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Effect of Endophytic Treatments on Plant Growth Performance and Disease Incidences in Soybean (*Glycine max* (L.) Merril) Cultivar JS-335 against Challenge Inoculation with *R. solani*

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Abstract: Endophytes could be efficient biological control agent in sustainable crop production and offer unique opportunity for crop protection and biological control. Present study was carried out to investigate the effects of indigenous endophytic microorganisms *Pseudomonas* sp., *Bacillus* sp., *Burkholderia* sp. *Streptomyces* sp., *Actinoplanes* sp., *Alternaria* sp. and *Fusarium sp.* on plant growth and disease control against challenge inoculation with *Rhizoctonia solani* in soybean (*Glycine max* (L.) Merril). It was observed that treatment with endophytes significantly ($p<0.05$) improved the seed germination, root, shoot length, Seedling Vigour Index (SVI), root nodulation in soybean. The significant increments were recorded fresh and dry weight, nitrogen, phosphorus and potassium (NPK) uptake and seed yield ($p<0.05$). The disease incidences were reduced significantly over control ($p<0.05$). Thus, present studies indicate that utilization of indigenous endophytes may exert more favorable effects on plant health, disease control which ultimately will enhance crop productivity.

Keywords: Endophytes, PGPRs, Biocontrol, Soybean (*Glycine max* (L.) Merril)

Introduction

Plant-associated microorganisms have been extensively examined for their roles in natural and induced suppressiveness of soil-borne diseases. Because, rhizobacteria and endophytes are part of the natural microflora of healthy plants, they may be considered to be important contributors to plant health and general soil suppressiveness. Biological control has been described as a non-hazardous strategy to reduce crop damage caused by plant pathogens when compared to the chemical control of plant diseases (Wang *et al*., 2010). A major factor influencing plant growth and health is the microbial population living both in the rhizosphere and as endophytes within healthy plant tissue. Plants may be considered complex microecosystems where, different niches are exploited by a wide variety of microbes. Such niches include not only the external surfaces of plants, but also the internal tissues which endophytic microbe inhabit without apparent harm to the host or external structures (Azevedo *et al*., 2000).

Even though some success has been achieved in controlling crop pathogens and plant growth promotion by supplementing the crop soil with Plant Growth-Promoting Rhizobacteria (PGPR) and other biocontrol microbial inoculants. However, large number of biocontrol agents fails to be effective due to the difficulty of manipulating the highly complex rhizosphere environment (Conn and Franco, 2004). Exotic strains from commercial inoculants may not survive in local soils due to different edaphic or climatic conditions or may be outcompeted by better adapted native strains during plant colonization resulting in poor performance of PGPR (Calvo *et al*., 2010). The efficacy of conventional control measures, however, is limited. Hence, there is an increasing need for novel and environmentally sound strategies to control the plant diseases.

Microbial endophytes are typically defined as plant associated microbes that colonize living internal tissues of plants without causing any visible symptoms or immediate over-negative effects and can be isolated from surface disinfected plant tissue (Wilson, 1995; Zinniel *et al*., 2002; Hung and Annapurna, 2004). Endophytic microbes include bacteria, actinomycetes and fungi are ubiquitous in most plant species. Endophytes exist in a range of tissue types within a broad range of plants, colonizing the plant systemically, residing latently in intercellular spaces, inside the vascular tissue or within cells (Khan and Doty, 2009). Relatively steady internal environment inside the plant tissues makes endophytes more bioactive than the rhizospheric or others plant associated microorganisms (He *et al*., 2009).

Endophytes might interact more closely with the host plant and therefore, could be efficient biological control agent in sustainable crop production and offer unique opportunity for crop protection and biological control (Melnick *et al*., 2008). The use of endophytes can be divided into two categories based on types of activity viz., growth promotion and disease control (Bacon and White, 2000).

Among the plant associated microorganisms, endophytes are regarded as a largely untapped resource for the discovery of isolates with novel antifungal and plant growth promoting traits (Mendes *et al*., 2007). Endophytic microorganisms have attracted the attention of researchers because of their potential to serve as biocontrol agents (Strobel and Daisy, 2003; Stein, 2005; Ryan *et al*., 2008). Endophytes living in the healthy tissues of plants are relatively unstudied and may be the potential source of novel natural products for exploitation in agriculture, medicine and other industries (Strobel and Daisy, 2003).

Although, the plant-endophyte interaction has not been fully understood, it has been reported that many isolates provide beneficial effects to their hosts like preventing disease development by synthesizing novel compounds and antifungal metabolites. Several endophytes have been shown to support plant growth and increase nutrient uptake by providing phytohormones, low molecular weight compounds, enzymes, antimicrobial substances like antibiotics and siderophores. Other beneficial effects of endophytes to plants include nitrogen fixation, increased drought resistance, thermal protection, survival under osmotic stress etc. (Khan and Doty, 2009).

