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Study of seed bacterization capacity of plant growth promoting endophytes from groundnut plant in *in vitro* and natural conditions

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Abstract
Among oilseeds crops in India, groundnut accounts for about 50% of area and 45 % of oil production**.** Groundnut is essentially a tropical plant and requires a long and warm growing season. Endophytes may benefit host plants by preventing pathogenic organisms from colonizing them. Seed bacterization has certain beneficial effect on plant growth response, induction of disease resistance and many more. Endophytic response was checked in *in vitro* and the natural condition. Seed bacterization creates competes with & eliminates pathogens in the rhizosphere. Plant growth promoting endophytes are beneficial in both in *invitro* and natural conditions.

Keywords: Groundnut, Endophytes, Seed bacterization, colonizing.

1. Introduction
Peanut is grown mostly in five states namely Andhra Pradesh, Gujarat, Tamil Nadu, Karnataka and Maharashtra and together they account for about 90 per cent of the crop's total area (Talawar S., 2004) [7]. The remaining peanut producing area is scattered in the states of Madhya Pradesh,
Uttar Pradesh, Rajasthan, Punjab, and Orissa. Although the crop can be grown in all the seasons, it is grown mainly in rainy season (*Kharif;* June-September). An endophytes is an endosymbiont, either bacterium or fungus. (A.P. Rao., G.K. Kishore, 2006) [4]. Endophytes are ubiquitous. Endophytic species are very diverse; it is thought that only a small minority of all existing endophytes have been characterized. A single leaf of a plant can harbor many different species of endophytes. Endophytes can be beneficial for plant growth, Endophytes have the root colonization capacity in which endophytes first establish contact and then penetrate young root epidermis and then spread into root tissues where they differentiate, according to species. Microorganisms that colonize the rhizosphere can be classified according to their effects on plants and the way they interact with roots, some trigger beneficial effects (B.S. Saharan, V. Nehra, 2011) [5]. It is reasonable to assume that PGPR must colonize the rhizosphere of the host plant to be most beneficial (De Weger *et al*., 1995) [2].
Seed bacterization usually means treatment of seeds with cultures of bacteria that will improve plant growth; such preparations are frequently called bacterial fertilizers. The classical example is treatment of legume seeds with *Rhizobium* whose value and mode of action is indisputable; preparations are on sale throughout the world. Seed bacterization has certain beneficial effect on plant growth response, induction of disease resistance and many more. Pot experiment was also performed to study the response of isolated endophytic cultures in natural conditions. Pot test is a quick and simple method. It helps to check the increase the yield of crop. Advantage of this test is its simplicity. This test can be done with replicates and defined controls.it can be done without the extensive effort required by field trials. This test helps to test the inoculation response of many different plants. Results of the pot test can be used for further data analysis. Endophytes may benefit host plants by preventing pathogenic organisms from colonizing them. Extensive colonization of the plant tissue by endophytes creates a "barrier effect", where the local endophytes outcompete and

Prevent pathogenic organisms from taking hold. Endophytic bacteria, along with rhizospheric bacteria contribute to plant growth. And it is not yet clear which of these two bacteria contributes more to plant.

2. Material and Method

2.1 Seed Bacterization assay

Seed bacterization of endophytic cultures was done (Sariah *et al*., 2012) [6]. The seeds were soaked in the culture having 0.4 OD for overnight at room temperature. Two such sets were prepared from the cultures that were isolated from seeds in which one set is having only culture while other has cultures with chitosan. The cultures were than centrifuged and pellet obtained was dissolved in sodium phosphate buffer. It was then plated on LB plates to count the initial endophytic population present in the solution. Plates were incubated overnight and then the colony forming units were counted. This count shows the initial number of endophytes that has sticked to surface of seeds.

2.2 Microcosm study/Pot assay experiment

Following the seed bacterization microcosm study was performed (Grenni *et al*., 2012) [3]. The seeds soaked in the cultures overnight were then transferred to the MS media for checking of the growth of plant in in-vitro conditions. Few seeds were transferred to the pot to observe the growth of plant from treated seeds in natural conditions. The sets of pot experiments were kept in which growth in some pots are started to appear. Seeds started germinating in the pots and in the MS media. After it grows in plantlet the root of plantlet should be cut and kept in sterile distilled water and from this dilutions should be prepared and then should be placed on the LB plates to count the number of endophytes colonized in the root of plantlet.

3. Result

3.1 Seed bacterization result

Table-1 shows the colony forming unit count of culture in which seed was soaked for overnight. In this data it can be seen that S-2b culture shows the highest colony forming units while S-1 culture shows the least colony forming units. Table-2 shows the colony forming unit count of dilution of distilled water in which seed was poured after soaking for overnight. In this data it can be seen that S-2b culture shows the highest colony forming units while S-1 culture shows the least colony forming units. Table-3 shows the initial stage of % colonization. In this data it can be seen that S-2b culture shows the highest colony forming units while S-1 culture shows the least colony forming units. Table-4 shows final stage of % colonization. In this data it can be seen that S-2b culture shows the highest colony forming units while S-1 culture shows the least colony forming units.

