting (Nowak and Pruski, 2002), a practice often referred as ''bio-priming''. It is an emerging trend in biotechnological approach aimed at reducing chemical input in plant production, while increasing plant fitness, productivity and resistance to pests and diseases in the context of sustainable horticulture. Upon exposure to stress, the primed plants adapt better and faster than non-primed plants (Conrath et al., 2002). The utilization of plant owns defense mechanism is a fascinating area of research which can be systemically activated upon exposure of plants to PGPR strains or infection by pathogen (Baker et al., 1997). This phenomenon is called induced systemic resistance (ISR) (Tuzun and Kuc, 1991). This mechanism

is facilitated by PGPR organism and operates through the activation of multiple defense compounds at sites distant from the point of pathogen attack (Dean and Kuc, 1985). In addition to disease control, it promotes the growth and development of crop plants. Pseudomonas strains 84 and 4B when introduced to banana roots of tissue-cultured plants at de-flasking stage significantly improved plant growth and reduced infection *of Fusarium oxysporum* f. sp. *cubense* in the rhizome under green house conditions (Smith et al., 2003). Hence, the present investigation was undertaken to study the importance of microbial inoculation in tuberose plants to enhance their resistance against peduncle blight disease.

2. Materials and Methods

2.1. Plant materials, bioagents and pathogen

Tuberose cultivar Prajwal was used in all experiments for evaluating the individual and consortial efficacy of bio formulations of *Pseudomonas fluorescens* Pf1, *Trichodema viride* Tv_1, *Bacillus subtilllis* Bs_{10} against peduncle blight disease. *P. fluorescens* Pf_1, *T. viride* Tv_1, *B. subtillis* Bs_{10} studied as biocontrol agents against various insect pests and diseases were obtained from the Culture Collection Section, Department of Plant Pathology, Tamil Nadu Agricultural University (TNAU), India. Strains of *P. fluorescens* Pf_1, *T. viride* Tv1, *B. subtillis* Bs_{10} used in this study were compatible with each other (data not shown). The virulent peduncle blight pathogen L. *theobromae* (ITCC NO 6751/11) was isolated from tuberose plants showing typical blight symptom.

2.2. Preparation of talc-based formulation of biocontrol agents

PGPR strains The talc-based formulation of each of the individual bacterial strains was prepared with some modification of method developed by Vidhyasekaran and Muthamilan (1995). A loopful of *P. fluorescens* strain was inoculated into the KB broth and incubated in a rotary shaker at 150 rpm for 48 h at room temperature (28±2 °C). One kilogram of talc powder was taken in a sterilized metal tray and its pH was adjusted to neutral by adding calcium carbonate at the rate of 15 g kg^{-1}. Ten gram of CMC was added to 1 kg of talc and mixed well and the mixture was autoclaved for 30 min on each of two consecutive days. The 400 ml of 48 h grown bacterial suspension containing 9 Í 10^8 cfu ml^{-1} were mixed with carrier-cellulose mixture under aseptic conditions. After drying (approximately to 35% moisture content) for overnight, it was packed in polypropylene bag, sealed and stored at room temperature (28±2 °C). At the time of application, the population of *P. fluorescens* in the formulation was 3 Í 10^8 cfu g^{-1} of talc powder.

T. viride Tv_1 were cultured in sterilized molasses yeast medium for 10 days. The fungal biomass and broth containing spore concentration of 1×10^7 cfu ml^{-1} were mixed with talc at 1:2 ratio. The formulations were air dried and packed in polythene covers (Jeyarajan et al., 1994) and used for further study.

2.3. Mixtures of PGPR strains

Bioformulations containing mixtures of fluorescent pseudomonads were prepared by growing the *Pseudomonas* strains separately in KB broth, mixing equal volumes of each and finally blending with talc powder, calcium carbonate and CMC. Same method was followed to the *Pseudomonas* strains with *B. subtilis* strain mixtures. The talc-based bioformulation mixtures were stored at 28±2 °C and used for further applications .

