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Efficacy of bioformulations of indigeneous bacterial bioagents strains against bacterial wilt of *Curcoma longa* L.

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The present study was undertaken to explore the possibility of using the formulation of indigenous strains of *Bacillus megaterium, Bacillus subtilis, Pseudomonas putida*, and consortia of these for evaluating the efficacy of these bio-agents on turmeric plant to manage the bacterial wilt of the economically important crop turmeric caused by *Ralstonia solanacearum*. In an attempt to evolve a biological management of the disease, the formulation of the antagonistic strains of *Bacillus megaterium, Bacillus subtilis*, *Pseudomonas putida*, and consortia of these was used for enrichment of farm yard manure for field application. The population dynamics of *Ralstonia solanacearum* in turmeric rhizosphere soil showed that the crop receiving T1+ application of 5 kg enriched farm yard manure with *Bacillus subtilis* treatment had the lowest population recovery of the pathogen at 75 Days after transplantation ($13.42x10^{-4}$ cfu/g) and at the termination of the experiment ($4.99\pm0.11x10^{-4}$ cfu/g). The percent leaf spot incidence at different days after transplanting (DAT) was found to be lowest at 75 Days after transplantation (7.56±0.022%) and termination (10.0±0.023%) in T1+ application of 5 kg enriched farm yard manure with *Pseudomonas putida* treatment. The yi)eld and yield attributes were found to be best performing in almost all the treatments of the antagonist formulations indicating their potential as PGPM.

Keywords: Bacterial wilt, *Ralstonia solanacearum*, *Pseudomonas putida*, *Bacillus megaterium, Bacillus subtilis,* PGPR.

Introduction

Turmeric is one of the major species cultivated for its underground rhizome, which is also called as hidden lily or turmeric of commerce (Suresh Muthukulam, 2001). It has versatile uses in flavoring, dye making, drug

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preparation, cosmetics and medicine (Kallupurachkal and Ravindran, 2002). So far, around 100 active constituents have been recorded from turmeric (Ross,1999; Roth, 1998). The Jaintia Hills District of Meghalaya, India produces some of the finest turmeric in the world with its "Lakadong" (*Curcuma longa*) variety. Due to its high curcumin content, robust aroma, colour and organic nature, this variety could have good market potential in India and possibly abroad. This herbal plant is highly prone to several phytopathogens. *Ralstonia solanacearum* is an aerobic non-sporing, Gram-negative plant pathogenic bacterium and highly heterogeneous bacterial pathogen that causes severe wilting in many economically important crops (Smith *et al.,* 1995). Due to its devastating lethality, *R. solanacearum* is now one of the more intensively studied phytopathogenic bacteria. The present investigation was, therefore, undertaken to evaluate the Bio-efficacy of talc formulation of bioagents in the management of *Ralstonia solanacearum* (bacterial wilt) infecting turmeric.

Materials and methods

Study sites, land preparation and transplanting

To evaluate the bio-efficacy of various talc biopesticides against *Ralstonia solanacearum* infecting turmeric plant (*Curcuma longa* L.*)* a field experiment was conducted during April, 2014 in turmeric crop at Shangpung village of Jaintia Hills District Meghalaya. Shangpung village is situated in the eastern part of the state of Meghalaya and lies between 25^{o}18' N latitudes and 92^{o}22' E longitudes. Formulated talc biopesticides of *Bacillus megaterium*, *Bacillus subtilis*, *Pseudomonas putida*, and consortia of these were used for evaluating the efficacy of these bio-agents on turmeric plant by adopting randomized block design consisting of six treatments including an untreated control which was replicated thrice with plot size of 13.12 x 8.2 ft. and wide ridges (1 meter) were made in each (Table 1).

Table 1. Developed bioformulations and their mode of application.

