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Management of Pod Rot of French bean (*Phaseolus vulgaris* L.) incited by *Sclerotium rolfsii* Sacc.

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ABSTRACT: *S. rolfsii* Sacc. is a soil borne pathogen with wide host range and difficult to control by fungicides. Excessive use of fungicides in agriculture has led to deteriorating human health, environmental pollution and development of pathogen resistance to fungicide. Hence, biocontrol agents are getting momentum in recent years due to an increasing awareness of pesticide hazards, environmental pollution and higher cost of development. Endophytes as biocontrol agents are known to control the plant diseases and also helps in plant growth promotion. The present investigation has been carried out to manage the pod rot of French bean incited by *Sclerotium rolfsii* Sacc. *in-vitro* and field conditions. Three screened efficient bacterial endophytes bacterial *viz.*, BS80, BS178 and BS118 revealed significant inhibition of radial mycelial growth ranging 29.92 to 65.18% when subjected to dual culture test against *S. rolfsii*. All these bacterial endophytes were found to be compatible and were used to applied in field in alone and in consortia (MC1, MC2, MC3 and MC4) on the basis of antagonistic and PGP properties. The PGP traits *in-vitro* also revealed the highest seed germination percentage of 88.67 when treated with MC4 whereas control showed germination percentage 78.33. *In-vitro* PGP activities in French bean found out that vigour index (VI) of 2470.33 by seed treated with combination of isolates BS80 + BS178 + BS118 (MC4) whereas control gave vigour index of 1534.67. Field experiment was conducted in which treatment consisting of seed + soil application with microbial consortia 4 showed PDI of 15.87 ± 0.43 as compared to control 37.30 ± 1.26. The microbial consortia was found significant in managing the disease incidence under field condition.

Keywords: *Sclerotium rolfsii*, French bean, Endophytes, Microbial Consortia.

INTRODUCTION

French bean is an important vegetable food and also an export crop in India. French beans are grown throughout the world and contribute nearly 30% of the total production of food legumes (Vasishtha and Srivastava, 2012). French bean is a rich source of nutrients and minerals and crude fibre are concentrated in seed while crude protein and energy are stored in cotyledons (Singh *et al.,* 1997). India is one of the leading pulse growing countries in the world, sharing about 35-36% of the total production of the different pulse crops (Pradan, 2014). French bean crop prone to many diseases such as anthracnose, pod rot, bean rust, white mold and *Fusarium* rot. Among these, pod rot incited by *S. rolfsii* Sacc. causes 40-50% yield loss in India (Dasgupta *et al*., 2005). The fungus *S. rolfsii* was first observed in United States (Rolf, 1892). *S. rolfsii* incites pod rot diseases on many crops. *S. rolfsii* favours high temperature of 25-35°C and Relative humidity 85%. Under heavy soil moisture sometimes more than 60-70% crops have been damaged due to this disease. Symptoms are typified by the development of white fungal thread over the affected pods. The white profuse mycelial growth of fungus covers the entire pod. The pathogen attacks the germinating seedlings and causes wilt. The pathogen produces initially white coloured sclerotia, later turned to dark brown and the small round bodies about the size of mustard seed like sclerotia. The large number of sclerotia produced by *S. rolfsii* and their ability to persist in the soil for several years, as well as the profuse growth of the fungus make it well suited facultative parasite and a pathogen of major importance throughout the world (Punja, 1985). *S. rolfsii* is a soil borne pathogen which infects more than 500 plant species including brinjal, bean, cucumber, groundnut, maize, soybean, tomato and water milon (Sharma *et al.,* 2002). *S. rolfsii* has wide host range and difficult to control by chemicals alone, because the fungus produces sclerotia and survives in soil for a longer period of time. Hence, alternative method of using bacterial endophytes as biocontrol agents for managing the disease pathogen. Bacterial endophytes are those bacteria which asymptomatically inhabit the internal tissues of plants and they colonize the same ecological niches as disease causing

organisms (Chen *et al.*, 1995). Endophytes are ubiquitous, colonize most of the plants and have been isolated from all the plants till date. Endophytes are biocontrol agents often effective against plant diseases (Hultberg *et al*., 2010).