2.4. Survival of biocontrol agents in talc based formulations

The talc based products of biocontrol agents were stored at room temperature (28±2 °C). One gram of sample was drawn at monthly intervals and the population was assessed by serial dilution using KB medium for *P. fluorescens*, NA medium for *B. subtilis* and special medium (Elad and Chet, 1983) for *Trichoderma.* The numbers of colonies were counted after 48 h for bacteria and 120 h for *Trichoderma*.

2.5. Glasshouse studies

2.5.1. Effect of PGPR strains against peduncle blight disease

Pot culture studies were conducted to test the efficacy of *P. fluorescens*, *B. subtilis*, *T. viride* strain mixtures as bulb and foliar applications separately in controlling peduncle blight disease incidence. Tuberose bulbs cv. Prajwal were treated with talc based formulations of effective bio control agents singly as, Tv_1, Pf_1, Bs_{10} or in combinations as Tv_1 with Pf_1, Tv_1 with Bs_{10} and Pf_1 with Bs_{10}. They were planted in pots containing potting medium. After 30 days the plants were sprayed with the biocontrol agents and challenge inoculated with the pathogen after two days. The treatments also included foliar spray of biocontrol agents at 30 days after planting (DAP) without challenge inoculation. The virulent isolate of *L. theobromae* was mass multiplied in the sand-maize medium, mixed with the sterilized pot soil @ 15 g kg^{-1} of soil and filled in earthen pots. Ten gram of the talc based bioformulation mixture was given as bulb treatment per kilogram of bulbs. The experiment was conducted in a completely randomized block design with each treatment replicated thrice. The peduncle blight disease incidence was calculated using the formula (Number of infected plants/total number of plants) Í 100.

2.6. Assay of defense-related enzymes

2.6.1. Colorimetric assay

The PGPR strains treated tuberose plants challenged with peduncle blight pathogen were collected for the estimation of defense-related enzymes. The leaf samples were collected at 0 h, 24 h, 48 h, 72 h, 96 h, and 120 h up to 7 days at 24 h interval. The leaves were homogenized in liquid nitrogen and stored at -70 °C. One gram of powdered sample was extracted with 2 ml of sodium phosphate buffer 0.1 M (pH 7.0) at 4 °C. The homogenate was centrifuged for 20 min at 10,000 rpm and the protein extract were used for estimation of defense enzymes. Phenylalanine ammonia-lyase (PAL) (EC 4.3.1.5) activity was

determined as the rate of conversion of 1-phenylalanine to transcinnamic acid at 290 nm Dickerson et al. (1984). Assay of peroxidase (PO) (EC 1.11.1.7) activity was carried out as per the procedure described by Hammerschmidt et al. (1982). The activity was expressed as the increase in absorbance at 470 nm min^{-1} mg^{-1} of protein. Polyphenol oxidase (PPO) (EC 1.14.18.1) activity was determined as per the procedure given by Mayer et al. (1965) and the activity was expressed as change in absorbance min^{-1} mg^{-1} of protein. Chitinase (EC 3.2.1.14) assay was carried out according to the procedure developed by Boller and Mauch (1988). The enzyme activity was expressed as n moles GlcNAc equivalents min^{-1} g^{-1} fresh weight. b-1, 3-glucanase activity (E.C. 3.2.1.39) was assayed by the laminarin-dinitrosalicylic acid method (Pan et al., 1991). The enzyme activity was expressed as mg glucose released min^{-1} mg^{-1} of sample. Catalase (EC 1.11.1.6) activity was assayed spectrophotometrically as described by Chaparro-Giraldo et al. (2000). The activity was calculated using the extinction coefficient (ε240 nm ¼ 40 mM cm^{-1}) for H_2O_2 and expressed in mmol min^{-1} mg^{-1} of sample. Superoxide dismutase (SOD) (EC 1.15.1.1) enzyme activity was expressed in SOD units mg^{-1} tissue (50% NBT inhibition ¼ 1 unit). The spectrophotometric assay of lipoxygenase (LOX) was carried out through the procedure developed by Kermasha and Metche (1986) and the activity was expressed as OD234 min^{-1} mg^{-1} of protein. Phenol content was estimated as per the procedure given by Zieslin and Ben-Zaken (1993) and expressed as catechol equivalents mg^{-1} tissue weight.