Within the framework of integrated plant disease management (IDM) the use of indigenous bacterial endophytes with biocontrol activity is environment friendly and ecologically efficient approach (Prieto *et al*., 2011). In spite of the great importance of endophytic microorganisms in agricultural ecosystems, only a very small part of the microbial diversity relevant to agriculture was carefully described. The great amount of information regarding the key role of endophytic microbes in agriculture is yet to be explored.

Soybean (*Glycine max* (L.) Merril) is an Asiatic leguminous plant, occupying large acres of land worldwide for its oil and protein. In recent years, soybean has assumed important position in India. It has well adapted to black soils of central and peninsular India. Major soybean producing states in India including, Madhya Pradesh, Maharashtra and Rajasthan contribute about 97% to total area and 96% production of soybean in the country (Namrata *et al*., 2012). Maharashtra is the second largest soybean producing state in India. It accounts for 34% of the India's bean production. Soybean is gaining popularity on account of its unique characteristics and adaptability to varied agro-climatic conditions (Pawar *et al*., 2011).

Washim is an important soybean producing area of Vidarbha region of Maharashtra, occupying 2095 ha of area with production of 2987 tons during 2010-2011 (Crop Production Statistics, Department of Agriculture, Government of Maharashtra). However, due to extreme diversity of pathogens and serious diseases severe plant losses and yield reductions are common in susceptible cultivars of soybean (Zivkovic *et al*., 2010). The soybean fungal pathogens are prevailing and chiefly intricate to control.

Inspite of increased numbers of reports about beneficial traits of endophytic microbes to crop plants protecting their host against predators and pathogens and promotion of plant growth, there is dearth of information regarding use of different endophytic microorganisms for the management of soil-borne fungal pathogens and growth promotion in soybean. Hence, with the view of plant health and productivity the proposed studies with special reference to indigenous endophytic microbes for soybeans crop cultivar JS-335, as model phytosystem, have been carried out.

Materials and Methods

Endophytic Microorganisms and R. solani

In present investigation indigenous endophytic bacteria, actinomycetes and fungi isolated from soybean were utilized to study their effects on plant growth performance and disease control against *R. solani* isolated from diseased Soybean plant. The isolated endophytes were initially screened for *in vitro* antagonistic activity against *R. solani* (Zivkovic *et al*., 2010; Yuan and Crawford, 1995). The antagonist thus obtained were further screened for the ability to exhibit plant growth promoting ability viz., secretion of plant growth regulators (auxins (indole-3-acetic acid (IAA) and indole-3-pyruvic acid (IPyA), gibberellins (GA3) and cytokinins [isopentenyl adenine (iPa), isopentenyl adenosine (iPA) and Zeatin (Z)), HCN and siderophore conditions adopting standard biochemical methodology (Strzelczyk and Pokojska, 1984; Shirling and

Gottlieb,1966; El-Tarabily *et al*., 2009 Tien *et al*., 1979; Thimmaiah, 2004; Lorck, 2006; Castric and Castric, 1983; Samuel and Muthukkaruppan, 2011; Neilands, 1981; Coleman, 1995; Wijesundera *et al*., 1995; Logeshwaran *et al*., 2009).

Studies on Interaction between the Isolated, Pathogens and Endophytes having Dual Attributes on the Growth Performance and Disease Incidences of Soybean

Field experiments were conducted to study the effect of interaction between the isolated pathogens and endophytes with dual attributes (Table 1) on growth performance and disease incidences in soybean cultivar JS-335.

Experimental Site and Soil

The experiment was conducted at Agriculture Research Farm, Microbiology Research Laboratory, Tondgaon Dist. Washim (MS) India. It is approximately 22 Km away from Washim city. The soil resembled to be the vertisol type (Fig. 1). Vertsol soil in which there is high content of expansive clay and is usually very dark in color.

Climatic Conditions

The climate of the district is characterized by hot summer and general dryness throughout the year except during the south-west monsoon season, i.e., June to September. The mean minimum temperature is 12°C and mean maximum temperature is 42°C.

Experimental Details

The experimentation was carried out during Kharif season of 2012. Micro plots of size 1 m^2 were prepared and used further for experimentation adopting randomized block design with three replications the layout of the plan is presented in Fig. 2A, B and C and details of the experiments are presented in Table 2A and B. All the experimentation was carried out in plots amended with fungal pathogen *R. solani* sick soil with soybean cultivar JS-335 as the test crop.

