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Development of Bioformulations for the Management of Blackgram Dry Root Rot Caused by *Rhizoctonia bataticola* (Taub Butler)

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Authors' contributions

This work was carried out by the combined effort of all the authors. Author PL designed the study wrote the protocol, carried out the statically analysis and wrote the first draft of manuscript. Authors MK and ER managed the analyses of the study. Author ER managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

An attempt was made to control dry root rot using consortia of bioinoculants. A total of 10 fungal (*Trichoderma)* and 30 bacterial (*Pseudomonas and Bacillus*) isolates were collected and screened for their antagonistic activity against mycelial growth of *Rhizoctonia bataticola* under *in vitro* condition. Among these, *Trichoderma* (TL1), *Pseudomonas fluorescens* (PfUL(A)) and *Bacillus subtilis* (BsOP2) isolates exhibited maximum inhibition. As results of the compatibility of the biocontrol agents revealed that *P. fluorescens* strains were compatible with *B. subtilis* and *Trichoderma* but *B. subtilis* strains were not compatible with *Trichoderma* strains. The biocontrol consortia consisting of *P. fluorescens* (PfUL(A)) and *B. subtilis* (BsOP2) + Farm Yard Manure (FYM) + Neem cake was found to be promising in reducing dry root rot incidence under field conditions. The biocontrol consortia also induced high level of defense - related enzymes *videlicet* phenylalanine ammonia lyase, catalase, peroxidase and polyphenol oxidase activity.

Keywords: Black gram; dry root rot; Bacillus; Pseudomonas; biocontrol consortia.

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1. INTRODUCTION

The productivity of blackgram or urdbean (*Vigna mungo* (L.) Hepper) was reduced due to various diseases with an estimated yield loss of 20 to 30 percent. Fungicides are widely used as seed or soil treatment to combat various root diseases. However, use of fungicides causes environmental hazards and development of resistance in pathogen. In recent years, more emphasis has been given to the use of bioagents and organic amendments. Several antagonistic organisms have been successfully used as biocontrol agents for controlling soil borne pathogens [1,2,3]. At present most of the biocontrol agents are applied singly to combat the growth of the pathogens. Although the potential benefits of a single biocontrol agent application has been demonstrated in many studies, it may also partially account for the inconsistent performance because a single biocontrol agent is not likely to be active in all kinds of soil environment and all agricultural ecosystems [4]. One of the strategies for overcoming such inconsistent performance is to combine two or more beneficial microbes in a biocontrol formulations. Combinations of biocontrol agents have the potential for more extensive colonization of the rhizosphere, more consistent expression of beneficial traits under a wider range of soil conditions and antagonistic to a larger number of plant pathogens than biocontrol strains applied individually. Thus, more emphasis was laid on the combined use of two or more strains of biocontrol agents, which turned out to be more successful than either of them alone, as reported by several workers [5,6,7,8]. Therefore, the present study was undertaken to evaluate the efficacy of biocontrol consortia consisting organic amendments *viz*., neem cake and FYM against root rot disease of blackgram.

2. MATERIALS AND METHODS

2.1 Isolation of Pathogen and Biocontrol Agents

The dry root rot pathogen *R. bataticola* was isolated from infected black gram plants using potato dextrose agar (PDA) medium. The biocontrol agents *Trichoderma*, *Pseudomonas and Bacillus* were isolated from rhizosphere soils of black gram using *Trichoderma* selective medium (TSM) [9], King's B medium (KB) [10] and Nutrient Agar (NA) medium [11], respectively. The individual colonies of *Trichoderma* were identified based on the morphological characters [12]. Similarly, the bacterial isolates were characterized based on standard biochemical tests [13]. Antagonism of *T. viride* against *R. bataticola* was assayed with the dual-culture [14].

2.2 Compatibility of Biocontrol Agents

The bacterial strains were tested for their compatibility with each other following the method [15]. The compatibility of the fungal biocontrol agent with the bacterial strains was tested by their mycelial overgrowth on the bacterial strains without any inhibition zone, using the dual culture technique [14].

2.3 Seed Treatment

Blackgram seeds (ADT5) were surface sterilized with 2% sodium hypochlorite for 30 sec. then rinsed in sterile distilled water and dried overnight. One gram of seeds was soaked in 10 ml of bacterial suspension (containing 3 x 10^8 cfu/ml) for 2 h and dried overnight in a sterile Petri plate. *Trichoderma* isolates multiplied in *Trichoderma* special broth were harvested for mycelial mats along with spores, then the contents were mixed with sterile distilled water and (20 x 10^8 cfu/ml) was checked through dilution plate technique and subjected to seed treatment as above.