Plot size : 4x 2.5m=10sq.m (13.12 x 8.2ft = 107.58sq.ft) **Total area** = T2+T3+T4+T5+T6+T7= 6 x 3 replicates =180sq.mt (or) 1936.51 sq.ft				
No. of Plots	**Replicate 1**	**Replicate 2**	**Replicate 3**	**Total**
	(13.12 x 8.2ft =107.58sq.ft)	(13.12 x 8.2ft =107.58sq.ft)	(13.12 x 8.2ft =107.58sq.ft)	
T-2	**T1** + Application of 2.5kg of enriched FYM	**T1** + Application of 2.5kg of enriched FYM	**T1** + Application of 2.5kg of enriched FYM	7.5Kg
T-3	**T1** + Application of 5kg of enriched FYM	**T1** + Application of 5kg of enriched FYM	**T1** + Application of 5kg of enriched FYM	15Kg
T-4	Application of 2.5kg of FYM	Application of 2.5kg of FYM	Application of 2.5kg of FYM	7.5Kg
T-5	Application of 5kg of FYM	Application of 5kg of FYM	Application of 5kg of FYM	15Kg
T-6	Chemical treatment: Mix carbofuran-33g +streptocyclin-9g in 30 litres of water and used for drenching.	Chemical treatment: Mix carbofuran-33g +streptocyclin-9g in 30 litres of water and used for drenching.	Chemical treatment: Mix carbofuran-33g +streptocyclin-9g in 30 litres of water and used for drenching.	99g+27g
T-7	Control	Control	Control	

Process of enrichment of organic manure for field application

2kg of talc formulation of bioagents viz., *Bacillus subtilis, Pseudomonas putida, Bacillus megaterium* and consortia of these was mixed with 2000 kg of farm yard manure (FYM) and kept for 18 days maintaining optimum moisture conditions. Every 2 days once the manure was mixed well.

Treatment of turmeric rhizome

5g of talc formulation of bioagents viz., *Bacillus subtilis, Pseudomonas putida, Bacillus megaterium* and consortia of these was added to 1 liter of water. The rhizome were treated by drenching in these suspensions for 30 minute and dried before plantation.

Transplantation of Seedlings into the Experimental plot

The enriched manure was used for field application to beds according to the treatments given in Table 1.

Estimation of Ralstonia solanacearum population density

The rhizosphere soil after 75 days and after termination of the experiment was enumerated by serial dilution technique and spread plate method on triphenyl tetrazolium chloride (TTC) agar. Three replicates were maintained for each of the dilutions. The cfu from each plate were counted out and population density of *R. solanacearum*/g rhizosphere soil was calculated as follows:

No. of org/g rhizosphere soil = Average No. of colonies in a dilution x Dilution factor / Dry weight of soil (g).

Measurement of disease incidence

After 75 days and termination of the experiment, the leaf spot incidence was recorded using the following formula:

The % leaf spot incidence = No. of occurrence of leaf spot in a treatment x 100 / Total no. of plants receiving that treatment.

Analysis of yield and yield attributes of treated crops to evaluate the* efficacy *of the biocontrol agent as PGPM

Data were recorded on Average fruit weight (g)/plant, No. of branches/plant, No. of fruits/plant, Yield/plant, Plant height and Mean leaf area of the treated turmeric plants to evaluate the efficacy of bioagents of these as plant growth promoting microorganisms using the method of Gargi *et. al.* (2012).

Results and discussion

The population density (cfu/g rhizosphere soil) of *R. sonalacearum* of rhizosphere soil of turmeric was calculated at 75 days after transplanting (DAT) and at the termination of the experiment and the results is represented in Table 1. Among the different treatments, the lowest population of the pathogen recorded in turmeric rhizosphere was in T1+ application of 5 kg enriched farm yard manure with *Bacillus subtilis* treatment i.e., 13.42×10^{-4} cfu/g and the