MATERIALS AND METHODS

Collection and maintenance of bacterial endophytes: The bacterial endophytes were collected from the repository of School of Crop Protection laboratory, School of Crop Protection, College of Post graduate Studies in Agricultural Sciences, Umiam, Meghalaya. The bacterial isolates were further streaked in a fresh Nutrient Agar (NA) medium with the help of sterile inoculating loop to obtain pure culture. They were incubated at 28 ± 1°C for 24 h. For further maintenance of pure culture, the bacterial endophytes were transferred to NA slants by regular sub-culturing under aseptic condition and were maintained at 4°C in refrigerator.

In vitro* evaluation of bacterial endophyte (*Bacillus* sp.) against *Sclerotium rolfsii

Six bacterial endophytes *viz*., BS80, BS118, and BS178, BS 1032, BS78 and BS179 were tested for their biocontrol potential against the pathogen on PDA medium using the Dual culture technique (Ganasen and Gnanamanickam 1987).

Dual culture Assay: The fungal culture was grown on PDA plate for 2-3 days. With the help of sterilized cork borer 5 mm diameter fungal discs were cut from the periphery of the culture plate and placed at the center of the fresh PDA plates. 24 h old culture of bacterial strains were then streaked parallel on both the side of the fungal disc 1 cm away from the disc. Three replications were maintained. The plates were then kept for incubation at 28 ± 1°C for 3 days. Visual observations on the inhibition of the growth of fungal pathogen were recorded 3rd day after incubation and compared with the PDA plate simultaneously inoculated with only the fungal pathogen and inhibition per cent was recorded by following the method described by Vincent, (1947) as

$$\text{Inhibition (\%)} = \frac{C-T}{C} \times 100$$

where, "C" is the maximum growth of the fungal mycelia under control

"T" is fungal mycelia growth in dual culture.

Experimental design and statistical analysis: Data analysis for the dual culture study against the pathogen was done by using One-way ANOVA. The significant difference, if any, among the treatment means were compared using critical difference (CD) at p = 0.05

Compatibility test among the potential bacterial endophytes: The potential bacterial endophytes were tested for their compatibility among each other following the method of Fukui *et al.* (1994). The bacterial strains were streaked horizontally and vertically to each other. The plates were incubated at room temperature (28±1°C) for 72 h and observed for the inhibition zone. Absence of inhibition zone indicates the compatibility with respective bacterial strains and the presence of inhibition zone indicated the incompatibility.

Preparation of the Microbial Consortia (MC): The preparation of the formulation was done following the method described by Nandakumar *et al.*, (2002). The three screened potential bacterial endophytes *viz.*, BS80, BS178, and BS118 were separately grown in LB broth. The bacterial suspension were mixed (v/v) to make the microbial consortia. BS80 and BS178 were mixed to make microbial consortium (MC1). Likewise BS178 and BS118 were mixed to make another microbial consortium (MC2), BS118 and BS80 were also mixed to make microbial consortium (MC3) and combination of all bacterial antagonists i.e. BS80, BS178, and BS118 together to make microbial consortium (MC4). Each microbial consortium containing 1×10^8 CFU ml^{-1} was used for seed treatment and soil application respectively.

Evaluation of Microbial consortia for the PGP activities on French bean *in vitro*

The French bean seeds (15 numbers) were soaked in 50 ml of the microbial consortia (10^8 cfu/ml) of BS80 + BS178, BS178 + BS118, BS80 + BS118 and BS80 + BS178 + BS118 respectively and the treated seeds were kept inside the Petri plates lined with the moistened filter paper. The seeded plates were kept in room temperature. Each treatment was replicated into 3 times, seeds soaked in sterile water only served as control. Observation were taken after 21 days of incubation for germination per cent, shoot length (cm) and root length (cm) and Vigour Index (VI) by following the methods described by Gopalakrishnan *et al.*, (2012) as

Vigour index (Vi) = (RL + SL) × GP

Where,

RL = mean root length (cm), SL = mean shoot length (cm), GP = Germination per cent

Evaluation of the MC against *Sclerotium rolfsii* Sacc. under field condition: Field experiment was conducted to manage Pod rot of French bean with the total area of 100 m^2 and each plot was having the size of 1.2×1.2 m^2. The experiment was laid out in randomized block design with three replications using the local cultivar of French bean.