2.4 Assessment of Plant Growth Promotion

Plant growth-promoting activity of the best isolates of *Pseudomonas* sp. (PfUL(A), PfAL1 and PfCBE9), *Bacillus* sp. (BSOP2, BCBE1 and BKK3) and *Trichoderma* sp. (TL1, TCBE3 and TOKK1) were assessed based on the seedling vigour index by the standard roll towel method [16].

2.5 Preparation of Biocontrol Consortia

Talc based formulation of *Pseudomonas, Bacillus* and *Trichoderma* were prepared as the methods discussed by [17,18]. The biocontrol consortia of fungal and bacteria was prepared by mixing equal quantity of talc based formulation both the biocontrol agents w/w.

2.6 Effect of Talc-based Bioformulations with Organic Amendments

Potting medium (red soils and: cowdung at 1:1:1, w/w/w) was autoclaved for 1 hour for two consecutive days and filled in pots and

incorporated with sand: maize inoculum (50 g/pot) of *R. bataticola*. Ten gram of talc based bioformulation was mixed with ten gram of FYM and neem cake then applied per pot as soil application (capacity of pot 10% of potting medium filled). Seeds of black gram cv. ADT-5 were surface sterilized with 2% sodium hypochlorite, seed treated with sown at 20 seeds per pot. Carbendazim at the rate of 2 g kg^{-1} of seed was applied as a chemical check. Ten seedlings were maintained per pot up to 20 days. Further, five seedlings were maintained until harvest. The pathogen inoculated and uninoculated served as control. Soil drenching of 0.1 per cent carbendazim was included as chemical check. Three replications (three pots per replication) were maintained and the pots were arranged in a randomized manner. The treatments of experiment were, T1 – *Pseudomonas* sp (PfUl(A)) + Neem cake + Farm Yard Manure, T2 – *Bacilllus* sp (BsOP2) + Neem Cake + FYM, T3– *Trichoderma* (TL1) + Neem cake + FYM, T4 – PfUl(A)+TL1+ Neem cake + FYM, T5 – BsOP2+ TL1+Neem cake + FYM, T6 –PfUl(A)+BsOP2+ + TL1+Neem cake + FYM, T7 – Carbendazim (0.1%), T8 –Inoculated control and T9–Healthy control. The incidence of root rot was recorded and expressed as percentage of disease incidence.

2.7 Assay of Defense Enzymes

Samples of blackgram were collected from individual treatments at 2 days interval starting from zero, 1, 3, 5, 7 and 9th days after challenge inoculated with the pathogen to study the induction of defence related enzymes in response to treatment and inoculation of pathogen in black gram plants under glass house conditions. One g of root sample was homogenized with 2 ml of 0.1 M sodium phosphate buffer (pH 7.0) at 4℃. The homogenate was centrifuged for 20 min at 10,000 rpm and the supernatant was used to determine Phenylalanine ammonia lyase (PAL), Catalase (CL), Peroxidase (PO) and Polyphenol oxidase (PPO). Peroxidase activity was assayed as described by [19] and was expressed as changes in absorbance at 470 nm min^{-1} g^{-1} of fresh tissue. PPO activity was determined following the procedure given by [20] and was expressed as changes in absorbance at 470 nm min^{-1} g^{-1} of fresh tissue. PAL activity was assayed following the method of [21] and was expressed as nmoles of cinnamic acid min^{-1} g^{-1} of fresh tissue. Catalase activity was determined following the procedure given by [22] and was expressed as changes in absorbance min^{-1} $g^{-1}$of fresh tissue.

2.8 Field Studies

Two field experiments were conducted to evaluate the efficacy of bioformulation on dry root rot disease incidence with organic amendments. The experiments were laid out in a randomized block design (RBD) with three replications. The treatments consisted of,T1 – *Pseudomonas* sp (PfUl(A)) (10 g ST + 2.5 kg SA) + Neem cake (150 kg/ha)+ Farm Yard Manure (2 ton/ha), T2 – *Bacillus* sp (BsOP2) (10 g ST + 2.5 kg SA) + Neem cake + FYM, T3 – *Trichoderma* (TL1) (4 g ST + 2.5 kg SA) + Neem cake + FYM, T4 – PfUl(A) (5 g ST + 1.25 kg SA) +TL1 (5 g ST + 1.25 kg SA) + Neem cake + FYM, T5 – BsOP2 (5 g ST + 1.25 kg SA) + TL1 (5 g ST + 1.25 kg SA) + Neem cake + FYM, T6 – PfUl(A) (5 g ST + 0.85 kg SA) + BsOP2 (5 g ST + 0.85 kg SA) + TL1 (2 g ST + 0.85 kg SA) + Neem cake + FYM, T7 – Carbendazim (2 g ST + 0.1% Soil drenching), T8 – Control. All the treatments were given as seed treatment and soil application. Seeds were soaked in double the volume of sterile distilled water containing the talc-based formulation (10 g kg^{-1} of seed) [23]. In the field, the biocontrol consortia applied at 2.5 kg/ha along with 2 tons of FYM and 150 kg neem cake. Seed treatment at 2 g/kg of seed and soil drenching at 0.1 percent carbendazim was used as the chemical check for comparison. The observations were recorded on dry root rot incidence, plant height, number of pods per plant and number of seeds per plant.