Table 1. Screened endophytic isolates with dual ability of antagonism against *R. solani* and plant growth promotion

		PGP trait							
		Plant growth regulators							
		Auxins		Gibberellins		Cytokines			
Endophytic isolates		IAA	IPyA	GA3	iPa	iPA	Z	HCN production	Siderophore production
JDB3	*Pseudomonas sp*	+	-	+	+	-	+	+	-
JDB9	*Bacillus sp.*	+	-	+	+	-	-	+	-
JDB23	*Burkholderia sp.*	+	-	-	-	-	-	-	+
JDA5	*Streptomyces sp.*	+	-	-	-	-	+	+	+
JDA6	*Streptomyces sp.*	+	-	-	-	-	-	-	+
JDA9	*Streptomyces sp.*	+	-	-	-	-	-	+	-
JDA15	*Actinoplanes sp.*	+	-	-	-	-	-	-	-
JDF3	*Alternaria sp*.	-	-	-	-	-	-	-	+
JDF12	*Fusarium sp*	+	-	-	-	-	+	+	+

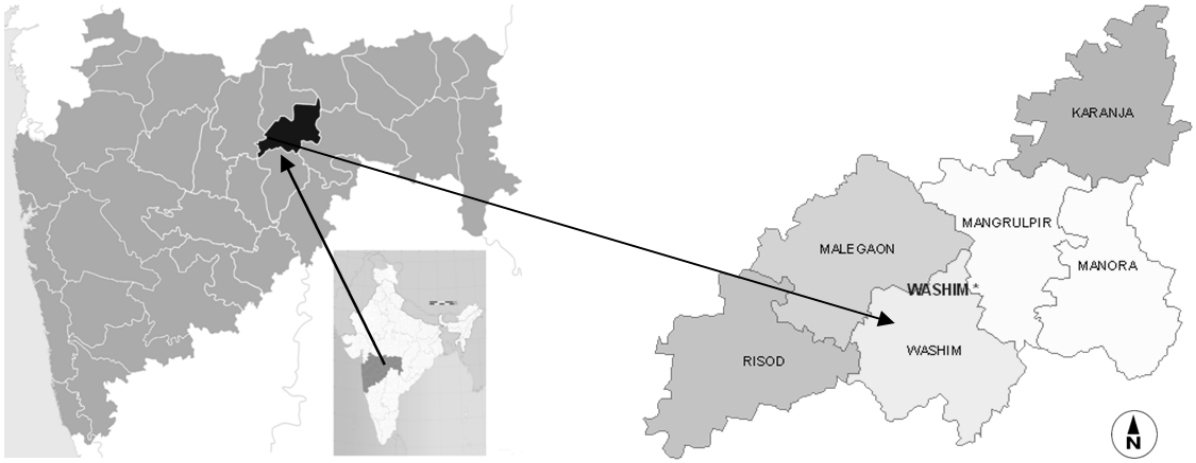

Fig. 1. Location of study area

(A) (B) (C)

Fig. 2. Plan of layout of the experimental sites

Table 2A. Details of experiments

Particulars	
Ploughing	03.06.2012
Harrowing	05.07.2012
Layout of the field	05.07.2012
Treatments	11
Replications	3
Design of experiment	Randomized block design
Microplot size (m^2)	1
Spacing (cm)	30
Crop variety	JS-335
Date of sowing	06.07.2012
Method of sowing	Drilling
Seed rate (kg/ha)	75
Recommended NPK dose and date of application	30:75:30
Harvesting of crop	15.10.2012

Table 2B. Treatment details *R. solani* sick soil

Treatment	Details
RT1	Seed treatment with bacterial isolateJDB3
RT2	Seed treatment with bacterial isolateJDB9
RT3	Seed treatment with bacterial isolate JDB23
RT4	Seed treatment with actinomycete isolate JDA5
RT5	Seed treatment with actinomycete isolate JDA6
RT6	Seed treatment with actinomycete isolate JDA9
RT7	Seed treatment with actinomycete isolate JDA15
RT8	Seed treatment with fungal isolate JDF3
RT9	Seed treatment with fungal isolate JDF12
RT10	RT1+RT2+RT3+RT4+RT5+RT6+RT7+RT8+RT9
RT11	Seed treatment with sterile dist. water (Control)

Preparation of R. solani Sick Soil

The fungal pathogen sick soil was prepared as described by Totawar (2001) with slight modifications. *R. solani* was enriched separately in 250 mL of potato dextrose broth and the inoculum was build upto 500 mL each. The inoculum treatment was separately given to cultivated seedlings at 15 DAS. Further the seedlings were examined for disease development at 30 DAS. The screened diseased plants were again processed for isolation of fungal pathogen. Thereafter, the process from the inoculum build up was repeated for six months so as to get the virulent soil. The virulent soil was further fortified manually (10% per kg) on the surface of experimental plots. The virulent soil fortified experimental plots were further considered as sick soil microplots. Whereas, microplots without fortification of fungal pathogens were maintained as control.

