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Application Effect Of Exopolysaccharide (Eps) Rich, Pgpr Coaggregates On The Enhancement Of Isr Mediated Biocontrol In Groundnut Sclerotium Rolfsii Pathosystem Under Rainfed Condition

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Abstract: The bioinoculation effect of different bioformulations, viz.,vegetative cell application, co-inoculation and co-aggregates application, of efficient PGPR cells viz., Methylobacterium (MB-5) and Rhizobium sp. (RM-5), together with challenge inoculation of Sclerotium rolfsii on the induction of induced systemic resistance (ISR) in groundnut-Sclerotium rolfsii pathosystem was studied under pot culture condition with groundnut cv.JL-24.

It was observed that the application of, Methylobacterium e and Rhizobium, as co-aggregates, positively altered the biochemical and physiological parameters viz., reducing and non-reducing sugars, total and OD phenol content and defense enzymes activities, such as, peroxidase (PO) and polyphenol oxidase (PPO), of groundnut plant to the highest level followed by co-inoculation and single strain inoculation treatment. The application of PGPR cells, as co-aggregates, was found to augment the total and OD phenol content and defense enzyme activities, such as, PO and PPO content of groundnut plant to a higher level whereas a reduction in reducing and non-reducing sugar level was recorded, which ultimately lead to a reduction in Sclerotium rolfsii incidence in rainfed groundnut.

It has been postulated that the EPS biosynthesis of PGPR cells during coaggregation processes, might act as an elicitor for the enhancement of ISR in groundnut-Sclerotium rolfsii pathosystem whereas application the vegetative cell and co-inoculation formulations, without any involvement of EPS, responded poorly for the enhancement of ISR in the same pathosystem. This is the first comprehensive report on the positive role of bacterial EPS, as a determinant of ISR, in groundnut-Sclerotium rolfsii pathosytem.

Keywords:PGPR, Microbial coaggregates, Physiological and Biological parameters, EPS-ISR, Sclerotium rolfsii, Rainfed groundnut

INTRODUCTION:

Groundnut (Arachis hypogoea .L.) as an important oil seed crop,widely distributed in the tropical, sub tropical and warm temperature zones of the world covering in over 80 countries, including India. In India, groundnut is cultivated both under irrigated as well as rainfed conditions. However, the rainfed ecology is the largest one in terms of area (8.0 Mha) and production (7.4 MT) but with least productivity (0.93 MT) (ICRISAT, 1999). Of the several biotic constraints, the incidence of diseases is considered to be the major constraint in limiting the yield of rainfed groundnut whereas the major yield loss (upto 70%) in rainfed groundnut has been attributed to collar rot disease, caused by the fungal phytopathogen viz., Sclerotium rolfsii (Ghewande et al ., 1983; Subramanyam et al .,1984.,). Collar rot disease is now severe in all major groundnut growing states of India, viz., Andhra Pradesh, Karnataka, Orissa, Gujarat, Maharashtra and Tamil Nadu and it was estimated that over 5,00,000 hectares of groundnut fields were infected by the fungal pathogen (Siddanagoudar,2005).

Now-a-days, rainfed groundnut production management strategies mainly focus on the use of synthetic chemical pesticides to suppress the phytopathogen and there by enhance the per hectare yield of the crop. However, the inherent limitations associated with synthetic chemical pesticides, such as, high cost, unavailability, toxicity, development of resistant strains, environmental pollution and adverse effect on beneficial soil microflora and fauna has compounded the problem in India and all other countries producing groundnut. In the present situation, the use of "plant growth promoting Rhizobacteria" (PGPR), as a biological approach, might be an alternative and suitable strategy to overcome the biological and environmental hazards posed by the persistent use of synthetic chemical pesticides.