The data were statistically analyzed using the IRRISTAT version 92 developed by the International Rice Research Institute Biometrics unit, the Philippines [24].

3. RESULTS AND DISCUSSION

3.1 *In vitro* Screening of Biocontrol Agents

A total of 10 *Trichoderma,* 20 *Pseudomonas* sp and 10 *Bacillus* sp isolates were screened for their antagonistic activities against mycelial growth of *R. bataticola*. All the ten *Trichoderma* isolates are inhibited the mycelial growth of *R. bataticola*. Among them, TL1 recorded the least mycelial growth (4 cm) with 55.6% inhibition over control. This was followed by TCBE3 and TOKK1 isolates with 4.4 cm and 4.6 cm mycelial

growth and 51.10 and 48.9% inhibition over control, respectively (Table 1). Out of twenty *P. fluorescens* isolates tested, PfUL(A) recorded maximum inhibition of 4.7 cm mycelial growth (41.1%). This was followed by isolates *viz*., PfAL1 and PfCBE9 isolates recorded 41.10% reduction over control (Table 2). In the case of *Bacillus* isolate (BSOP2) recorded maximum inhibition of 5 cm mycelia growth (44.4%). This was followed by isolates *viz*., BCBE1 andBKK3 which recorded 33.33% and 31.10% reduction, respectively (Table 3). Our results are in conformity with the findings of many workers [25,26].

Table 1. Efficacy of different *Trichoderma* isolates against the growth of *M. phaseolina in vitro*

***Trichoderma* isolates**	**Growth of *M. phaseolina***	**Per cent inhibition over control**
TCBE3	4.4[b]	51.1[b] (45.6)
TTV1	8.2[f]	8.9[e] (16.9)
TMDU1	5.5[c]	38.9[bc] (38.6)
TSOP4	5.8[cd]	35.6[c] (36.6)
TVL1	4.0[a]	55.6[a] (48.2)
TVL2	6.8[e]	24.4[d] (29.6)
TOR1	6.2[d]	31.3[cd] (33.9)
TKM2	5.4[c]	40.0[bc] (39.2)
TOKK1	4.6[b]	48.9[ab] (44.4)
TTNL1	6.3[de]	30.0[cd] (33.2)
Control	9.0[g]	0.00

Values are mean of three replications. In a column, means followed by a common letter(s) are not significantly different (P = 0.05) by DMRT

Table 2. Efficacy of different *Pseudomonas* isolates against the growth of *M. phaseolina in vitro*

***Pseudomonas* isolates**	**Growth of *M. phaseolina***	**Per cent inhibition over control**
PfCBE3	8.5[gh]	5.6[h] (12.4)
PfPPN3	8.1[fg]	10.0[fgh] (18.1)
PfPPN4	6.8[d]	24.4[cde] (29.5)
PfUL(A)	4.7[a]	44.4[a] (41.8)
PfKK1	7.7[ef]	14.4[efg] (22.1)
PfKK2	5.6[bc]	37.8[\]7b] (37.9)
PfAL1	5.3[b]	41.1[b] (39.9)
PfOKK2	7.4[e]	17.8[def] (24.8)
PfCBE2	8.5[gh]	5.6[h] (12.4)
PfCBE9	5.3[b]	41.1[b] (39.9)
PfV1	6.6[d]	26.7[bcd] (31.1)
PfKB1	8.6[gh]	4.8[h] (11.4)
PfKB3	8.3[g]	7.8g[h] (15.6)
PfVT1	7.4[e]	17.8[def] (24.8)
PfSOP2	6.8[d]	24.4[cde] (29.5)
PfTVL1	6.0[c]	33.3[bc] (35.2)
PfTVL4	5.6[bc]	37.8[b] (37.9)
PfMDU1	8.1[fg]	10.0[fgh] (18.1)
PfMDU2	7.5[e]	16.7[def] (29.0)
PfPPOZ1	8.2[fg]	8.9[fgh] (16.9)
Control	9.0[h]	0.00

Values are mean of three replications. In a column, means followed by a common letter(s) are not significantly different (P = 0.05) by DMRT

Table 3. Efficacy of different *Bacillus* isolates against the growth of *M. phaseolina in vitro*