2.7. Activity gel electrophoresis

2.7.1. Peroxidase

To study the expression pattern of different isoforms of peroxidases in different treatments, activity gel electrophoresis was carried out. For native anionic polyacrylamide gel electrophoresis, resolving gel of 8% and stacking gel of 4% were prepared. After electrophoresis, the gels were incubated in the solution containing 0.15% benzidine in 6% NH_4Cl for 30 min in dark. Then drops of 30% H_2O_2 were added with constant shaking till the bands appear. After staining, the gel was washed with distilled water and photographed (Sindhu et al., 1984).

2.7.2. Poly phenol oxidase (PPO)

Enzyme was extracted by homogenizing 1 g of leaf tissue in 0.01 M potassium phosphate buffer (pH 7.0). The homogenate was centrifuged at 20,000 g for 15 min at 4 °C and the supernatant was used as enzyme source. After native electrophoresis, the gel was equilibrated for 30 min in 0.1% p-phenylene diamine in 0.1 M potassium phosphate buffer (pH 7.0) followed by 10 mM catechol in the same buffer. The addition of catechol was followed by a gentle shaking which resulted in appearance of dark brown discrete bands (Jayaraman et al., 1987).

2.8. Statistical analyses

The data were statistically analyzed using IRRISTAT version 92 developed by the International Rice Research Institute Biometrics unit, the Philippines (Gomez et al., 1984). Prior to statistical analysis of variance (ANOVA) the percentage values of the disease index were arcsine transformed and the means were compared by Duncan's multiple range test (DMRT).

3. Results and Discussion

The application of talc-based formulation of fluorescent pseudomonad, *T.viride*, *B. subtilis* strain mixtures amended significantly reduced the incidence of peduncle blight disease under glasshouse conditions. Lowest incidence was observed in plants treated with Tv_1+Pf_1+Bs_{10} (85.58) comparable with the chemical treatment (95.55%) whereas in untreated control recorded the maximum damage of 31.5%. Further, the growth and yield attributing characters viz., germination percent, root and spike length and number of spikelet's per plant, No of flowers were significantly higher in the Tv_1+Pf_1+Bs_{10} treated plants challenged with peduncle blight pathogen compared to control plants (Table 1).

Table 1: Effect of consortial bioformulation of PGPR on growth and disease incidence of tuberose

Sl. No.	Treatments	Disease incidence (%)	Reduction over control (%)	Flower spike no. $plant^{-1}$
1.	Tv_1 (BT+FS)	42.0	54.44	13.25
2.	Pf_1 (BT+FS)	40.5	52.75	10.56
3.	Bs_{10} (BT+FS)	38.0	57.25	11.56
4.	Tv_1+Pf_1 (BT+FS)	37.4	57.92	11.98
5.	Tv_1+Bs_{10} (BT+FS)	35.1	60.51	12.87
6.	Pf 1+Bs_{10} (BT+FS)	31.6	64.45	13.24
7.	Tv_1+Pf_1+Bs_{10} (BT+FS)	30.5	65.68	14.70
8.	Carbendazim (0.1%)-FS	4.0	95.50	13.00
9.	Tebuconazole (0.1 %)-FS	8.0	91.00	13.85
10.	Healthy control	0.0	-	-
11.	Infected control	88.89	-	8.54
CD (p=0.05)		2.34		

Biological control is an attractive and alternative approach to control plant diseases. Management plant diseases by different PGPR strains either as suspension or through different formulations has been reported by many workers (Saravanakumar et al., 2007; Karthiba et al., 2010). However, ISR by PGPR against various diseases was considered as the most desirable pproach in crop protection (Ramamoorthy et al., 2001). Recently, Manikandan et al. (2014) demonstrated the PGPR mediated induction of defense responses in

tomato plants against *Fusarium* wilt disease. In our study, we concentrated on biotic inducers (plant growth promoting fluorescent pseudomonads) for inducing the defense molecules (defense enzymes and PR-proteins) in tuberose plants against the peduncle blight pathogen