Several mechanisms of plant- microbe interaction may participate in the PGPR association and affect plant growth, including , N-fixation, hormonal interaction,

improvement in root growth, solubilistation of nutrients, alleviation of salinity and biocontrol against phytopathogens. Thus , the PGPR affect the plant growth directly by producing and secreting plant growth substances or by elicting root metabolic activities by supplying biologically fixed nitrogen and indirectly by changing the microbial balance in the rhizosphere (Kloepper and Schroth, 1981), production of iron chelating siderophores, antibiotics, hydrogen cyanide and through induction systemic resistance (ISR) in host plants which ultimately implicated in reduction of plant pathogens and deleterious rhizobacteria with a corresponding improvement in plant growth. The well known PGPR, include, bacteria belonging to the genera, namely, Azospirillium, Azotobacter, Pseudomonas, Bacillus, Azoarcus, Klebsiella, Arthrobacter, Enterobacter, Serratia and Rhizobium on non-legumes (Burdman et al., 2000).

Induced resistance is defined as an enhancement of the plant's defensive capacity against a broad spectrum of phytopathogens that is acquired after appropriate stimulation. The resulting elevated resistance due to an inducing agent upon infection by a pathogen is called " induced systemic resistance (ISR) (Hammerschmidt and Kuc,1995). The induction of systemic resistance by rhizobacteria is commonly referred as ISR whereas the same induction by other agencies is called "systemic acquired resistance" (SAR) (Van Loon et al.,1998).

PGPR induce resistance in plants against different fungal, bacterial and viral diseases (Liu et al., 1995a,b; Maurhofer et al., 1998), insect (Zehnder et al., 1997) and nematode pest (Sikora, 1988). Induction of systemic resistance by selected strains of PGPR against plant diseases and insect pest has been proved by spatially separating the pathogen and PGPR in the plants (Van Peer et al., 1991). The PGPR mediated ISR against different fungal, bacterial and viral disease has been reported by many authors in different host plants (Liu et al.,1995; Wei et al., 1996; Maurhofer et al., 1998). Several bacterial of PGPR strains viz., lipoploysaccharides,(Van Peers and Schippers,1992), siderophores (Leeman et al.,1996; Usharani,2005), salicylic acid (van Loon 1997; Chaudhary and Johri,2008) and EPS (Kyungseok et al.,2005;Haggag,2007; Guzzo et al., 2008) have been reported to be responsible for the induction of systemic resistance in host plants.

The synergistic effect of PGPR strains mixture in augmenting the ISR mediated biocontrol of phytopathogens in different crop plants has been reported by Duffy and and Weller,(1995). Rhizobium mediated ISR against different fungal phytopathogens in legume crops has been reported by many authors (Schwinghamer and Belkengren, 1968; Breil et al., 1993, 1996 Chakraborty and Purkayastha, 1984; Deshwal et al., 2003). Methylobacterium mediated ISR against different fungal phytopathogens in different legume crops has been reported by (Lee et al., 2006; Madhaiyan et al., 2004,2006). Rhizobium and Methylobacterium sp. have been widely uses as agricultural bioinoculant in many developing countries (Thangamani and Sundram., 2005) for the enhancement of growth and yield of commercially important crops. However, there were no earlier reports, regarding the interaction effect of Rhizobium and Methylobacterium coinoculation on the ISR mediated biocontrol of collar rot phytopathogen available.

Further, the introduced bioinoculant exhibited poor performance in natural environments and in the rhizosphere of host plants due to lack of stress tolerance and poor survivability in soils. Okon and Lanbandera –Gonzalez (1994) stressed the importance of the physiological status of microorganisms in any agricultural bioinoculant preparation rather than their cell numbers to ensure more stress tolerance and survival in soil, van-Veen et al.(1997) suggested that instead of trying single strain with single trait, as agricultural bioinoculant,trying to use microbial consortia for harnessing multiple benefits.

In the recent years, several new agricultural bioinoculant formulations have been proposed of which EPS mediated "Intergeneric Microbial Coaggregates" proposed by Neyra et al.,(1999) as a novel bioformulation, seems to be a promising one for the production of multipurpose agricultural bioinoculant with multiple benefits. Hence, the present research work has been undertaken with an aim to exploit the positive role of EPS rich "Intergeneric microbial coaggregates", comprising the genera of Rhizobium and Methylobacterium, on ISR mediated biocontrol against Sclerotium rolfsii in groundnut crop grown under rainfed condition.