Bacillus isolates	Growth of *M. phaseolina*	Per cent inhibition over control
BCBE1	6.0[b]	33.33[ab] (35.2)
BCBE2	8.2[de]	8.9[d] (16.9)
BPPN5	7.3[c]	18.9[c] (25.6)
BKK3	6.2[b]	31.1[ab] (33.9)
BOKK3	8.3[e]	7.8[d] (15.6)
BV2	7.4[c]	24.4[bc] (29.6)
BKB3	8.2[de]	8.9[d] (16.9)
BSOP2	5.0[a]	36.7[a] (37.2)
BV3	7.6[c]	15.6[cd] (23.1)
BMDU2	7.7[cd]	14.4[cd] (22.1)
Control	9.0[f]	0.00

Values are mean of three replications. In a column, means followed by a common letter(s) are not significantly different (P = 0.05) by DMRT

3.2 Plant Growth Promotion

The biocontrol strains of *Pseudomonas* (PfUL(A)), *Bacillus* (BsOP2) and *Trichoderma* (TL1) produced black gram seedlings with a significantly higher vigour index, 3943.3, 3825.6 and 3706.1, respectively, than the control. Interestingly, *Pseudomonas* (PfUL(A)), *Bacillus* (BsOP2) and *Trichoderma* (TL1) also produced higher germination percentage 98.6%, 98.6%, 98.6% and seedling length 41.2cm, 39.5cm, 40 cm, respectively. The untreated control seedlings had the lowest vigour index (2409.4) (Table 4). [6] evaluated the efficacy of 13 plant growth promoting rhizobacterial strains against chilli fruit rot and dieback incited by *Colletotrichum capsici*. The results reveal in this study corroborate earlier studies and indicate a future possibility that plant growth promoting rhizobacteria bioformulations can be used to promote growth and health of economic crops [2,27].

3.3 Compatibility of Biocontrol Agents

Strains of *Pseudomonas* (PfUL(A)), *Bacillus* (BsOP2) and *Trichoderma* (TL1) were tested *in vitro* for compatibility. Strains that overgrew each other were compatible with each other, whereas strains that were separated by an inhibition zone were incompatible. No inhibition zone formed between PfUL(A) + TL1, PfUL(A) + BsOP2 indicating that these strains were compatible. Inhibition in growth was found between BsOP2 + TL1 indicating that these strains were incompatible. Several authors have suggested that combinations of introduced biocontrol agents have to be compatible with each other for better and more consistent disease suppression [28]. Several authors have suggested that combinations of introduced biocontrol agents have to be compatible with each other for better and more consistent disease suppression [28]. In the current study, the isolates of *T. viride* (TVL1), *P. fluorescens* (PfUL(A)) and *B. subtilis* (BSOP2), organic amendments, such as neem cake and FYM showed greater antagonistic activity against *M. phaseolina in vitro*. The results are consistent with the findings of several research workers who demonstrated the use of antagonistic microorganisms (*T. viride*, *P. fluorescens* and *B. subtilis*), organic amendments against various soil borne fungal pathogens [7,29].

3.4 Glasshouse Study

Those treatments that had been most effective in inhibiting the mycelial growth of *M. phaseolina* were selected for pot culture studies. Of these treatments, a combination of fungal and bacterial strains reduced the incidence of root rot more strongly than did the individual strains. The result from the pot culture experiment revealed that among different treatments received individual and combinations of biocontrol agents along with FYM+Neem cake, the treatment combination of PfUL(A)+ BSOP2 performed better in 257 reducing root rot incidence of blackgram. The recorded disease incidence was 20 per cent, this was followed by 26.7% incidence was observed in the combination of PfUL(A)+TVL1. The untreated check was recorded 66.75 per cent incidence. The highest germination was also recorded in seeds treated with the mixture of PfUL(A)+ BSOP2 + neem cake + FYM (95%). The treatment differed significantly from all other treatments, as well as from the untreated control which was only 71.7 per cent (Table 5). Several authors have suggested that combinations of

Table 4. Growth promotion activities of biocontrol agents on blackgram seedlings

Biocontrol agents	Seedling length	Germination %	Vigour index
TL1	40.0^{ab}	98.6^{a} (83.28)	39440^{b}
TCBE3	38.6^{bcd}	98.6^{a} (83.28)	38260^{d}
TOKK1	38.6^{bcd}	94.6^{d} (76.57)	36710^{g}
PfUL(A)	41.2^{a}	98.6^{a} (83.28)	40820^{a}
PfAL1	38.1^{cde}	97.3^{b} (80.57)	37270^{f}
PfCBE9	36.8^{ef}	96.0^{c} (78.48)	35520^{i}
BSOP2	39.5^{bc}	98.6^{a} (83.28)	39140^{c}
BCBE1	38.2^{cde}	97.3^{b} (80.57)	37360^{e}
BKK3	36.2^{f}	97.3^{b} (83.28)	35420^{j}
Carbendazim (2 g/kg)	37.5^{def}	97.3^{b} (80.57)	36680^{h}
Control	34.6^{g}	93.3^{d} (75.0)	32420^{k}

Values are mean of three replications. In a column, means followed by a common letter(s) are not significantly different (P = 0.05) by DMRT. Values in parentheses are arcsine transformed.