Treatment Details

Soybean seeds were treated with endophytes alone and in combination. Test crops without endophyte treatment were maintained as control. The charcoal based endophytic bio-inoculants were produced (Chandrashekhara *et al*., 2007; Gopalakrishnan *et al*., 2012; Sudisha *et al*., 2006) and used for seed treatments.

Seed Treatment with Endophytic Bio-Inoculants

The seeds were surface sterilized with 2% sodium hypochlorite for 2 min and washed with sterile distilled water and further blotted dry with sterile blotting paper. Seeds were treated with 10% (w/v) jiggery solution and allowed to dry for 5 min. Seed treatment was done using charcoal based inoculants (25 gm/kg of seeds). The charcoal based inoculants were then added to seeds and mixed uniformly so as to achieve a homogenous coat over seed. Treated seeds were stored in cool and dry place at room temperature away from sunlight. The treated seeds were sown in respective microplots. Seeds without endophytic treatments were maintained as control. The treatments were designated as $RT_{1\text{-}11}$, representing treatments in the *R. solani* sick soil. Necessary agricultural operations viz., thinning, hoeing and weeding were carried out as and when required with the help of local labors.

Studies on Effect of Endophytic Treatments on Growth Performance and Disease Incidences

Three plants from each plot in the net plot area were selected randomly and tagged for recording different biometric observations. Mean of three plants was considered for analysis. Germination count of each plot was taken and per cent germination was calculated on 15th Days After Sowing (DAS). Vegetative growth parameters viz., per cent germination, root-shoot length, Seedling Vigor Index (SVI), fresh and dry weight and NPK uptake was recorded at 30th DAS for both treated and untreated control plant. Seedlings were uprooted from each treatment plot on 30th DAS without disturbing the root system and root-shoot length (cm) were measured. Seedling Vigor Index (SVI) was calculated by the formula (Hatwalene, 1993):

$$S.V.I = \{(\text{Root length}) + (\text{Shoot length})\} \times \text{Germination \%}$$

Root Nodulation Count

Randomly selected plants were uprooted at 45th DAS along with soil mass. The root system was dipped in water to remove adhering soil and enough care was taken to keep the root system and nodules intact so that none of the nodules were lost. The nodules were separated from roots, washed, counted and further recorded as number of nodules per plant (Meenakshi, 2008).

Estimation of Fresh and Dry Weight

The fresh and dry weight were recorded on 30th DAS and expressed in gram per plant (g/plant).The fresh weight of the plants was determined by weighing the individual plants immediately after harvesting. The dry weight was estimated after drying the plants at 65°C in an oven for 12 hr.

Estimation of NPK Uptake

The collected plant samples were processed for the estimation NPK uptake adopting standard methods. Estimation of Nitrogen (N) by following the Microkjeldhal method (Jackson, 1973), Phosphorus (P) uptake by Vanadomolybdate reagent and potassium (K) was estimated by atomizing the diluted plant extract in the flame photometer as described by Jackson (1973).

Yield

At harvest, yield of soybean seeds per net plot was recorded (kg/plot) and expressed in kg/ha (Meenakshi, 2008).

Disease Incidences

Observations were also made for the existence of number of healthy plant units; number of infected plant units from each plot was recorded at 30th DAS and per cent disease incidence was calculated using formula (Gilligan, 1983):

$$\text{Disease incidence } (\%) = \frac{\text{No. of infected plants units}}{\text{Total No. of } (\text{healthy} + \text{infected}) \text{ plant units assessed}} \times$$

Results

Effect of Endophytic Treatments on Plant Growth Performance of Soybean Cultivar JS-335 against Challenge Inoculation with R. solani

Data on effect of endophytic treatments on plant growth parameters viz., germination, root and shoot length, Seedling Vigor Index (SVI), root nodulation fresh and dry weight, NPK uptake and seed yield was recorded against challenge inoculation with *R. solani.*

Germination

Results on the influence of endophytic treatments on germination of soybean in *R. solani* sick soil are presented in Table 3. The endophytic treatments significantly improved the germination of soybean over uninoculated control. Maximum germination was recorded at consortial treatment RT10 (79.25%) over uninoculated control RT11 (57.03 %). Among the individual treatments the maximum germination was recorded in bacterial treatment RT1 (73.33%) followed by treatment RT2 (71.85%). However, germination was observed to be minimum in case of actinomycete treatment RT5 (50.37%) and fungal RT8 (54.07%) lower germination was recorded as compared to uninoculated control. Endophytic treatment RT10 was found to be significantly higher and was at par with treatment RT1 and RT2. Whereas, other treatments viz., RT3, RT5, RT6, RT7 and RT9 were found to be insignificant in improving germination as compared to control RT11.

Root Length

Results on the influence of endophytic treatments on root length of soybean at 30th DAS in *R. solani* sick soil are presented in Table 3.

