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Different Formulation of Pgpb Cells and Challenge Inoculation of *Sclerotium rolfsii* on the Enhancement of Growth and Yield Parameters and Induced Systemic Resistance (ISR) in Groundnut (*Arachis hypogaea* L.)

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ABSTRACT

The effect of different bioformulations of viz., single strain inoculation, co-inoculation and natural co-aggregates application of PGPB cells viz., Bradyrhizobium and Methylobacterium together with supplementation of salicylic acid and challenge inoculation of *Sclerotium rolfsii* on the enhancement of growth and yield in rainfed groundnut with special emphasis to biocontrol against stem rot disease (*Sclerotium rolfsii*) the application of co-aggregates (natural) along with the salicylic acid could augment the plant height, root, shoot dry weight, nodules number and weight, disease incidence, seed weight and haulm yield of groundnut to a higher level followed by co-aggregates (natural), co-inoculation with salicylic acid, co-inoculation, vegetative cells with salicylic acid and vegetative cells alone treatments. Moreover the "Intergeneric PGPB co-aggregates with salicylic acid application" and augmented the ISR mediated biocontrol of stem rot pathogen (*Sclerotium rolfsii*) a destructive fungal disease of groundnut crop and thus reduced the biological and environmental hazards and improved the crop productivity of rainfed groundnut.

Keywords: Groundnut, PGPB, CO-Inoculation, *Sclerotium rolfsii*, Stem rot

INTRODUCTION

Stem rot disease of groundnut, caused by *Sclerotium rolfsii* sacc., is one of the most destructive fungal disease of groundnut, causing pod yield losses upto 90% under severe condition [1] and has a ubiquitous occurrence in almost all the groundnut growing countries of the world, including, India. Infection is usually restricted to plant parts that are indirect contact with the soil, usually *S. rolfsii* attacks stems, roots, leaves, pegs and pods of groundnut plant. Initial disease symptoms comprise small, water-soaked lesions on the lower stem or near the soil surface followed by yellowing and wilting of the lateral branches, main stem and eventually the entire plant. Diagnostic signs of the fungus, include, characteristic white myeclial growth and brown sclerotia extending from the infected tissues.

Induced systemic resistance (ISR) has emerged as a potential tool in crop protection practices and a suitable alternative strategy for the reduction in the use of synthetic chemical pesticides and purely based on biological control. Induced systemic resistance can be defined as the phenomenon by which plants exhibit increased level of resistance to broad spectrum of phytopathogens by the prior activation of genetically programmed defense pathways [2]. The colonization of phyllosphere and rhizosphere with selected PGPB [3] strains can also lead to induction of systemic resistance, commonly denoted as ISR. Induction of systemic resistance by selected strains of PGPB has been proved by spatially separating the pathogen and PGPB in plants [4]. Pieterse et al. [5] critically reviewed the triggering; signaling and expression of ISR mediated by PGPB and suggested the use of combination of different rhizobacterial strains that triggers different signaling pathways in the host plant for the maximization of ISR.

MATERIALS AND METHODS

A pot culture experiment was conducted to study the effect of different bioformulations of *viz.*, single strain inoculation,

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co-inoculation and natural co-aggregates application of PGPB cells viz., Bradyrhizobium and Methylobacterium together with supplementation of salicylic acid and challenge inoculation of *Sclerotium rolfsii* on the enhancement of growth and yield in rainfed groundnut with special emphasis to biocontrol against stem rot disease (*Sclerotium rolfsii*). The study was conducted during winter season (August to November, 2011) with groundnut cv. (JL-24), at the polyhouse of Department of Microbiology, Faculty of Agriculture, Annamalai University, Annamalai Nagar, India.

Rectangular cement pots with 18" × 12" × 12" size were filled with 45 kg of groundnut field soil flooded with water for 2 days and brought to fine puddle condition. After draining the excess, water, groundnut seeds were sown in rows in the pots, separately. The age of the seedlings were counted from the time of sowing. The experiment was arranged in randomized block design (RBD) with three replications and the following were the treatments viz.,

T1 - Control (100% NPK)

T2 - Bradyrhizobium alone

T3 - Methylobacterium alone

T4 - Bradyrhizobium+Salicylic acid

T5 - Methylobacterium+Salicylic acid

T6 - Bradyrhizobium+Methylobacterium Co-I

T7 - Bradyrhizobium+Methylobacterium Co-I+Salicylic acid

T8 - Bradyrhizobium+Methylobacterium CoA

T9 - Bradyrhizobium+Methylobacterium CoA+Salicylic acid

Groundnut plants were challenge inoculated by spraying *S. rolfsii* spore suspension (50,000 spore mL^{-1} inoculum level) on 10^{th} DAS with an atomizer and the control plant was sprayed with sterile water. High humidity was created by sprinkling water frequently in the polyhouse. The experiment was maintained under limited water supply as per the conditions prevailing in rainfed ecosystem. Five representative samples of plant hills in each pot were pegmarked for periodical observations.

Growth parameters

Effect on plant growth: The height of the plant from each treatment was measured at 30^{th} and 45^{th} days after sowing (DAS). The mean value of plants from three replications was recorded.

Effect on dry weight of root and shoot: The dry weight of the root and shoot was taken at 30^{th} and 45^{th} days after sowing (DAS). Three plant samples were drawn, washed, air dried and later dried to a constant weight in an oven at 60°C. The oven dried weight of the root and shoot a sample was recorded.

N content of plant: The plant samples were collected at 30^{th} and 45^{th} day after sowing (DAS), washed in water, air dried and later dried to a constant weight in an oven at 60°C. Then, they were powdered, sieved and 100 mg of sample was taken for analysis. The total nitrogen content was estimated by Microkjeldahl method.