highest population of the pathogen was recorded in the control (without any treatment) i.e., $63.35x10^{-4}$ cfu/g. These results show that *Bacillus subtilis* is potentially a potent bio control agent for use in controlling bacterial wilt caused by *R. solanacearum.* This behavior represents an important approach for controlling wilt disease in turmeric. The potentialities of the used strains could be attributed to their effect to secrete hydrolytic enzymes or antifungal metabolites. Ongena and Jacques (2008) reported that *B. subtilis,* and other members of the genus *Bacillus,* have long been used as biological control agents (BCAs) in agriculture. How *B. subtilis* exerts strong biocontrol activities in the rhizosphere is not well understood. Nagorska *et al.* (2007) reported that production of antimicrobial agents, biofilm formation, and triggering of host systemic resistance contribute to the biocontrol activities of *B. subtilis*.

Table 2. Population density of *R. solanacearum* in turmeric rhizospheric soil at different DAT.

		Population density of *Ralstonia solanacearum* (10^{-4} cfu/g rhizosphere soil)		
		Days after transplantation (DAT)		
Plots	**Treatments**	**75 Days**	**Termination**	**Mean**
T-2	T1+ application of 2.5 kg enriched farm yard manure with *Bacillus megaterium*	52.56±0.12	35.12±0.83	43.84
T-2	T1+ application of 2.5 kg enriched farm yard manure with *Bacillus subtilis*	27.37±0.09	4.99±0.11	16.18
T-2	T1+ application of 2.5 kg enriched farm yard manure with *Pseudomonas putida*	62.20±0.25	3.40±0.17	32.8
T-2	T1+ application of 2.5 kg enriched farm yard manure with *Bacillus megaterium + Bacillus subtilis + Pseudomonas putida*	61.58±0.09	11.13±0.23	36.36
T-3	T1+ application of 5 kg enriched farm yard manure with *Bacillus megaterium*	50.23±0.11	6.95±0.07	28.59
T-3	T1+ application of 5 kg enriched farm yard manure with *Bacillus subtilis*	22.39±0.58	4.44±0.08	13.42
T-3	T1+ application of 5 kg enriched farm yard manure with *Pseudomonas putida*	29.70±0.18	5.12±0.09	17.41
T-3	T1+ application of 5 kg enriched farm yard manure with *Bacillus megaterium + Bacillus subtilis + Pseudomonas putida*	23.63±0.14	8.41±0.44	15.89
T-4	Application of 2.5 kg farm yard manure	84.60±0.33	10.55±0.02	47.58
T-5	Application of 5 kg farm yard manure	48.21±0.54	9.47±0.22	25.84
T-6	Chemical treatment: 33g of carbofuran + 9g of streptocyclin by drenching	59.27±0.22	16.87±0.04	38.07
T-7	Control	90.55±0.01	36.14±0.8	63.35

Table 2 represents the effect of different treatments of bioformulation on percentage leaf spot incidence at 75 days after transplanting (DAT) and at the termination of the experiment. The percent leaf spot incidence at different DAT was found to be lowest at 75 Days after transplantation (7.56±0.022%) and termination (10.0±0.023%) in T1+ application of 5 kg enriched farm yard manure with *Pseudomonas putida treatment.* However all the bioformulations tested showed their ability to reduce the disease incidence. Burr *et al*. (1978) reported that *Pseudomonas* spp. can aggressively colonize root systems and are metabolically very active and have a high growth. *Pseudomonads* spp. can also produce plant hormones and other growth promoting substances such as auxins (Loper and Schroth, 1986), gibberellins (Ramamoorthy *et al.,* 2002) and 1-aminocyclopropane-1-carboxylate deaminase (Jacobson *et al.,* 1994). Thus they play a role in growth promotion of the plant. Priou *et al.* (2005) also recorded 80% reduction of the tomato bacterial wilt disease using *Pseudomonas putida*.