Significant increase in root length was recorded in endophytic treatments over uninoculated control. Maximum root length was recorded at individual bacterial treatment RT2 (7.73 cm) over uninoculated control RT11 (4.30 cm). Root length values were on par at individual bacterial RT1 (7.50 cm), RT3 (7.43 cm) and consortial treatment RT10 (7.50 cm). However, root length in actinomycete treatment RT4 (4.06 cm) was observed to be lower as compared to control RT11.

Table 3. Effect of endophytic treatments on growth parameters of soybean in *R. solani* sick soil

Treatment details	Germination		Mean root length (cm)	Mean shoot length (cm)	Mean seedling vigor index (SVI)
	Mean	%			
RT1	33.00	73.33	7.50	12.60	1474.00
RT2	32.33	71.85	7.73	14.53	1599.90
RT3	27.33	60.74	7.43	13.96	1299.85
RT4	22.66	50.37	4.06	9.40	678.32
RT5	26.33	58.51	4.63	10.60	891.432
RT6	28.00	62.22	4.30	9.56	862.81
RT7	29.00	64.44	5.10	8.23	859.25
RT8	24.33	54.07	5.73	10.63	885.01
RT9	26.33	58.51	4.30	6.70	643.70
RT10	35.66	79.25	7.50	13.20	1640.67
RT11	25.66	57.03	4.30	7.56	676.83
F-Test	Sig		Sig	Sig	Sig
SE (m)	1.55		0.21	0.20	21.48
CD (5%)	4.57		0.61	0.60	63.19

Whereas, root length in actinomycete treatment RT6 (4.30 cm) and fungal treatment RT9 (4.30 cm) was observed to be at par with control treatment RT11. Endophytic treatments RT1, RT2, RT3, RT7, RT8 and RT9 significantly increased root length as compared to uninoculated control RT11 whereas RT5 was found to be statistically insignificant in improving root length.

Shoot Length

Data on the influence of endophytic treatments on shoot length of soybean at 30th DAS in *R. solani* sick soil are presented in Table 3. Endophytic treatments significantly increased shoot length over control. Maximum shoot length was recorded in individual bacterial treatments RT2 (14.53 cm) followed by treatment RT3 (13.96 cm), RT1 (12.60 cm) and consortial treatment RT10 (13.20 cm) over uninoculated control RT11 (7.56 cm). However, shoot length in individual fungal treatment RT9 (6.70 cm) was found to be lower as compared to control RT11. Endophytic treatments RT1, RT2, RT3, RT10 were significantly high in improving shoot length whereas treatment RT5 was insignificant as compared to control.

Seedling Vigor Index (SVI)

Data on the influence of endophytic treatments on SVI of soybean at 30th DAS in *R. solani* sick soil is presented in Table 3. Endophytic treatments significantly enhanced SVI of soybean. However, the degree of enhancement varied treatments. Maximum SVI was recorded in bacterial treatment RT2 (1599.90) followed by consortial treatment RT10 (1640.67), bacterial treatment RT1 (1474.00) over uninoculated control RT11 (676.83). However, SVI in actinomycete treatment RT4 (678.32) and in fungal treatment RT9 (643.70) was found to be lower as compared control RT11.

Endophytic treatments RT1, RT2, RT3 and RT10 were significantly higher in improving SVI of soybean whereas treatments RT4 and RT9 were insignificant as compared to control.

Root Nodulation

The data on nodulation as influenced by endophytic treatments at 45th DAS in *R. solani* is presented in Table 4. The nodulation was significantly increased in all endophytic treatments. However, nodulation was varied between individual and consortial treatments. Maximum no. of nodules were recorded in consortial treatment RT10 (59) followed by bacterial RT1 (46), RT4 (43) and RT2 (42) as compared to uninoculated control RT11 (18). All the endophytic treatments significantly improved root nodulation. Endophytic treatments RT1, RT2, RT4 and RT10 were significantly higher in improving nodulation as compared to control RT11.

Fresh Weight and Dry Weight

Results on the influence of endophytic treatments on fresh and dry weight (g) of soybean in *R. solani* sick soil are presented in Table 5. Significant improvement was observed in fresh and dry weight in endophytic treatments over uninoculated control. However, the degree of improvement varied between treatments. Maximum fresh weight was recorded in consortial treatment RT10 (18.40 g) and minimum fresh weight was recorded in individual actinomycete treatment RT6 (11.50 g) as compared to control RT11 (8.40 g). Fresh weight values recorded were on par at bacterial treatments RT1 (16.93 g), RT2 (17.86 g) and RT3 (17.73 g). All the endophytic treatments significantly improved fresh weight of soybean however treatments RT1, RT2, RT3 and RT10 were highly significant as compared to other treatments.