Field preparation: Power tiller was used for making soil of good tilth and field was exposed to bright sunshine and final ploughing was done. Clods and stubbles were also removed from the field to ensure the proper growth of crop and the field was incorporated with well decomposed FYM to maintain its soil fertility.

The Microbial Consortia (MC) which were prepared from the 3 screened potential endophytes.

Where MC 1 = combination of two efficient screened endophytes (E1+E2)

MC 2 = combination of two efficient screened endophytes (E2+E3)

MC 3 = combination of two efficient screened endophytes (E3+E1)

MC 4 = combination of three efficient screened endophytes (E1+E2+E3)

Table 1: Treatments details.

T_1	Seed treatment + soil application with endophyte 1 (E1)
T_2	Seed treatment + soil application with endophyte 2 (E2)
T_3	Seed treatment + soil application with endophyte 3 (E3)
T_4	Seed treatment + soil application with MC 1
T_5	Seed treatment + soil application with MC 2
T_6	Seed treatment + soil application with MC 3
T_7	Seed treatment + soil application with MC 4
T_8	Seed treatment + soil application with fungicide
T_9	Control

Experimental design and statistical analysis: The experiment was laid out in a Randomized Block Design (RBD). Data were analysed using Two-way analysis of variance (ANOVA) with three replication and control. (Gomez and Gomez, 1984).

RESULTS

Six screened bacterial endophytes *viz.,* BS80, BS178, BS179, BS118, BS1032 and BS78 were collected from the repository of SCP laboratory. Out of which BS80, BS178 and BS118 showed more effective against the pathogen.

Compatibility test among the potential bacterial endophytes: For the development of microbial consortia formulation and evaluating PGP traits *in-vitro* were conducted by following the method described by Fukui *et al.,* (1994). Among six bacterial endophytes tested, E1, E2 and E3 exhibited highest inhibition. The three potential screened endophytes (E1, E2 and E3) were streaked vertically and horizontally on NA plates in such a way that the bacterial endophytes meet the other isolates. After the incubation it was observed that, all the bacterial antagonists selected for making the consortia were compatible to each other showing no zone of inhibition at the point of contact between the bacterial antagonists.

Table 2: *In-vitro* evaluation of bacterial endophytes against *S. rolfsii* Sacc.

Bacterial isolates	Mycelial growth (cm)	Inhibition of growth over control (%)
E1	3.13±0.15^{e}	65.18±1.01(53.82^{a})
E2	3.36±0.25^{e}	62.59±1.65(52.28^{a})
E3	3.86±0.25^{d}	57.03±1.62(49.02^{b})
E4	4.16±0.15^{d}	53.70±0.97(47.10^{b})
E5	5.36±0.15^{c}	40.37±0.99(39.43^{c})
E6	6.30±0.54^{b}	29.92±3.82(33.07^{d})
Control	9.00±0.00^{a}	0.00±0.00(0.95^{e})
SEm (±)	**0.15**	**1.05**
CD (0.05)	**0.46**	**3.17**

Data in parentheses are arc sine transformed values. Data followed by different small letters after mean values within each column indicate significant difference among treatments using ANOVA p=0.05

(a) (b) (c) (d)

(a) E1 (BS80) + *Sclerotium rolfsii* (b) E2 (BS118) + *Sclerotium rolfsii;* (c) E3 (BS178) + *Sclerotium rolfsii* (d) *Sclerotium rolfsii*

Plate 1: Dual culture assay.

Table 3: Compatibility test of the endophytes.