Table 5. Effect of biocontrol consortia and organic amendments on germination and dry root rot incidence in blackgram

Treatments	Germination %	% increase over control	Per cent disease incidence	% decrease over control
PfUL(A)	83.3^{d} (65.9)	16.3	33.3^{cd} (35.2)	50
BSOP2	86.7^{c} (68.6)	20.9	30.0^{bc} (33.2)	55
TL1	81.7^{e} (64.7)	14.0	36.7^{d} (37.3)	45
PfUL(A)+ BSOP2	95.0^{a} (77.1)	32.6	20.0^{a} (26.5)	70
PfUL(A) + TL 1	91.7^{b} (73.3)	28.0	26.7^{b} (31.0)	60
PfUL(A) + BSOP2 + TL1	83.3^{d} (65.9)	16.3	43.3^{e} (41.1)	35
Carbendazim (0.1%)	81.7^{e} (64.7)	14.0	20.0^{a} (26.5)	70
Control	71.7^{f} (57.9)	-	66.7^{g} (54.8)	-

Values are mean of three replications. In a column, means followed by a common letter(s) are not significantly different (P = 0.05) by DMRT. Values in parentheses are arcsine transformed.

introduced biocontrol agents have to be compatible with each other for better and more consistent disease suppression [28]. Similarly, the incorporation of biocontrol agents with organic amendments and the efficacy of neem and FYM in fungal disease management have been reported by many workers [30,7]. An important prerequisite for the effectiveness of strains appears to be the compatibility of the co312 inoculated microorganisms [28]. In the present study, the isolates of *T. viride* (TVL1), *P. fluorescens* (PfUL(A)) and *B. subtilis* (BSOP2) were compatible with each other and with neem cake.

3.5 Induction of Defense Related Enzymes

The peroxide activity increased significantly up to seven days in all the treatments (treated with biocontrol agents, organic amendments and inoculated with the pathogen) and thereafter it declined. Among the various bioformulations, application of treatment of PfUl(A)+BsOP2+ Neem cake + FYM followed by challenge inoculation and showed higher induction of peroxidase (1.678 changes in absorbance min^{-1} g^{-1} of fresh tissue). The induction reached a maximum level on 7 days after challenge inoculation. The activity of the enzyme thereafter declined with a decreasing rate than the inoculated control. Plants treated with bioformulation PfUl(A)+BsOP2+ Neem cake + FYM also recorded a higher level of PO activity throughout study period than the other treatments. The inoculated control showed reduction of PO activity starting from 7th day and then decreased to lower level than uninoculated control (Fig. 1). The same trend was observed in the PPO, PAL and Catalase (Figs. 2, 3 and 4). These three enzyme activity was increased significantly up to seventh day in all the treatments and thereafter declined. Combined application of PfUl(A)+BsOP2+ Neem cake + FYM and challenge inoculated with the pathogen recorded higher PPO (1.38 changes in absorbance min^{-1} g^{-1} of fresh tissue), PAL (1.235 changes in absorbance min^{-1} g^{-1} of fresh tissue) and Catalase (0.912 changes in absorbance min^{-1}

g^{-1} of fresh tissue) activity respectively than individual applications. The next highest activity was observed in the plants treated with the combinations of BsOP2+ TL1+Neem cake + FYM. Peroxidase considered as an important PR proteins [31] and a key enzyme in the biosynthesis of lignin and other oxidative phenols. Increase in peroxidase expression in combined biocontrol agent treated test plants was significant, compared to untreated (absolute) and negative control (pathogen infested) plants. Some workers have reported the role of peroxidase in cell wall-building processes by oxidation of hydroxyl cinnamyl alcohols into free radical intermediates, phenol oxidation, polysaccharide cross linking, cross linking of extension monomers, lignification and suberization. These defense related genes are sleeping genes and it is needed to activate them by appropriate stimuli. *P. fluorescens* has been used in induced systemic resistance by some earlier workers [32]. [33] noticed that rhizosphere colonization of various bacteria induced PO activity in bean. The higher PO activity was observed in cucumber roots treated with *Pseudomonas corrugate* challenged with *Pseudomonas aphanidermatum* [34] and seedlings treated with *Pseudomonas* spp. challenged with similar pathogen in chilli [35].