Table 4. Effects of endophytic treatments on root nodulation in soybean in *R. solani* sick soil

Treatment details	Average no. of nodules/plant
RT1	46
RT2	42
RT3	36
RT4	43
RT5	33
RT6	32
RT7	35
RT8	36
RT9	36
RT10	59
RT11	18
F-Test	Sig
SE(m)	1.60
CD(5%)	4.70

Table 5. Effects of endophytic treatments on fresh and dry weight of soybean DAS in *R. solani* sick soil

Treatment details	Mean fresh weight (g)	Mean dry weight (g)
RT1	16.93	7.20
RT2	17.86	7.86
RT3	17.73	7.60
RT4	12.83	6.36
RT5	13.43	6.20
RT6	11.50	5.30
RT7	14.26	6.26
RT8	13.46	5.53
RT9	12.26	4.66
RT10	18.40	8.80
RT11	8.40	4.56
F-Test	Sig	Sig
SE(m)	0.64	0.26
CD (5%)	1.87	0.76

Table 6. Effects of endophytic treatments on NPK uptake in soybean in *R. solani* sick soil

Treatment details	Average N uptake (kg/ha)	Average P uptake (kg/ha)	Average K uptake (kg/ha)
RT1	31.50	5.86	30.66
RT2	30.33	5.26	30.20
RT3	29.00	4.93	29.33
RT4	28.16	6.00	28.23
RT5	26.73	6.20	29.80
RT6	27.00	5.13	27.56
RT7	26.10	6.16	27.00
RT8	27.80	6.66	27.36
RT9	26.16	5.43	28.26
RT10	32.63	6.73	30.63
RT11	23.50	3.56	21.03
F-Test	Sig	Sig	Sig
SE(m)	2.89	2.46	2.52
CD (5%)	8.51	7.23	7.40

Maximum dry weight (8.80 g) was recorded in consortial treatment RT10 whereas it was minimum at actinomycete treatment RT6 (5.30 g) over uninoculated control (4.56 g). Whereas, dry weight values were on par at bacterial treatments RT2 (7.86 g) and RT3 (7.60 g). Significant improvement was recorded at all endophytic treatment except treatment RT9. Treatments RT1, RT2, RT3 and RT10 were highly significant in improving dry weight over other treatments.

NPK Uptake

The data on NPK uptake at 30th DAS as influenced by endophytic treatments is presented in Table 6. NPK uptake was significantly increased in all endophytic treatments. Maximum N uptake was recorded in consortial treatment RT10 (32.63 kg/ha) followed by bacterial RT1 (31.50 kg/ha) and RT2 (30.33 kg/ha) whereas minimum N uptake was recorded at actinomycete RT7 (26.10 kg/ha) followed by fungal RT9 (26.16 kg/ha) as compared to uninoculated control RT11 (23.50 kg/ha). Endophytic treatments RT1, RT2 and RT10 were highly significant in improving N uptake as compared to other treatments.

Maximum P uptake was recorded in consortial treatment RT10 (6.73 kg/ha) followed by fungal RT8 (6.66 kg/ha) whereas it was minimum at bacterial treatment RT3 (4.93 kg/ha) and actinomycete RT6 (5.13 kg/ha) as compared to control RT11 (3.56 kg/ha). P uptake was significantly higher at treatments RT8 and RT10 as compared to other treatments.

Maximum K uptake was recorded at bacterial treatment RT1 (30.66 kg/ha) followed by consortial RT10 (30.63 kg/ha) and bacterial RT2 (30.20 kg/ha) whereas, it was minimum at actinomycete treatment RT7 (27.0 kg/ha) as compared to control RT11 (21.03 kg/ha). All endophytic treatments significantly improved K uptake. Treatments RT1, RT2 and RT10 were significantly higher as compared to other treatments.

Seed Yield

The data on seed yield (kg/ha) as influenced by endophytic treatments is presented in Table 7. The seed yield was significantly increased in endophytic treatments. However, seed yield was varied among the treatments. Maximum seed yield was recorded in consortial treatment RT10 (1380.33 kg/ha) followed by bacterial RT2 (1221.00 kg/ha), RT3 (1180 kg/ha) and RT1 (1129.33 kg/ha) as compared to uninoculated control RT11 (809.67 kg/ha).

Endophytic treatments RT1, RT2, RT3, RT4 and RT10 were significantly higher in improving seed as compared to other treatments and uninoculated control RT11. However, actinomycete treatments RT6 and fungal RT9 were found to be statistically insignificant as compared to control.