MATERIALS AND METHODS:

Strains of Methylobacterium and Rhizobium viz., Methylobacterium. extorquens (MB-5) and Rhizobium sp. (RM-5), were isolated from the phyllosphere and rhizosphere of rainfed groundnut respectively grown at different locations of cuddalore district and maintained in methanol mineral salt medium(MMS) and yeast extract mannitol agar (YEMA), respectively at 35ºC with monthly transfer and used throughout the study.Sclerotium rolfsii AU-1 (provided by Department of plant pathology, Annamalai University) was used as reference strain for the biocontrol study and the same was maintained in oat meal agar (OMA) medium and examined periodically for its virulence.

Preparation of inoculum: All the two isolates viz., Rhizobium sp. (RM-5) and Methylobacterium extorquens (MB-5) were grown in YEMA and MMS medium, respectively under shaking culture condition at 30ºC ± 2ºC for 24 h. Then, the media was centrifuged at 5000 × g for 10 min,separately, to harvest the log phase cells and the pellets obtained after centrifugation, washed three times with 0.1 M phosphate buffer (pH 6.8). Finally, the cells were resuspended in the same buffer to a cell concentration of 1 × 107 CFU/mL-1 by measuring the OD at 420 nm and used as inoculum.

Preparation of cofloc of PGPR cells:All the two isolates viz., Methylobacterium (MB-5) and Rhizobium sp. (RM-5),were grown in MMS and YEMA broth, respectively, duly supplemented with 0.05% yeast extract in a shaking bath at 30±2ºC for 5 days. Then, the media were centrifuged, separately, at 5000x g for 10 min to get stationary phase cells and the pellets, obtained after centrifugation, washed three

times in phosphate buffer (pH 6.8) and finally the cells were resuspended in the same buffer to a cell concentration of 1 x 107 CFU/mL-1 by measuring the absorbance at 420nm. The preparation of Co-AG buffer was done according to the specification of Grimaudo and Nesbitt (1997). The co-aggregates was prepared according to Jabra-Rizk et al.(1997), as stated herewith. One ml aliquot of each PGPR isolate viz., Methylobacterium extorquens (MB-5) and Rhizobium sp. (RM-5) was mixed together with 10 ml Co-AG buffer, vortexed for 10s, shaken on a rotary platform shaker for 3 min and left undisturbed at room temperature for 24 h. After the incubation period, the coaggregates, settled at bottom of the tube were obtained after decanting the buffer.

Pot Culture Experiment

A pot culture experiment was conducted to study the bioinoculation effect of different bioformulations of viz., single strain inoculation, co-inoculation and co-aggregates application of PGPR cells viz., Rhizobium and Methylobacterium together with challenge inoculation of Sclerotium rolfsii on the enhancement of ISR mediated biocontrol against collar rot disease (Sclerotium rolfsii) in rainfed groundnut. The study was conducted during winter season (Aug to Nov, 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"x12"x12" size were filled with 45 kg of groundnut field soil, flooded with water for 2 days and brought into 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 treatments viz .,Control, Rhizobium alone , Methylobactrium alone, Rhizobium + Methylobacterium co-inoculation and Rhizobium + Methylobacterium co-aggregates application, were maintained. During the experimental period, the annual mean minimum and the maximum temperature of experimental area was 25ºC and 30ºC, respectively and the mean highest and lowest humidity were 96 and 78 percent, respectively. The mean annual rain fall of this area was 1500 mm. A fertilizer schedule of 25: 50: 75 NPK ha-1 was followed. Regarding the 'N' fertilization, 50 per cent of the same was given as basal dose while the other 50 per cent was given as top dressing in two split doses. The entire dose of P2 O5 and K2O has been applied basally as super phosphate and muriate of potash, respectively. Groundnut plants were challenge inoculated by spraying S.rolfsii spore suspension (50,000 spore mL-1 inoculum level) on 10th 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 crop was given a hand weeding on 30th DAS and well protected against pests and diseases. 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 and same were recorded on 0, 7, 14, 21days after challaenge inoculation. The reducing and non- reducing sugar was estimated according to Mahadevan and Sridhar, (1986) whereas,the total and OD phenol content was assayed according to Malik et al.,(1997). The defense enzyme activities such as peroxidase (PO),Polyphenol oxidase (PPO) was assayed according to Putter,(1974)and Ester-Bauer, (1977), respectively.