Performance of yield and yield attributes of treated crops to evaluate the efficacy of the antagonists formulations as PGPR

Table 3 reveals the average size of rhizome (g), yield/plant (kg) and the number of rhizomes/plant. The average size of rhizome ranges from 0.10 to 0.25 grams, the number of rhizomes per plant ranges from 2 to 6 and the yield per plant ranges from 1.0kg to 3.20kg. The yield and yield attributes were found to be best performing in almost all the treatments of the antagonist formulations indicating their potential as PGPR. Jinnah *et al.* (2002) reported that the biocontrol agent *Pseudomonas fluorescens* produced positive effect on the plant growth characters such as plant height, number of branches / plant and yield characters such as fruit yield, total fruit weight / plant and number of fruits/ plant. Kumar *et al.* (2001) also reported that the potential use of five plant growth promoting fluorescent *Pseudomonas* strains isolated from Indian and Swedish soils suggested that these bacteria induce plant growth and disease suppression in sustainable agriculture production systems.

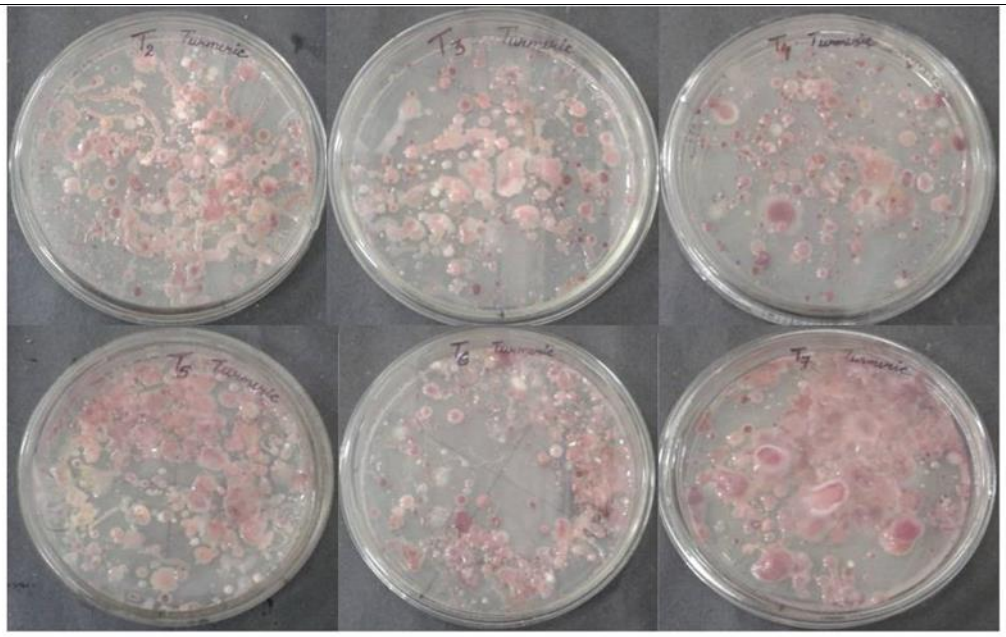

Plate 1. Colony Forming Unit of *Ralstonia solanacearum* from the treated plots.

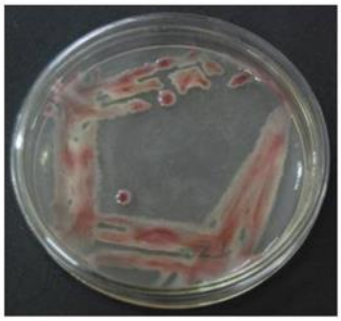

Plate2. Pure culture of *Ralstonia solanaecearum*.