Nodule numbers and dry weight: The rhizosphere soil (loosely adhered soil with groundnut roots after shaking-off) was collected and air dried for 2 days. After air drying, the nodules were removed from the root and they were counted and weighed.

Yield parameters

Pod and seed weight: The pod and seed weight per plant was recorded. The thousand grain weight was taken at 14% moisture level for all the samples and recorded in grams.

Haulm yield of groundnut: The matured crop was harvested, hand threshed and sun dried. The dried grains from each treatment were weighed and recorded. After threshing, groundnut stalk was subjected to sun drying and the weight was recorded.

STATISTICAL ANALYSIS

The experimental results were statistically analyzed in randomized block design (RBD) and in Duncan's multiple range test (DMRT) as per the procedure described by Gomez and Gomez [6].

RESULTS AND DISCUSSION

Regarding the different formulations of Bradyrhizobium and Methylobacterium cells, the application of co-aggregates (natural) along with the salicylic acid could augment the plant height, root, shoot dry weight, nodules number and weight, disease incidence, seed weight and haulm yield of groundnut to a higher level followed by co-aggregates (natural), co-inoculation with salicylic acid, co-inoculation, vegetative cells with salicylic acid and vegetative cells alone treatments.

The results of the present study clearly indicated the ability of Bradyrhizobium and Methylobacterium co-aggregates (natural) together with salicylic acid application in augmenting the pod weight, seed weight and haulm yield of groundnut plant when compared to other formulations and the results presented in **Table 1**.

Table 1: Effect of different bioformulations of PGPR cells on the enhancement of plant growth promotion.

Treatments	Plant Height (CM)	Dry Weight of Root (g/plant)	Dry Weight of Shoot (g/plant)	N Content (%)	Nodule		Disease Incidence (%)	Seed weight (g/plant)	Haulm yield (kg/ha)
					No.	Weight (mg)			
Control+100 % N	28.40 ± 0.3	1.00 ± 0.3	2.05 ± 0.6	0.95	38.1	10.5	86.8 ± 1.61	6.52 ± 0.20	3201
BR-5+75% N	33.10 ± 0.5	1.20 ± 0.2	2.18 ± 0.5	1.26	46.7	12.9	61.3 ± 0.78	7.24 ± 0.54	3703
MB-5+75% N	30.42 ± 0.2	1.17 ± 0.4	2.11 ± 0.7	1.20	41.3	11.5	70.4 ± 0.51	6.80 ± 0.4	3459
BR-5+75% N+SA	38.26 ± 0.6	1.30 ± 0.5	2.40 ± 0.4	1.40	47.5	14.5	44.6 ± 0.85	8.54 ± 0.41	3941
MB-5+75% N+SA	36.36 ± 0.4	1.25 ± 0.1	2..25 ± 0.3	1.33	44.8	13.6	50.2 ± 0.23	7.96 ± 0.69	3802
BR-5+MB-5-Co-I+75% N	39.86 ± 0.1	1.35 ± 0.7	2.46 ± 0.1	1.47	50.4	15.9	36.8 ± 0.31	8.95 ± 0.35	4105
BR-5+MB-5-Co-I+75% N+SA	42.10 ± 0.7	1.41 ± 0.4	2.51 ± 0.2	1.55	53.2	16.2	20.6 ± 0.56	9.65 ± 0.32	4335
BR-5+MB-5-Co-A(N)+75% N	44.05 ± 0.4	1.46 ± 0.1	2.54 ± 0.4	1.64	56.3	17.1	15.9 ± 0.43	10.15 ± 0.4	4668
BR-5+MB-5-Co-A(N)+75% N+SA	46.52 ± 0.5	1.51 ± 0.3	2.60 ± 0.7	1.71	60.7	18.0	19.8 ± 0.22	10.90 ± 0.5	5990
LSD (p>0.05)	0.96	0.019	0.08	0.06	0.96	0.83	0.60	0.50	0.27

Abbreviations: BR-5: Bradyrhizobium; MB-5: Methylobacterium; Co-I: Coinoculation; Co-A: Coaggregates; SA: Salicylic Acid; DAS: Days after Sowing

The effect of Bradyrhizobium inoculation in augmenting the growth parameters of groundnut has been studied by many authors [7-10]. The effect of Methylobacterium inoculation in augmenting the growth and yield parameters of groundnut has already been reported [11-13]. The co-inoculation effect of *Bradyrhizobium* sp. and *Methylobacterium* sp. has also been reported so far. The increase in the total N content of groundnut due to the inoculation of Bradyrhizobium and Methylobacterium has been reported. Madhaiyan et al. [14]. The augmentation of disease incidence, seed and haulm yield due to Bradyrhizobium and Methylobacterium inoculation has been reported by Bremer [15].

CONCLUSION

It was concluded that "intergeneric PGPB co-aggregates with salicylic acid" application together with 75% recommended N level could augment the growth and yield parameters of groundnut when compared to the groundnut crop grown in 100% recommended 'N' level without any bioinoculation and thus a saving of 25% recommended 'N' level could be possible through the application of "intergeneric PGPB co-aggregates" in rainfed groundnut crop. Moreover the "intergeneric PGPB co-aggregates with salicylic acid application" and augmented the ISR mediated

biocontrol of stem rot pathogen (*Sclerotium rolfsii*) a destructive fungal disease of groundnut crop and thus reduced the biological and environmental hazards and improved the crop productivity of rainfed groundnut.

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