3.1. Phenylalanine ammonia lyase PAL

Activity of PAL was induced in tuberose treated with biocontrol agents upon challenge inoculation with *L. theobromae* (Table 2). In general, the PAL activity reached maximum at six DAI with the pathogen and declined thereafter. The enzyme activity was significantly higher in plants treated with Tv_1 and Bs_{10} when compared to other biocontrol agents without challenge inoculation. Plants treated with biocontrol agents alone also showed enhanced PAL activity as against healthy control. PAL plays an important role in the biosynthesis of various defense chemicals in phenyl propanoid metabolism (Dayyf et al., 1997). PAL activity could be induced during plant-pathogen or plant insect interactions (Ramamoorthy et al., 2002a; Harish et al., 2009). In the present study, increased activity of PAL was recorded in plants treated with Tv_1 and Bs_{10} mixture inoculated with *L. theobromae*. Induction of PAL by

Table 2: Induction of phenylalanine ammonia lyase in tuberose treated with biocontrol agents

Sl. No.	Treatments	Change in absorbance min^{-1} g^{-1} of fresh tissue				
		0 DAI	3 DAI	6 DAI	9 DAI	12 DAI
1.	TV_1	156^{cd}	158^{gh}	163^{gh}	152^{f}	124^{f}
2.	TV_1+pathogen	158^{cd}	218^{cd}	196^{de}	190^{c}	194^{c}
3.	Pf_1	151^{d}	153^{h}	157^{h}	140^{g}	124^{f}
4.	Pf_1+pathogen	152^{d}	184^{e}	200^{d}	170^{d}	113^{g}
5.	Bs_{10}	163^{bc}	165^{fg}	171^{fg}	156^{f}	145^{e}
6.	Bs_{10}+pathogen	162^{bc}	225^{bc}	232^{b}	200^{b}	188^{c}
7.	Tv_1+Pf_1	153^{d}	155^{h}	160^{h}	151^{f}	123^{f}
8.	Tv_1+Pf_1+ pathogen	155^{cd}	215^{d}	196^{de}	159^{f}	193^{c}
9.	Tv_1+Bs_{10}	181^{a}	183^{e}	188^{e}	197^{bc}	179^{d}
10.	Tv_1+Bs_{10}+ pathogen	183^{a}	243^{a}	243^{a}	237^{a}	229^{a}
11.	Pf_1+BS 10	168^{b}	171^{f}	177^{f}	161^{d}	138^{e}
12.	Pf_1+BS_{10}+ pathogen	170^{b}	233^{b}	210^{c}	199^{bc}	208^{b}
13.	Healthy control	80.1^{e}	82.8^{i}	84.9^{j}	83^{i}	81^{i}
14.	Control (Pathogen alone)	83.7^{e}	169^{f}	157^{h}	130^{h}	95^{h}

Means in columns followed by the same letter are not significantly different (p<0.05) according to DMRT

fluorescent pseudomonads was reported in cucumber against Pythium aphanidermatum (Chen et al., 2000) and tomato against *Fusarium oxysporum* f.sp. *lycopersici* (Ramamoorthy et al., 2002a).

3.2. Peroxidase

Enhanced peroxidase activity was noticed in plants due to treatment with bioagents (Table 3). However, additional increase in PO activity was observed in plants inoculated with the pathogen. The activity reached its peak at sixth day in treated plants compared to healthy ones. In pathogen inoculated plants also the activity of PO attained its peak at 6 DAI and declined steeply at 12 DAI. Native PAGE analysis showed the presence of the two isoforms (PO_1 and PO_2) of peroxidase in plants treated with Pf_1+Bs_{10} upon challenge inoculation with the pathogen (Plate 1). Peroxidases have been found to play a major role in the regulation of plant cell elongation, phenol oxidation, polysaccharide cross-linking, IAA oxidation, and wound healing (Vidhyasekaran et al., 1997). Saravanakumar et al., 2006) reported that rice plants treated with fluorescent pseudomonad strain mixtures and challenged with leaffolder larvae or sheath rot pathogen resulted in increased accumulation of peroxidase isoforms. In the present study, plants treated with the combination of Pf_1 and Bs_{10} followed by challenge inoculation with the pathogen showed enhanced induction of peroxidase than individual bioformulation treatments and pathogen inoculated controls. Moreover, two isoforms *viz.,* PO_1 and PO_2 were also observed in such plants. Peroxidases have been implicated in phenol oxidation, IAA oxidation, polysaccharide cross linking, cross linking of extension monomers, oxidation of hydroxyl-cinnamyl alcohols into free radical intermediates and wound healing (Vidhyasekaran et al., 1997a).