Table 7. Effects of endophytic treatments on seed yield of soybean in *R. solani* sick soil

Treatment details	Seed yield (kg/ha)
RT1	1129.33
RT2	1221.00
RT3	1180.30
RT4	1016.00
RT5	954.33
RT6	825.00
RT7	884.33
RT8	979.66
RT9	887.00
RT10	1380.33
RT11	809.66
F-Test	Sig
SE(m)	27.99
CD (5%)	82.35

Table 8. Effects of endophytic treatments on disease incidences in soybean in *R. solani* sick soil

Treatment details	No. of infected plants	Disease incidence (%)
RT1	5.33	16.16
RT2	6.66	20.62
RT3	5.66	20.73
RT4	5.333	23.53
RT5	8.00	30.38
RT6	6.00	21.42
RT7	7.33	25.28
RT8	7.00	28.77
RT9	5.667	21.52
RT10	4.667	13.08
RT11	13.00	50.66
F-Test	Sig	
SE(m)	0.51	
CD (5%)	1.5	

Studies on Interaction between the Isolated Pathogens and Endophytes with Dual Attributes on Disease Incidences in Soybean

Endophytic microbes with antagonistic and plant growth promoting activity were utilized for interaction studies between the isolated fungal pathogens of soybean. The effect of endophytic microbes on disease incidences was evaluated in soybean cultivar JS-335 against challenge inoculation with *R. solani.*

Disease incidences on soybean against challenge inoculation with *R. solani* were recorded from endophytic treatment at 30th DAS and the results are presented in Table 8. All the endophytic treatments were found effective in reducing the disease incidences as compared to uninoculated control (50.66%). However, the degree of disease incidences varied between the treatments and ranged between 13.08-30.38%. Maximum protection was offered by the consortial treatment RT10 (13.08%) followed by bacterial RT1 (16.16%) as compared control RT11. All endophytic treatments significantly reduced disease incidences however; treatment RT10 was highly significant in reducing the disease incidences.

Discussion

Endophytic microorganisms promote the growth of host plant in various ways and they protect the host plant from pathogens. Our findings are in support with various reports. Endophytic bacteria enhance plant growth by producing plant growth regulators such as gibberellins, cytokinins and indole acetic acid, which directly or indirectly promote plant growth and development (Holland, 1997; Barka *et al*., 2002). Bhowmik *et al*. (2002) reported that cotton seed bacterization with the endophyte Endo PR8 was highly effective in reducing cotyledonary infection with *Xam*. Bacterized grapevines had a greater fresh weight of the shoots and roots and faster growth with more lignin deposits (Barka *et al*., 2002).

Endophytic bacteria from cotton tissues led to better seed germination and better control of cotton wilt caused by *V. dahliae* (Fu *et al*., 1999). Mondal *et al*. (1999) found that five strains of *Pseudomonas* inhibited *Xam,* increased cotton seed germination by 12.8% and improved normal seedling growth by 22.4%. In two field trials, treatment with *Bacillus pumilus* strain INR7, isolated from a surface-sterilized stem of a surviving cucumber plant in a field heavily infested with cucurbit wilt disease, caused by *Erwinia tracheiphila,* resulted in significant growth promotion relative to the nontreated control in cucumber (Wei *et al*., 1996).

Endophytic fungi, residing in the root tissues can play pivotal role in host-plant growth by influencing mineral composition, plant hormonal balance, chemical composition of root exudates, soil structure and plant protection against biotic and abiotic stresses (Waller *et al*., 2005; Rodriguez *et al*., 2008; Redman *et al*., 2011). Previous studies have shown that endophytic fungal association can significantly increase plant biomass and growth and also elaborated the beneficial effects of endophytic fungi on the growth responses of host-plants under various stress conditions (Waller *et al*., 2005; Hamilton *et al*., 2010; Redman *et al*., 2011; Khan *et al*., 2012).

Plant-fungus relationship has been proclaimed a pivotal source for plant growth and development (Rodriguez and Redman, 2008). Endophytic fungi have been regarded as plant protectant and growth regulator during normal and extreme environmental conditions. Various novel endophytic fungal species like *Piriformospora indica, Neotyphodium* sp*., Curvularia protuberate* and *Colletotrichum* sp. etc have been known to improve plant growth during abiotic stress conditions. *Penicillium* species have been known as a vital source for bioactive secondary metabolites. Some strains of this genus also produce plant growth regulators like gibberellins, auxin, etc. (Khan *et al*., 2013).

Rajendran *et al*. (2006) studied the effect of indigenous bacterial endophytic strains on plant growth promotion of cotton. 133 endophytic bacteria were isolated from the healthy roots, stems, leaves and seeds of cotton plants. Endophytic *Bacillus* isolates EPCO 102 (leaf isolate) and EPCO 16 (root isolate) were found to increase the vigour index of cotton seedlings significantly, with a maximum vigour index of 1404.55 for cotton seedlings treated with EPCO 102 suspension, compared with a vigour index of 226.4 with the untreated controls.

Growth-promoting activity of the endophytic fungus *Piriformospora indica*, resulted in enhanced barley grain yield (Waller *et al*., 2005). During the first 4 weeks of barley development, shoot fresh weight of infested plants was up to 1.65 times higher compared with control plants. *P. indica*-infested Annabell showed an increase in grain yield of 11per cent, mainly because of a higher number of ears per plant. In cultivar Ingrid, the grain yield increase was 5.5%.