RESULTS AND DISCUSSION:

The application effect of different bioformulations viz., single strain inoculation, co-inoculation and co-aggregates application of PGPR cells viz., Rhizobium and Methylobacterium on the enhancement of different biochemical parameters viz.,reducing, non-reducing sugar levels, total and OD phenol content, starch content and different physiological parameters viz., peroxidase(PO) and polyphenol oxidase (PPO) in rainfed groundnut cultivar JL-24 was studied under pot culture condition and results presented in Fig.1to Fig 7.

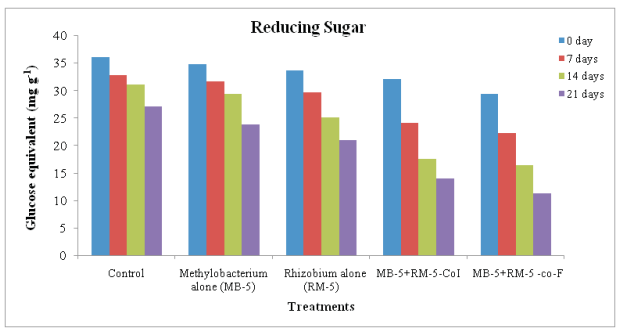

Fig.1. Changes in reducing sugar content of groundnut as influenced by different formulations Methylobacterium and Rhizobium cells and challenge inoculation of Sclerotium rolfsii

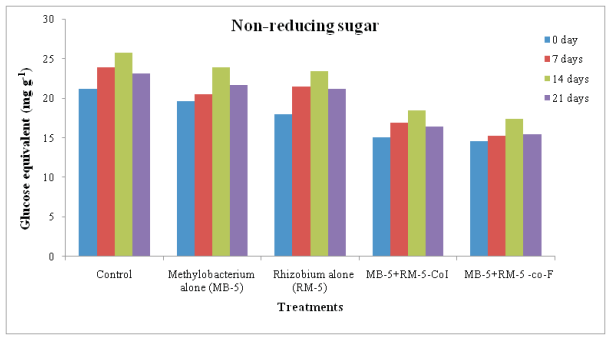

Fig.2-Changes in non-reducing sugar content of groundnut as influenced by different formulations Methylobacterium and Rhizobium cells and challenge inoculation of Sclerotium rolfsii

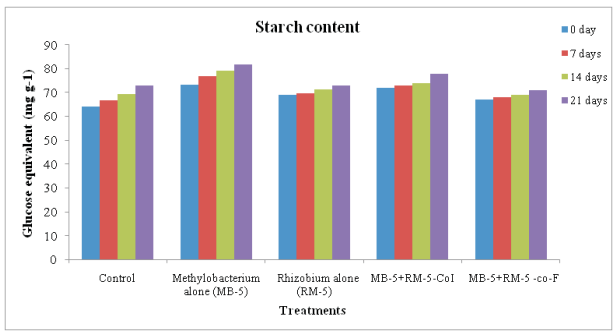

Fig.3-Changes in starch content of groundnut as influenced by different formulations Methylobacterium and Rhizobium cells and challenge inoculation of Sclerotium rolfsii