Isolates	E1	E2	E3
E1	+	+	+
E2	+	+	+
E3	+	+	+

where '+' indicates compatibility and '-' indicates incompatibility

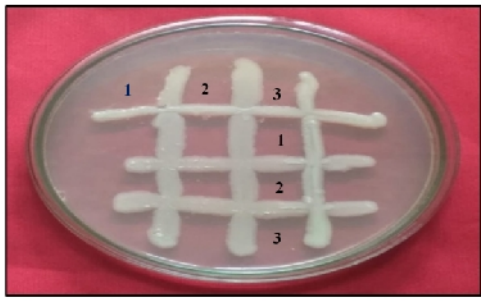


Plate 2: Compatibility test among three potential isolates 1. E1 2. E2 3. E3

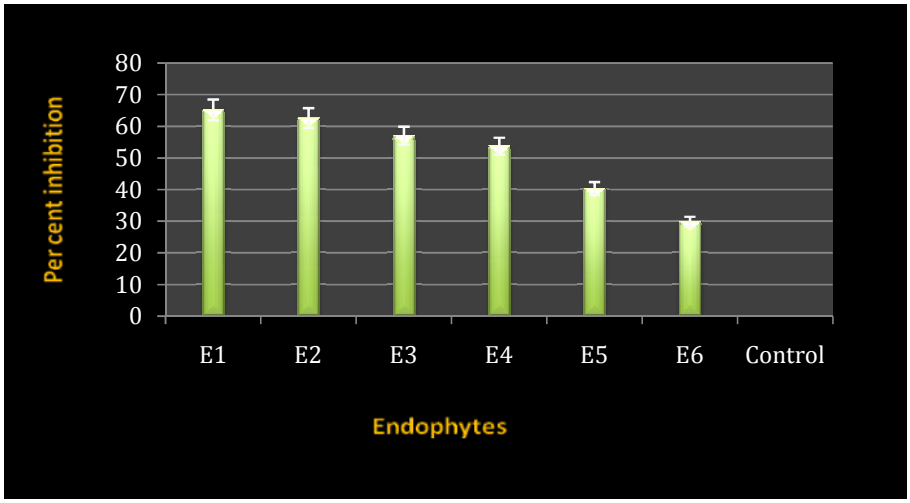


Fig. 1. *In-vitro* evaluation of bacterial endophyte (*Bacillus* spp.) against *S. rolfsii* Sacc.

Table 4: Evaluation of endophytes alone and in consortia on French bean seeds under *in-vitro* conditions.

Isolates	Germination (%)	Shoot length (cm)	Root length (cm)	Vigour
E1	83.33(64.67±1.98)^{b}	14.40±0.43^{cde}	8.37 ± 0.57^{d}	1860.00^{bc}
E2	82.67(63.27± 2.18)^{b}	14.20 ± 0.23^{de}	8.43 ± 0.52^{cd}	1770.00^{c}
E3	81.00(62.27± 1.98)^{b}	14.17 ± 0.40^{de}	8.43 ± 0.11^{cd}	1769.67^{c}
MC1 (E1+E2)	86.67(66.93± 2.18)^{b}	15.17± 0.56^{b}	8.90 ± 0.45^{bc}	2007.33^{b}
MC2(E2+E3)	84.33(64.67± 3.18)^{b}	14.63 ± 0.32^{bcd}	8.89 ± 0.15^{b}	1930.67^{bc}
MC3(E3+E1)	85.00(65.93± 2.18)^{b}	14.97 ± 0.52^{bc}	8.87 ± 0.05^{b}	1994.33^{b}
MC4(E1+E2+E3)	88.67(75.21 ± 2.18)^{a}	16.13 ± 0.35^{a}	10.33 ± 0.45^{a}	2470.33^{a}
Control	78.33(56.82 ± 3.13)^{c}	13.80 ± 0.52^{e}	8.10 ± 0.10^{d}	1534.67^{d}
SEm (±)	**1.40**	**0.25**	**0.18**	**57.04**
CD (0.05)	**4.19**	**0.75**	**0.53**	**170.99**