Table 3. Effect of formulated talc bioagents applied in different methods on % leaf spot incidence of turmeric at different DAT

		% leaf spot incidence	
		Days after transplantation (DAT)	
Plots	**Treatments**	**75 Days**	**Termination**
T-2	T1+ application of 2.5 kg enriched farm yard manure with *Bacillus megaterium*	24.53±0.01	44.4±0.011
T-2	T1+ application of 2.5 kg enriched farm yard manure with *Bacillus subtilis*	14.55±0.02	30.0±0.01
T-2	T1+ application of 2.5 kg enriched farm yard manure with *Pseudomonas putida*	25.22±0.08	36.4±0.03
T-2	T1+ application of 2.5 kg enriched farm yard manure with *Bacillus megaterium + Bacillus subtilis + Pseudomonas putida*	13.87±0.021	30.7±0.06
T-3	T1+ application of 5 kg enriched farm yard manure with *Bacillus megaterium*	21.22±0.024	30.0±0.01
T-3	T1+ application of 5 kg enriched farm yard manure with *Bacillus subtilis*	11.53±0.023	30.0±0.06
T-3	T1+ application of 5 kg enriched farm yard manure with *Pseudomonas putida*	7.56±0.022	10.0±0.023
T-3	T1+ application of 5 kg enriched farm yard manure with *Bacillus megaterium + Bacillus subtilis + Pseudomonas putida*	10.0±0.023	25.0±0.035
T-4	Application of 2.5 kg farm yard manure	35.54±0.011	83.3±0.016
T-5	Application of 5 kg farm yard manure	23.88±0.025	42.8±0.025
T-6	Chemical treatment: 33g of carbofuran + 9g of streptocyclin by drenching	15.77±0.014	22.23±0.22
T-7	Control	59.56±0.011	100 ±0.0

Table 4. Performance of yield and yield attributes of the treated crops.

Plot	Treatment	Average size of rhizome in grams	Yield in kg	Number of rhizome per plant
T-2	T-1+ 2.5 kg FYM enriched with *Bacillus megaterium*	0.20±0.05	1.00±0.11	3
T-3	T-1 + 5 kg FYM enriched with *Bacillus megaterium*	0.20±0.02	1.50±0.23	2
T-4	2.5 kg FYM	0.20±0.03	1.25±0.12	3
T-5	5 kg FYM	0.15±0.01	2.75±0.56	5
T-6	Chemical pesticides	0.15±0.02	1.50±0.13	3
T-7	Control	0.10±0.02	1.20±0.11	3
T-2	T-1+ 2.5 kg FYM enriched with *Bacillus subtilis*	0.20±0.01	2.75±0.12	4
T-3	T-1 + 5 kg FYM enriched with *Bacillus subtilis*	0.20±0.05	2.75±0.25	4
T-4	2.5 kg FYM	0.15±0.13	2.00±0.22	4
T-5	5 kg FYM	0.15±0.03	2.25±0.33	6
T-6	Chemical pesticides	0.20±0.06	2.00±0.22	4
T-7	Control	0.15±0.11	2.00±0.82	3
T-2	T-1+ 2.5 kg FYM enriched with *Pseudomonas putida*	0.20±0.06	1.00±0.10	2
T-3	T-1 + 5 kg FYM enriched with *Pseudomonas putida*	0.15±0.05	2.75±0.17	3
T-4	2.5 kg FYM	0.15±0.01	1.50±0.23	2
T-5	5 kg FYM	0.20±0.09	3.20±0.42	5
T-6	Chemical pesticides	0.10±0.07	2.50±0.85	3
T-7	Control	0.15±0.22	1.75±0.34	4
T-2	T-1+ 2.5 kg FYM enriched with consortia	0.20±0.04	2.25±0.33	5
T-3	T-1 + 5 kg FYM enriched with consortia	0.25±0.03	1.25±0.25	3
T-4	2.5 kg FYM	0.20±0.11	1.25±0.22	2
T-5	5 kg FYM	0.15±0.09	2.50±0.23	4
T-6	Chemical pesticides	0.15±0.07	2.00±0.33	3
T-7	Control	0.15±0.12	2.25±0.22	3

Conclusion

Bacillus subtilis and Pseudomonas putida are potentially potent biocontrol agents for use in controlling bacterial wilt caused by *R. solanacearum.* Besides biocontrol properties, the bioformulations also show best performance in yield, yield attributes, physiological and biochemical parameters indicating their plant growth promoting potential.

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