3.4. Polyphenol oxidase (PPO)

The trend of increasing PPO activity was similar to that of PO in all the treatments (Table 4). The PPO activity reached its maximum in six days after spraying with Pf_1+Bs_{10} followed by challenge inoculation with *L. theobromae*. Native PAGE analysis revealed the presence of two isoforms (PPO_1 and PPO_2) upon challenge inoculation with biocontrol agents except Bs_{10} which showed PPO_2 only (Plate 2). Tuberose plants pretreated with biocontrol agents exhibited additional increase in PPO activity upon challenge inoculation with

B. theobromae and two isoforms viz., PPO_1 and PPO_2 were noticed. Similar reports of enhanced PPO activity in response to treatment of biocontrol agents and other pathogens have been reported in several crops (Saravankumar, 2002; Thilagavathy, 2005). Involvement of PPO in ISR mediated by PGPR in cucumber has been demonstrated by Chen et al. (2000). Application of bioformulation mixture induced several isoforms of PPO in cabbage and cauliflower against *P. brassicae* and *R. solani* (Loganathan, 2002).

3.5. Phenols

Prior application of effective biocontrol agents had a profound

Table 3: Induction of peroxidase in tuberose treated with biocontrol agents

Sl. No.	Treatments	Change in absorbance min^{-1} g^{-1} of fresh tissue				
		0 DAI	3 DAI	6 DAI	9 DAI	12 DAI
1.	TV_1	0.118^{fg}	0.129^{f}	0.186^{ef}	0.154^{ef}	0.131^{g}
2.	TV_1+pathogen	0.123^{f}	0.136^{f}	0.192^{e}	0.164^{e}	0.128^{g}
3.	Pf_1	0.259^{d}	0.264^{de}	0.292^{d}	0.276^{cd}	0.251^{f}
4.	Pf_1+pathogen	0.261^{d}	0.285^{d}	0.310^{d}	0.294^{c}	0.276^{e}
5.	Bs_{10}	0.573^{a}	0.581^{a}	0.592^{c}	0.565^{a}	0.570^{a}
6.	Bs_{10}+pathogen	0.578^{a}	0.584^{a}	0.645^{a}	0.577^{a}	0.534^{bc}
7.	Tv_1+Pf_1	0.213^{e}	0.242^{e}	0.298^{d}	0.267^{d}	0.252^{df}
8.	Tv_1+Pf_1+pathogen	0.224^{e}	0.248^{e}	0.305^{d}	0.274^{cd}	0.271^{ef}
9.	Tv_1+Bs_{10}	0.446^{c}	0.525^{c}	0.594^{c}	0.497^{b}	0.485^{b}
10.	Tv_1+Bs_{10}+pathogen	0.512^{b}	0.548^{b}	0.628^{ab}	0.509^{b}	0.496^{d}
11.	Pf_1+BS 10	0.530^{b}	0.578^{a}	0.592^{c}	0.563^{a}	0.554^{ab}
12.	Pf_1+BS_{10}+pathogen	0.558^{a}	0.582^{a}	0.612^{bc}	0.565^{a}	0.524^{c}
13.	Healthy control	0.095^{h}	0.102^{g}	0.113^{g}	0.103^{g}	0.091^{h}
14.	Control (Pathogen alone)	$.097^{gh}$	0.116^{fg}	0.164^{f}	0137^{f}	0.090^{h}

Means in columns followed by the same letter are not significantly different (p<0.05) according to DMRT

Table 4: Induction of polyphenoloxidase in tuberose treated with biocontrol agents