3.6 Field Study

The greatest reduction in dry root rot incidence was observed in plots treated with the mixture of PfUL(A)+ BsOP2 + neem cake + FYM (25.65 PDI) followed by PfUL(A)+ TL1 + neem cake + FYM (29.02PDI) as compared with the untreated control (55.85PDI). The biocontrol agents not only reduced disease incidence and also enhances the plant growth. Mixture application of PfUL(A)+ BsOP2 + FYM + neem cake recorded the maximum plant height of 103 cm with yield (785 kg/ha) and compared to control (64 cm, 580 kg/ha). This was followed by PfUL(A) + TL1 + FYM + neem cake recorded 98 cm plant height and 710 kg/ha yield (Table 6). Soil application of biocontrol agents *viz., T. viride, T. harzianum, P. fluorescens and B. subtilis* effectively reduced root rot caused by soil borne pathogens in several crops [36,7,27]. The *P. fluorescens* strains reduced the root rot infection through several mechanisms including production of lytic enzymes [37], siderophores [38], salicylic acid [39] and hydrogen cyanide [40]. *B. subtilis* strains known to inhibit several soil borne diseases such as *Fusarium* wilt of red gram [41] and *R. solani* (damping-off of peppermint) [42]. Organic amendments are recommended as biological means to reduce the incidence of

Table 6. Effect of biocontrol consortia and organic amendments on the incidence of dry root rot growth and yield parameters in blackgram

Treatment details	Shoot length (Cm)	Root length (Cm)	Plant height (Cm)	No. of pods/ plant	No. of seeds/pod	Percent disease incidence	Yield Kg/ha
(PfUl(A)) + Neem cake + Farm Yard Manure	61^{c}	35^{b}	96^{c}	100^{d}	8^{a}	31.52^{d}	650^{e}
(BsOP2) + Neem Cake + FYM	62^{b}	35^{b}	96^{c}	107^{b}	8^{a}	30.40^{c}	700^{c}
(TL1) + Neem cake + FYM	58^{d}	32^{c}	90^{d}	75^{e}	7^{b}	32.46^{e}	605^{f}
PfUl(A)+BsOP2+ Neem cake + FYM	66^{a}	37^{a}	103^{a}	110^{a}	8^{a}	25.65^{a}	785^{a}
PfUl(A)+ TL1+Neem cake + FYM	65^{a}	36^{a}	98^{b}	106^{b}	7^{b}	29.02^{b}	710^{b}
PfUl(A) + BsOP2 +TL1+Neem cake+ FYM	63^{b}	35^{b}	98^{b}	104^{c}	7^{b}	31.24^{d}	680^{d}
Carbendazim	53^{e}	25^{d}	78^{e}	65^{f}	8^{a}	24.85^{a}	700^{b}
Control	45^{f}	19^{e}	64^{f}	35^{g}	6^{c}	55.85^{f}	580^{g}

Values are mean of three replications. In a column, means followed by a common letter(s) are not significantly different (P = 0.05) by DMRT