A hyaline sterile fungus forming epiphyllous mycelial nets was isolated from meristem cultures of *Mentha piperita* (Mucciarelli *et al*., 2002). Histological studies indicated that the culture isolate is able to colonize stems and leaves with no damage to the host plant. In vitro grown peppermint plants displayed enhanced vegetative growth when infected by the fungus, with mycelium extending from green tissues to growing rootlets.

Hipol (2012) isolated 36 fungal endophytes from apparently healthy sweet potato plants from leaves, stems and roots collected from Baguio City. Among the isolates, only P3AL2c and P3BS1c significantly enhanced growth of paclobutrazol treated rice seedlings. They further demonstrated that the significant increase in plant length for the seedlings treated with the culture filtrates of P3AL2c and P3BS1c were due to the presence of growth promoting metabolites from these fungal endophytes. Treatment of the IR 64 seeds with paclobutrazol, a GA biosynthesis inhibitor, suppresses the endogenous GAs production by blocking its biosynthesis pathway in the plant. Also, the growth media were devoid of nutrients, it being water agar only. As such, growth promotion in the test seedlings can be attributed to the activity of plant growth promoting secondary metabolites from fungal culture filtrates.

Endophytic microbes secreting plant growth regulating compounds are of great agronomic importance to enhance crop yield and quality. These growth regulating compounds can affect plant development as well as support plant growth in instances of biotic and abiotic stress such as tolerance to herbivory, heat, salt, disease and drought and increased below and above ground biomass.

In present investigation the potential of endophytic microbes in reducing the disease incidences has been studied. Significant reductions in the diseases incidences were observed. Our findings correlate with reports of other workers. Application of strains *B. pumilus* strain SE34 and *Pseudomonas fluorescens* strain 89B-61 by incorporation into the potting medium at the time of planting elicited significant reductions in disease severity when *P. infestans* was inoculated onto leaves 5 weeks after planting in tomato (Yan *et al*., 2002).

Rajendran *et al*. (2006) tested endophytic bacterial strains for their effectiveness against *Xam* in potted cotton plants along with plantomycin as a chemical check. They found that with plantomycin at 100 ppm the lowest incidence (8.38 %) of BBC was recorded 60 DAS, followed by *Bacillus* isolate EPCO 102 + chitin (14.853%). *Bacillus* isolate EPCO 16 and *Pseudomonas fluorescens* Pf1 were similar in their effectiveness against *Xam*. Plants without any endophytic bacteria had the highest BBC incidence (40.56%).

Coombs *et al*. (2004) screened 38 actinobacterial strains isolated from wheat, representing *Streptomyces*, *Microbispora*, *Micromonospora* and *Nocardioides,* for their antifungal potential against *Rhizoctonia solani*, *Pythium* sp. and *Gaeumannomyces graminis* var *tritici* (the causal agent of take-all disease in wheat) both *in vitro* and by bioassays. The analyses revealed that 64% of the strains had antifungal properties in *in vitro* assays and 17 strains were efficient *in planta* (in steamed soil) against take-all disease. The active isolates were also effective under field conditions in the biocontrol against take-all as well as *Rhizoctonia* (Coombs *et al*., 2004).

The cumulative yield of marketable cucumber fruit was also significantly enhanced by endophytic *Bacillus pumilus* strain INR7 in both field trials. In the same study, strain 89B-61 also increased plant growth and yield and reduced the incidence of both angular leaf spot and anthracnose. In a subsequent field trial, INR7 reduced the severity of cucurbit wilt (Zehnder *et al*., 2001). In addition, the severity of angular leaf spot, following inoculation with *Pseudomonas syringae* pv. lachrymans and the severity of naturally occurring anthracnose were significantly reduced by INR7.

Conclusion

It was observed that endophytic treatments improved the growth performance of soybean against the challenge inoculation with *R. solani.* Plant growth parameters viz., per cent germination, root-shoot length, Seedling Vigor Index (SVI), root nodulation, fresh and dry weight and NPK uptake and yield were significantly enhanced over the uninoculated control. Diseases incidences in soybean were significantly reduced due to the endophytic treatments against all the six fungal pathogens of soybean. It was observed that endophytic treatments showed better plant growth and plant protection as compared to uninoculated control. Among the treatments, single treatment performed better than uninoculated control whereas consortial treatments

performed better over single treatment. Thus, present studies indicate that utilization of indigenous endophytes may exert more favorable effects on plant health, disease control which ultimately will enhance crop productivity.

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Author's Contributions

Jitendra Dalal and Nikhilesh Kulkarni: Designed and planned the current research work and analyzed and interpreted the results.

Jitendra Dalal: Conducted the experiments and collected the data.

Ethics

Authors declare no competing interest.

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