Sl. No.	Treatments	Change in absorbance min^{-1} g^{-1} of fresh tissue				
		0 DAI	3 DAI	6 DAI	9 DAI	12 DAI
1.	TV_1	0.226^{ef}	0.230^{gh}	0.242^{f}	0.179^{k}	0.129^{h}
2.	TV_1+pathogen	0.241^{cd}	0.418^{b}	0.432^{c}	0.323^{d}	0.288^{c}
3.	Pf_1	0.219^{g}	0.223^{h}	$0.235\underline{f}$	0.175^{k}	0.138^{h}
4.	Pf_1+pathogen	0.222^{fg}	0.307^{d}	0.361^{d}	0.256^{f}	0.207^{ef}
5.	Bs_{10}	0.247^{bc}	0.245^{f}	0.251^{f}	0.202^{ij}	0.176^{g}
6.	Bs_{10}+pathogen	0.250^{bc}	0.379^{c}	0.463^{b}	0.432^{b}	0.312^{b}
7.	Tv_1+Pf_1	0.245^{bc}	0.242^{gh}	0.247^{f}	0.235^{gh}	0.199^{f}
8.	Tv_1+Pf_1+pathogen	0.258^{ab}	0.429^{b}	0.446^{bc}	0.365^{c}	0.303^{b}
9.	Tv_1+Bs_{10}	0.235^{de}	0.243^{fg}	0.248^{f}	0.228^{hi}	0.198^{f}
10.	Tv_1+Bs_{10}+pathogen	0.239^{cd}	0.312^{d}	0.345^{d}	0.282^{e}	0.236^{d}
11.	Pf_1+BS 10	0.240^{cd}	0.274^{e}	0.283^{e}	0.260^{f}	0.227^{d}
12.	Pf_1+BS_{10}+pathogen	0.267^{a}	0.453^{a}	0.582^{a}	0.470^{a}	0.383^{a}
13.	Healthy control	0.221^{g}	0.226^{h}	0.238^{f}	0.214^{ij}	0.212^{e}
14.	Control (Pathogen alone)	0.223^{fg}	0.321^{d}	0.297^{e}	0.246^{fg}	0.204^{ef}

Means in columns followed by the same letter are not significantly different (p<0.05) according to DMRT

effect on the accumulation of phenol in tuberose plants upon challenge inoculation with the pathogen. The accumulation of phenol from the third day increased and attained a peak on six DAI and thereafter slowly declined. Treatment with consortial formulation of Tv_1+Bs_{10} challenged with the pathogen recorded maximum total phenol content on 6 DAI (428 µg g^{-1} tissue) followed by Pf_1+Bs_{10} inoculated with the fungus (410 µg g^{-1} tissue) (Table 5). Phenolic compounds enhance the mechanical strength of host cell wall and also inhibit the invading pathogenic organisms. Accumulation of phenolics by prior application of *P. fluorescens* in pea has been reported against *Pythium ultimum* and *F. oxysporum* f.

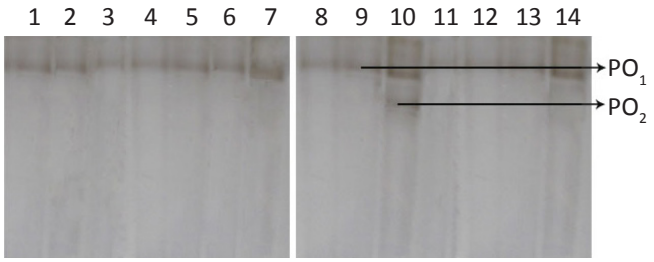
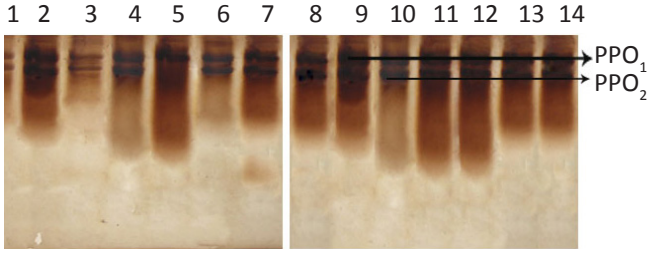


Plate 1: Induction of isoforms of peroxidase in tuberose plants treated with biocontrol agents



1.	TV_1	8.	Tv_1+Pf_1+pathogen
2.	TV_1+pathogen	9.	Tv_1+Bs_{10}
3.	Pf_1	10.	Tv_1+Bs_{10}+pathogen
4.	Pf_1+pathogen	11.	Pf_1+BS 10
5.	Bs_{10}	12.	Pf_1+BS_{10}+pathogen
6.	Bs_{10}+pathogen	13.	Healthy control
7.	Tv_1+Pf_1	14.	Control (Pathogen alone)