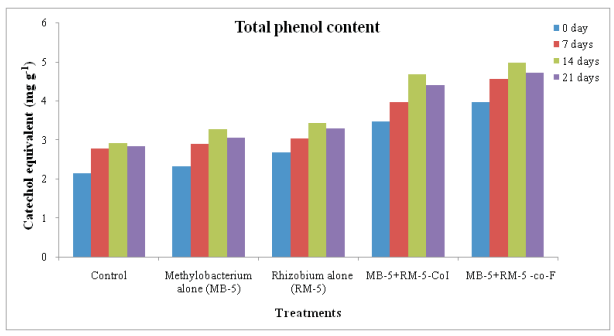

Fig.4-Changes in total phenol content of groundnut as influenced by different formulations Methylobacterium and Rhizobium cells and challenge inoculation of Sclerotium rolfsii

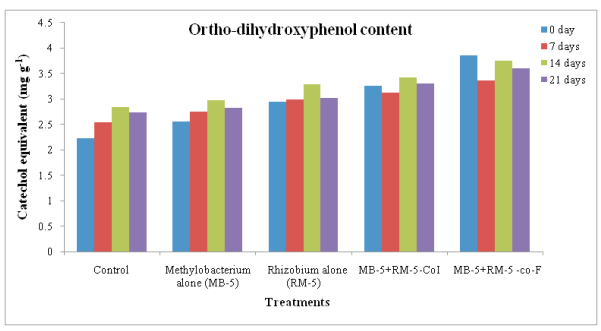

Fig.5-Changes in ortho-dihydroxyphenol content of groundnut as influenced by different formulations Methylobacterium and Rhizobium cells and challenge inoculation of Sclerotium rolfsii

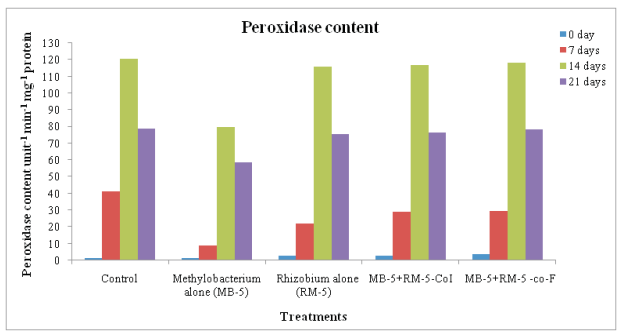

Fig.6-Changes in peroxidase of groundnut content as influenced by different formulations Methylobacterium and Rhizobium cells and challenge inoculation of Sclerotium rolfsii

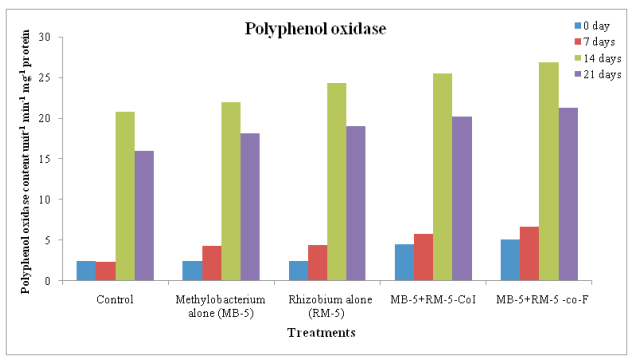

Fig.7-Changes in polyphenol oxidase content of groundnut as influenced by different formulations Methylobacterium and Rhizobium cells and challenge inoculation of Sclerotium rolfsii

It was observed that all the formulations of PGPR cells could augment the biochemical and physiological parameters of rainfed groundnut cv. JL 24 to a higher level when compared to control (without bioinoculation). These observations clearly revealed the positive effect of PGPR cells inoculation in augmenting the biochemical and physiological parameters of rainfed groundnut. Among the different bioformulations of PGPR cells, the application of "Intergeneric PGPR co-aggregates", consisting of Rhizobium and Methylobacterium,could augment the total and OD phenol content, starch content and reduction in the level of reducing and non reducing sugar and enhanced the PO and PPO activities in rainfed groundnut to a highest level followed by co-inoculation and single strain inoculation of PGPR cells. Between the two single strain inoculation treatments viz., Rhizobium and Methylobacterium, the inoculation of Rhizobium alone treatment recorded a higher value for the above parameters when compared to Methylobacterium alone treatment Many experimental data pertaining to pathophysiology support the occurrence of higher phenolics level in diseased plants when compared to healthy ones. Several workers endeavored to find out a correlation between increased levels of total and ortho-dihydroxy (OD) phenol with host resistance because the post