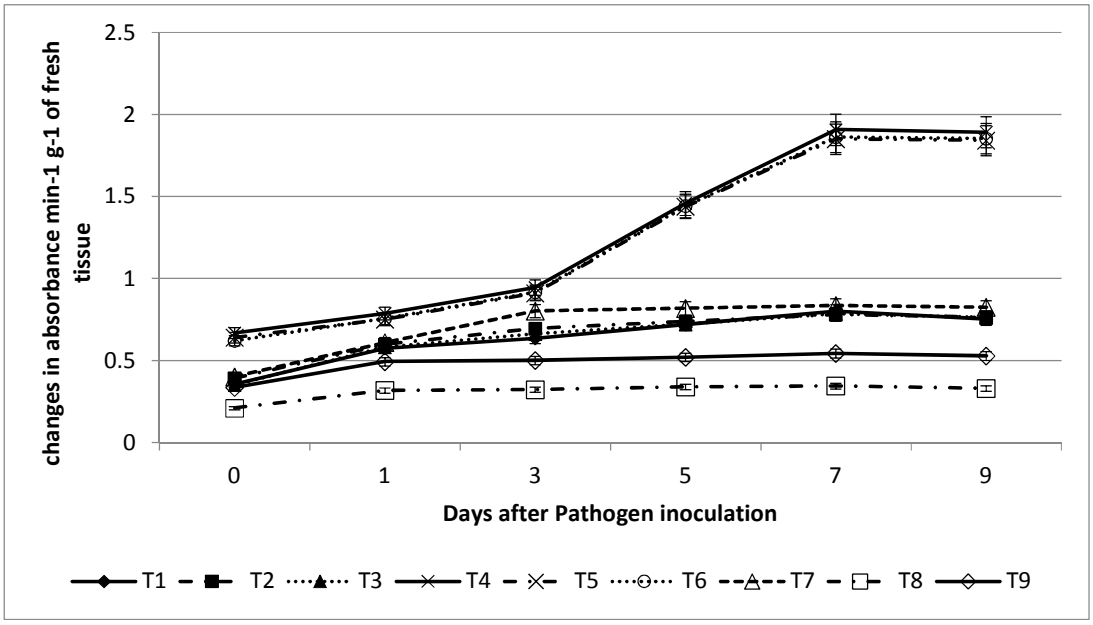

Fig. 1. Induction of peroxidise activity in blackgram plants treated with biocontrol consortia and organic amendments. T1–TL1, T2–PfUL(A), T3–BSOP2, T4–PfUL(A) + TL1, T5–PfUL(A)+ BSOP2, T6–PfUL(A)+BSOP2+TL1, T7–Carbendazim (0.1%), T8–Inoculated control, T9–Healthy control

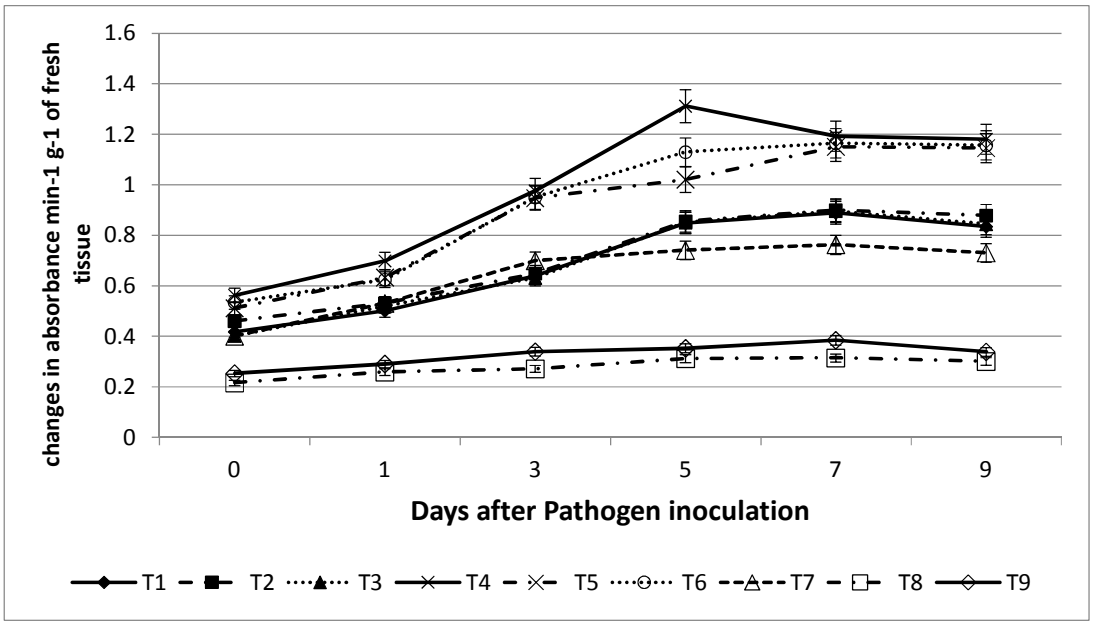

Fig. 2. Induction of polyphenol oxidase activity in blackgram plants treated with biocontrol consortia and organic amendments. T1–TL1, T2–PfUL(A), T3–BSOP2, T4–PfUL(A) + TL1, T5–PfUL(A)+ BSOP2, T6–PfUL(A)+BSOP2+TL1, T7–Carbendazim (0.1%), T8–Inoculated control, T9–Healthy control

several soil borne diseases. [43] reported that the activity of *R. solani* in organic amended soil was temporarily checked which was due to increase in CO_2 and decrease in N content of

soil. Soil amendment with FYM led to increased disease control efficacy of fungal antagonist *Trichoderma* spp. against *Fusarium* wilt of cumin [30]. Seed treatment with *P. fluorescens* along with soil amendment like mustard cake, vermicompost and FYM provided a better protection against *Macrophomina* root rot of chickpea [44].