Data in parentheses are arc sine transformed values. Data followed by different small letters after mean values within each column indicate significant difference among treatments using ANOVA at p=0.05

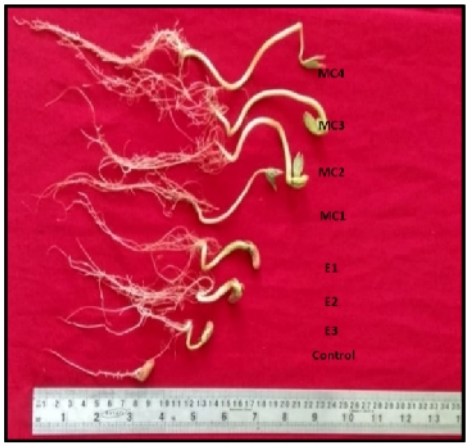


Plate 3: Vigour index of French bean seeds at 21th day.

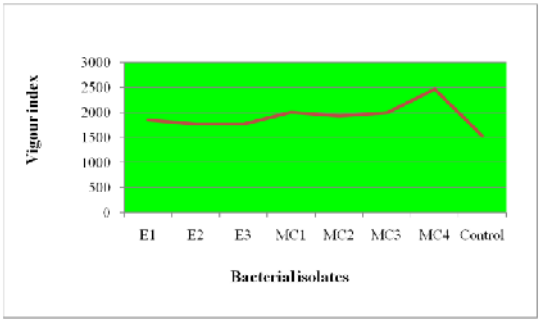


Fig. 2. Vigour index of French bean seeds.

Evaluation of the endophytes alone and in consortia against *Sclerotium rolfsii* Sacc. under field condition: The three screened potential bacterial endophytes were evaluated against pod rot of French bean under field conditions. Different treatments were followed *viz.*, seed treatment, soil application, seed + soil application with different strains *Bacillus subtilis*, mancozeb @0.2% as chemical check and control were followed during the field evaluation. The percent disease index was recorded with the method given by Mayee and Datar, (1986). The experiment revealed that the seed treatment + soil application with MC4 was found to be the best in managing the disease with lowest per cent disease index of 15.87 whereas control showed PDI of 37.30.

Table 5: Evaluation of endophytes against *S. rolfsii* Sacc. under field condition.

Treatments		PDI		Plant height(cm) 90 DAS	No. of branches/ plant 90 DAS	No. of pods/plant 90 DAS	Weight of 10 pod/plant(g) 90 DAS
		(75 DAS)	(90 DAS)				
T1	ST + SA with E1	24.40 ± 0.20 (29.59)^{b}	26.23 ± 0.86 (32.43)^{b}	30.97 ± 1.72^{a}	4.27 ± 0.37^{a}	18.03 ± 0.25^{a}	78..98 ± 0.25^{c}
T2	ST + SA with E2	25.43 ± 0.64 (33.10)^{ab}	28.23 ± 1.31 (33.17)^{ab}	29.91 ± 1.36^{b}	4.33 ± 0.11^{a}	17.50 ± 0.65^{b}	77.11 ± 0.65^{c}
T3	ST + SA with E3	26.60 ± 0.66 (27.60)^{a}	29.97 ± 1.29 (27.11)^{c}	28.97 ± 1.22^{b}	4.67 ± 0.57^{a}	17.07 ± 0.76^{a}	76.82 ± 0.76^{c}
T4	ST + SA with MC1	21.60 ± 0.78 (28.10)^{b}	23.57 ± 0.65 (29.96)^{b}	37.88 ± 2.06^{a}	4.57 ± 0.40^{a}	19.53 ± 0.20^{ab}	85.23 ± 0.40^{ab}
T5	ST + SA with MC2	22.77 ± 0.64 (31.74)^{b}	24.97 ± 0.80 (32.71)^{a}	36.63 ± 1.15^{a}	4.30 ± 0.51^{a}	18.90 ± 0.10^{a}	84.67 ± 0.18^{b}
T6	ST + SA with MC3	23.63 ± 0.66 (29.72)^{b}	25.13 ± 0.32 (30.08)^{b}	35 ± 1.95^{a}	4.87 ± 1.51^{a}	19.03 ± 1.02^{a}	84.12 ± 0.22^{b}
T7	ST + SA with MC4	13.30 ± 0.44 (21.69)^{c}	15.87 ± 0.43 (24.38)^{d}	43.12 ± 1.28^{a}	5.67 ± 0.23^{a}	23.43 ± 0.52^{a}	94.70 ± 0.36^{a}
T8	ST + SA with mancozeb	19.67 ± 0.46 (23.42)^{c}	20.53 ± 0.49 (25.30)^{d}	36.17 ± 0.90^{a}	4.70 ± 0.17^{a}	17.80 ± 0.52^{a}	74.18 ± 0.44^{a}
T9	Control	35.53 ± 0.71 (36.23)^{a}	37.30 ± 1.26 (37.63)^{a}	24.80 ± 0.30^{b}	3.87 ± 0.23^{b}	12.30 ± 0.26^{b}	62.37 ± 0.16^{d}
SEm(±)		1.06	1.09	2.44	0.05	0.92	1.98
CD (0.05)		3.06	2.89	1.06	1.64	3.09	2.09