inflectional accumulation of phenols in the host plant is considered to be one of the resistant reactions (Farkas and Kiraly,1962) It was well known that OD phenols are the most active forms of phenols and their oxidation is mediated by the enzyme polyphenol oxidase (PPO) and peroxidase (PO) and the resulting quinine production which are more toxic than phenolics and effective inhibitors of SH group of enzymes which might be inhibitor to pathogen (Goodman et al., 1962). Peroxidase is an oxido- reductive enzyme that involve in wall binding process. Peroxidsase also catalyzes the condestation of phenolic compounds into lignin and is associated with disease resistance in plants (Hammerschmidt et al., 1995). The increase in peroxidase activity has an important function in secondary wall biosynthesis by polymerizing hydroxyl and methylhydroxy cinnamic alcohols into lignin and forming rigid cross-linking between cellulose, pectin, hydroxyl proline and lignin (Griesbach, 1981). Polyphenol oxidase (PPO) is a widespread enzyme found in plant cells. This enzyme dehydrogenating ortho-dihydroxy phenols to produce ortho-quinines. It indicates the higher activity towards hydroxylation of mono phenol to diphenol. Carbohydrates are the major source of energy and have a great influence on the incidence and development of the disease. Plant tissues containing larger amount of oxidisable carbohydrate are more prone to the invasion of pathogens than lower amount of oxidisable carbohydrates. Altered carbohydrate metabolism of the host in response to infection was studied by several workers (Farkas and Kiraly,1962; Bhaskaran and Prasad, 1971).. The inoculation effect of Methylobacterium or Rhizobium in augmenting the different biochemical and physiological parameters related to ISR of host plant has already been reported (Madhaiyan et al 2006). The positive co-inoculation effect of Pseudomonas and Bacillus on PGPR mediated ISR has already been reported by (Li and Alexander 1988). in leguminous plants. Rubiya (2006), Kannan(2010) reported the positive effect of "Intergeneric Microbial Coaggregates" application on the alteration of biochemical and physiological parameters in lowland rice and rainfed maize, respectively which ultimately lead to ISR mediated biocontrol against Pyricularia oryzae and Helminthosporium turcicum, respectively. Vaidhei (2013) reported the positive effect of Methylobacterium coflocs bioinoculation on the enhancement of ISR mediated biocontrol against Pyricularia oryzae in lowland rice. The results of the present study are also in confirmity with the earlier findings of Rubiya (2006), Kannan (2010) and vaidhei(2013). The application effect of PGPR coaggregates consisting of Methylobacterium and Rhizobium, on the enhancement of ISR mediated biocontrol against Sclerotium rolfsii in rainfed groundnut crop has not been reported, so far. This is the first comprehensive report regarding the positive role of EPS rich, PGPR coagreggates in augumenting the ISR mediated biocontrol against Sclerotium rolfsii in rainfed groundnut JL-24.

CONCLUSION:

The application effect of PGPR cells viz.,Methylobacterium and Rhizobium, as natural coaggregates bioformulation, augumented the biochemical viz.,level of reducing and non-reducing sugar, total and OD phenol , starch content and physiological parameters viz., peroxidase, (PO) and poly phenol oxidase (PPO) to a higher level than other bioformulations, and which ultimately lead to ISR mediated biocontrol against Sclerotium rolfsii in rainfed groundnut cv.JL.24

Application:

Application of Methylobacterium and Rhizobium cells, as coaggregates bioformulation, augmented the ISR mediated biocontrol against Sclerotium rofsii in rainfed groundnut to the higher level through the modification of different biochemical and physiological mechanisms of the host plant.

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