Plate 2: Induction of isoforms of polyphenol oxidase in tuberose plants treated with biocontrol agents

sp. *pisi* (Benhamou et al., 1996b). Induction of enzymes such as PAL and PO leading to the accumulation of phenolics and lignin can occur in response to insect and pathogen attack (Ramamoorthy et al., 2001). In the present study, higher level of accumulation of phenolics occurred in treatment with consortial formulation of *Trichoderma viridae* (Tv_1)+*Bacillus subtilis* (Bs_{10}) challenged with the pathogen recorded maximum total phenol content on 6 DAI (428 µg g^{-1} tissue).

In the present study combination of three antagonists *viz.,* Tv_1, Pf_1 with Bs_{10} as bulb treatment and followed by foliar spray was found to be reduce peduncle blight of tuberose up to 85.68%, which was on par with the combination of two antagonists Pf_1 and Bs_{10} with 84. 45% disease reduction. Combined application of *B. subtilis* and *P.fluorescens* has been found to be control the post harvest fungal decay of citrus fruits caused by *B. theobromae* (Johnson et al., 2008) and stem end rot of Citrus caused by *B. theobromae* (Sharma et al., 2009).

In conclusion, from the above evidences, it is well known that defense enzymes are induced in tuberose plant by the application of bioformulation mixture containing fluorescent pseudomonad mixtures and *B. subtilis* (Bs_{10}) against L. *theobromae*. This offers a promising tool for enhancing pest and disease management in tuberose plants. The timing and expression patterns of the defense mechanisms are important for the reduced attack and suppression of pathogen respectively. Higher level expression of defense related proteins and timely accumulation of chemicals at the infection and feeding site certainly prevents the entry of pathogen and reduces the attack by insect in groundnut plants treated with mixture of plant growth promoting fungal and bacterial strains.

Table 5: Induction of phenols in tuberose treated with biocontrol agents

Sl. No.	Treatments	Change in absorbance min^{-1} g^{-1} of fresh tissue				
		0 DAI	3 DAI	6 DAI	9 DAI	12 DAI
1.	TV_1	188^{e}	200^{i}	217^{gh}	191^{ef}	174^{ef}
2.	TV_1+pathogen	200^{d}	360^{c}	389^{c}	378^{b}	318^{b}
3.	Pf_1	221^{b}	223^{gh}	230^{gh}	183^{gf}	143^{i}
4.	Pf_1+pathogen	228^{ab}	286^{d}	317^{d}	216^{d}	208^{d}
5.	Bs_{10}	177^{e}	181^{j}	183^{i}	167^{i}	159^{gh}
6.	Bs_{10}+pathogen	183^{e}	227^{gh}	238^{f}	310^{c}	198^{d}
7.	Tv_1+Pf_1	207^{cd}	213^{hi}	219^{gh}	182^{fg}	151^{gh}
8.	Tv_1+Pf_1+pathogen	212^{c}	236^{fg}	257^{fg}	198^{e}	177^{de}
9.	Tv_1+Bs_{10}	231^{ab}	246^{ef}	280^{e}	181^{fg}	161^{fg}
10.	Tv_1+Bs_{10}+pathogen	240^{a}	398^{a}	428^{a}	396^{a}	300^{c}
11.	Pf_1+BS 10	205^{cd}	203^{i}	207^{h}	174^{gh}	152^{gh}
12.	Pf_1+BS_{10}+pathogen	201^{cd}	377^{b}	410^{b}	368^{b}	337^{a}
13.	Healthy control	149^{f}	148^{k}	155^{j}	125^{j}	130^{j}
14.	Control (Pathogen alone)	157^{f}	178^{j}	214^{h}	169^{hi}	151^{hi}

Means in columns followed by the same letter are not significantly different (p<0.05) according to DMRT

4. Conclusion

Consortial bioformulated microbe inoculated bulblets showed sustained, timely induction and accumulation of these defense enzymes and PR-proteins which enhances the resistance in tuberose against peduncle blight disease incited by *Lasiodiplodia theobromae* and also improved the vegetative growth, physiological attributes, PR-proteins and phenol contents.

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