Data in parentheses are arc sine transformed values. Data followed by different small letters after mean values within each column indicate significant difference among treatments using ANOVA at p=0.05.

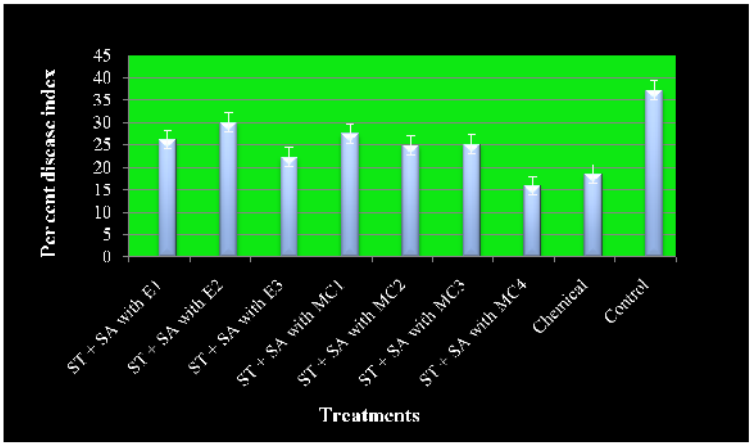
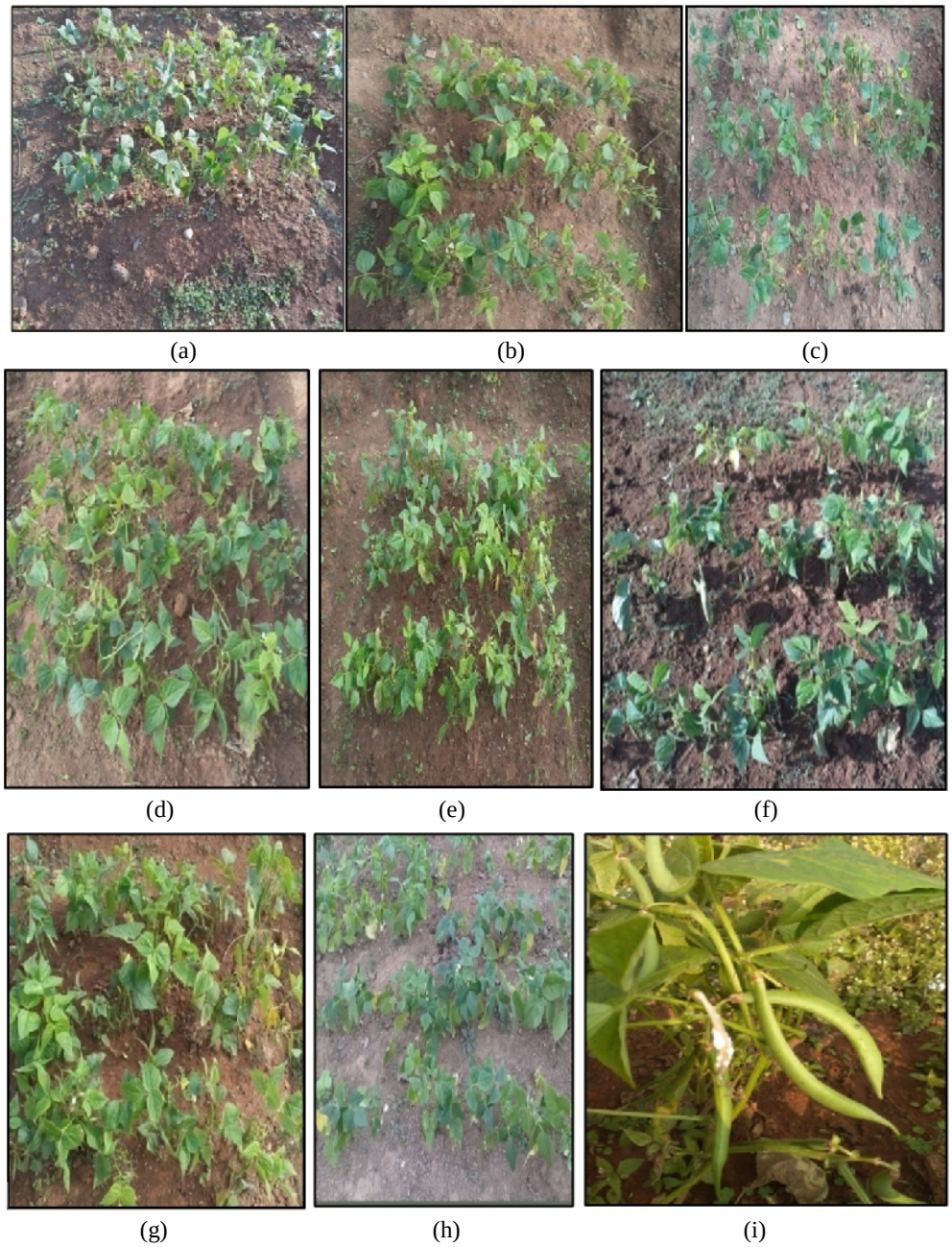