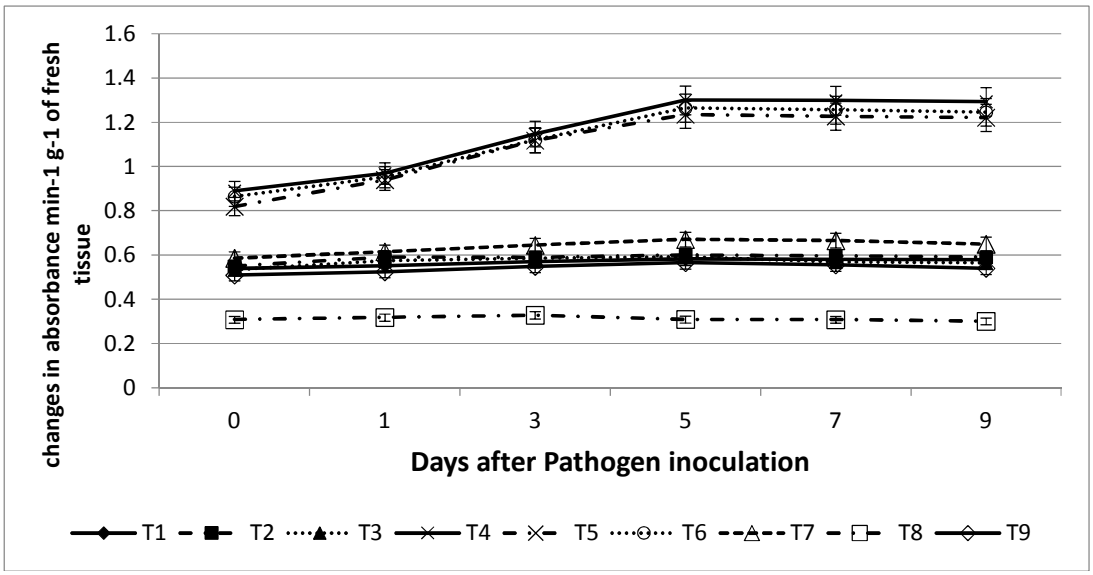

Fig. 3. Induction of phenylalanine ammonia lyase activity in blackgram plants treated with biocontrol consortia and organic amendments.T1–TL1, T2–PfUL(A), T3–BSOP2, T4–PfUL(A) + TL1, T5–PfUL(A)+ BSOP2, T6–PfUL(A)+BSOP2+TL1, T7–Carbendazim (0.1%), T8–Inoculated control, T9–Healthy control

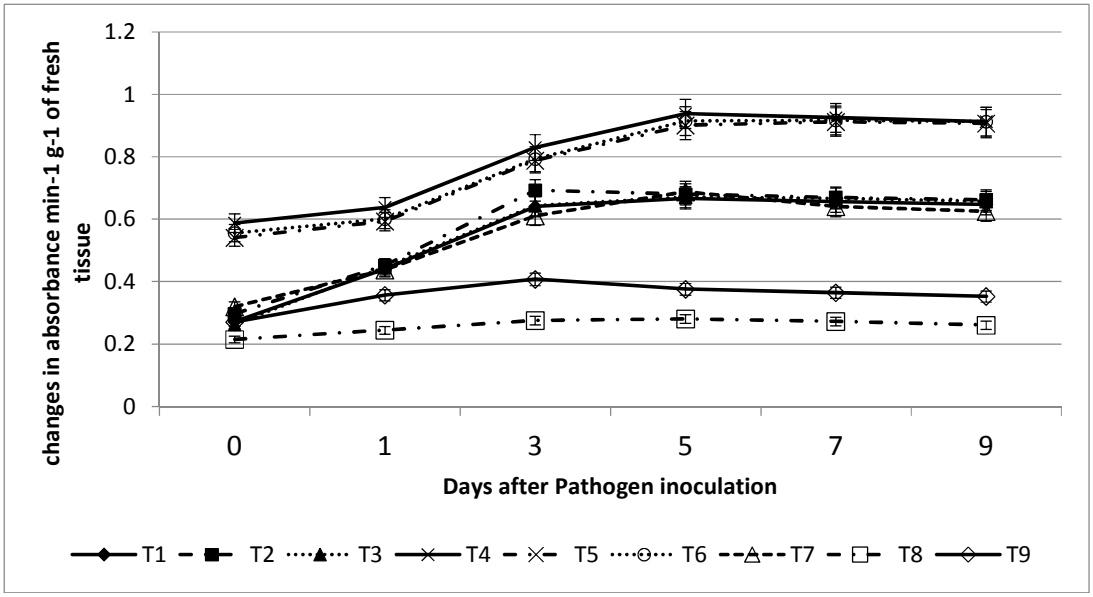

Fig. 4. Induction of catalase activity in blackgram plants treated with biocontrol consortia and organic amendments. T1–TL1, T2–PfUL(A), T3–BSOP2, T4–PfUL(A) + TL1, T5–PfUL(A)+ BSOP2, T6–PfUL(A)+BSOP2+TL1, T7–Carbendazim (0.1%), T8–Inoculated control, T9–Healthy control

4. CONCLUSION

The sclerotia of root rot pathogen is capable of living in the soil for very long time since it is very difficult to control with fungicides. Moreover, blackgram is mostly grown under rainfed condition farmers are not preferring synthetic fungicides. Hence, combined application of organic amendment and biocontrol agent is the ecofriendly sustainable management strategy for the root rot.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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