Fig. 3. Percent disease index of different treatments under field condition.

(a) Experimental plot of French bean. (b) Experimental field view during pod bearing stage.

Plate 4.



(a) (b) (c)

(d) (e) (f)

(g) (h) (i)

Plate 5: Growth of French bean in (a) E1 (b) E2 (c) E3 (d) MC1 (e) MC2 (f) MC3 (g) MC4 (h) Chemical (i) Control in field conditions.

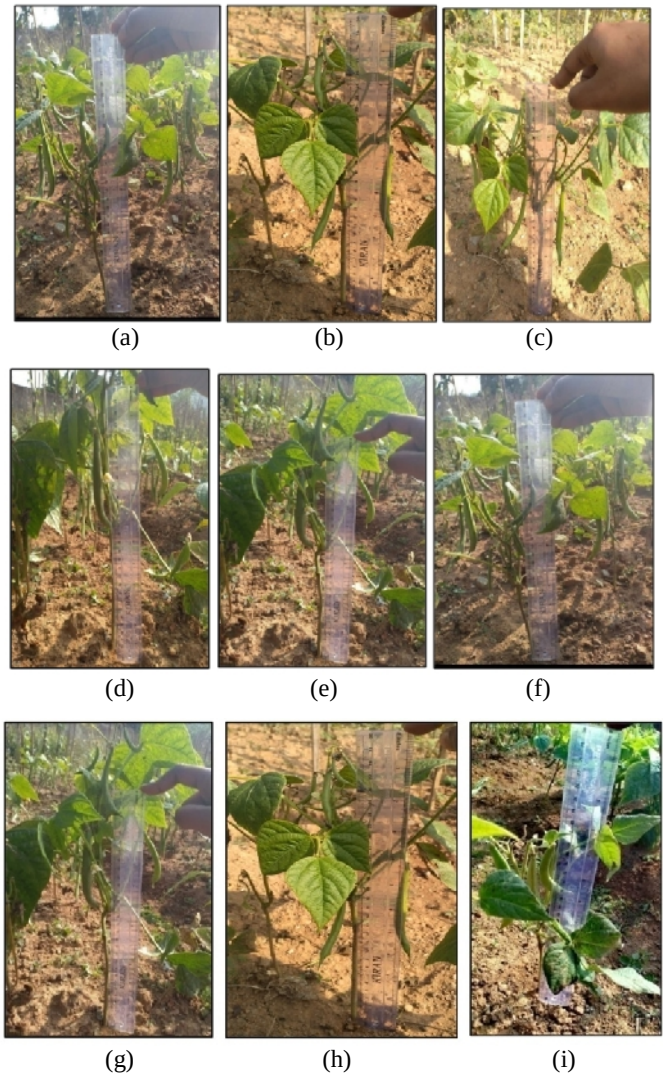


Plate 6: Plant height in - treatment with (a) E1 (b) E2 (c) E3 (d) MC1 (e) MC2 (f) MC3 (g) MC4 (h) Chemical (i) Control.

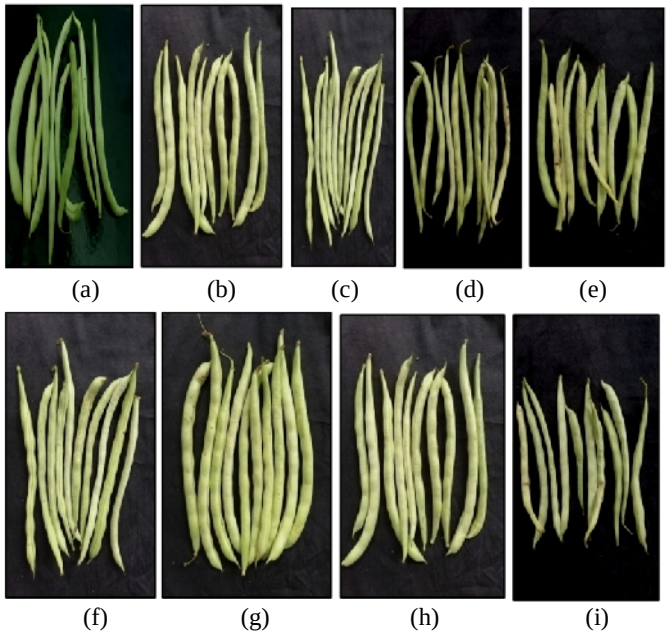


Plate 7: Yield in -treatment with (a) E1 (b) E2 (c) E3 (d) MC1 (e) MC2 (f) MC3 (g) MC4 (h) Chemical (i) Control.

CONCLUSION

From the present investigation, it may be concluded that pod rot of French bean incited by *S. rolfsii* Sacc. was found predominantly well distributed in different localities of Meghalaya. The microbial consortia efficacy was tested in field conditions based on their antagonism and *in-vitro* plant growth promotion studies. Seed treatment + Soil application of Microbial consortium (MC4) gave significant result with PDI of 15.87 ± 0.43 as compare to control which gave PDI of 37.30 ± 1.26. Thus it can be concluded that the microbial consortia as biocontrol agents can help to manage the disease incidence providing safer, eco-friendly and economical management. Thus these consortia can be evaluated for multi-location trial for further investigation in future and can implement for successful integrated disease management (IDM) in Meghalaya.

FUTURE SCOPE

Biological control is great aspect in lowering the utilization of pesticides for managing the plant disease. It often involves biocontrol agents that would able to interact with either a plant or pathogens of plant to reduce the growth of pathogen and limits its negative impact on the host plant. Bacterial endophytes promote plant growth and yield and can acts as biocontrol agents. Ultimately, exclusive studies on the endophytic bacteria would reveal useful information for effective disease management without causing harm to other biosystem.

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Conflict of Interest. None.

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