Table 1: Cultures in which seeds were soaked

Cultures	Colony Forming units (CFU)	Dilution	Aliquotes (ml)	CFU/ml
Control	-	-	0.1	-
Control + Chitosan	-	-	0.1	-
S-1	216 (2.16 $x10^{-2}$)	10-9	0.1	2.16 $x10^{12}$
S-1 + Chitosan	279 (2.79 $x10^{-2}$)	10-9	0.1	2.79 $x10^{12}$
S-2a	257 (2.57 $x10^{-2}$)	10-9	0.1	2.57 $x10^{12}$
S-2a + Chitosan	343 (3.43 $x10^{-2}$)	10-9	0.1	3.43 $x10^{12}$
S-2b	274 (2.74 $x10^{-2}$)	10-9	0.1	2.74 $x10^{12}$
S-2b + Chitosan	349 (3.49 $x10^{-2}$)	10-9	0.1	3.49 $x10^{12}$
S-3	305 (3.05 $x10^{-2}$)	10-9	0.1	3.05 $x10^{12}$
S-3 + Chitosan	336 (3.36 $x10^{-2}$)	10-9	0.1	3.36 $x10^{12}$

[Note: S= Endophytic cultures from Groundnut seeds]

Table 2: Dilution from seeds in distilled water

Cultures	Colony Forming units (CFU)	Dilution	Aliquotes (ml)	CFU/ml
Control	-	-	0.1	-
Control + Chitosan	-	-	0.1	-
S-1	208 (2.08 $x10^{-2}$)	10-4	0.1	2.08 $x10^{7}$
S-1 + Chitosan	256 (2.56 $x10^{-2}$)	10-4	0.1	2.56 $x10^{7}$
S-2a	240 (2.40 $x10^{-2}$)	10-4	0.1	2.40 $x10^{7}$
S-2a + Chitosan	320 (3.20 $x10^{-2}$)	10-4	0.1	3.20 $x10^{7}$
S-2b	268 (2.68 $x10^{-2}$)	10-4	0.1	2.68 $x10^{7}$
S-2b + Chitosan	332 (3.32 $x10^{-2}$)	10-4	0.1	3.32 $x10^{7}$
S-3	296 (2.96 $x10^{-2}$)	10-4	0.1	2.96 $x10^{7}$
S-3 + Chitosan	328 (3.28 $x10^{-2}$)	10-4	0.1	3.28 $x10^{7}$

Table-3 Initial stage % Colonization result

Cultures	CFU/ml (Dilution from cultures)	CFU/ml (Dilution from seed)	% Colonization
Control	-	-	-
Control + Chitosan	-	-	-
S-1	2.16 x 10^{12}	2.08 x 10^{7}	0.0963
S-1 + Chitosan	2.79 x 10^{12}	2.56 x 10^{7}	0.0917
S-2a	2.57 x 10^{12}	2.40 x 10^{7}	0.0934
S-2a + Chitosan	3.43 x 10^{12}	3.20 x 10^{7}	0.0933
S- 2b	2.74 x 10^{12}	2.68 x 10^{7}	0.0978
S- 2b + Chitosan	3.49 x 10^{12}	3.32 x 10^{7}	0.0951
S- 3	3.05 x 10^{12}	2.96 x 10^{7}	0.0970
S- 3 + Chitosan	3.36 x 10^{12}	3.28 x 10^{7}	0.0976

Table 4: Final stage %Colonization result

Cultures	CFU/ml (Initial count on seed)	CFU/ml (Final count on root)	% Colonization
Control	-	-	-
Control + Chitosan	-	-	-
S-1	2.08×10^7	1.90×10^2	0.0913
S-1 + Chitosan	2.56×10^7	1.96×10^2	0.0765
S-2a	2.40×10^7	1.59×10^2	0.0663
S-2a + Chitosan	3.20×10^7	2.57×10^2	0.0803
S- 2b	2.68×10^7	2.08×10^2	0.0776
S- 2b + Chitosan	3.32×10^7	2.89×10^2	0.0870
S- 3	2.96×10^7	2.01×10^2	0.0679
S- 3 + Chitosan	3.28×10^7	2.74×10^2	0.0835

3.2 Microcosm study/Pot Experiment result

Another set of above treated seeds were also grown in the pot in the natural conditions in the triplicates and also the control sets were kept in the triplicates. The earlier and improved growth was observed in the treated seed pots as compared to control pots. So the endophytes were also has positive effect on growth of groundnut plant in the natural conditions. The plant treated with the endophytic cultures showed early and healthy growth as compared to the control plants. So the isolated endophytes were also shown the positive effect on plant in the natural conditions.

4. Discussion

Seed bacterization of endophytic cultures from the groundnut seed was checked in which among all four cultures S-2b culture shows the highest colony forming units while S-1 culture shows the least colony forming units. Seed bacterization creates competes with & eliminates pathogens in the rhizosphere. Seed bacterization in this study was checked in both conditions *in-vitro* and in natural condition by pot experiment. Bhowmik *et al.* (2002) [1] reported that seed bacterization with one of the endophytes (Endo PR 8) reduced damping-off disease of cotton caused by *R. solani* and *S. rolfsii* Ziedan (2006) [8]. Revealed that bacterial treatment of peanut seeds before sowing (soaking of bacterial suspensions) resulted in reduced *Aspergillus Niger* and *F. oxysporum* colonization over peanut seed at 30 days after harvesting.

5. Conclusion

Seed bacterization was performed in which the initial and final % of endophytic culture colonization in both *in-vitro* and natural conditions. This work can be further carried out at the green house level and then can be checked at the field level. Many other future aspects are also there of this work as it can be proceeded by applying marker labeling to the endophyte and studying the pathway after it get colonized that is entry pathway and its life cycle in the plant. Also less research has been done on the plant growth promoting endophytes, so this work will have great importance in order to protect and increase the yield of the groundnut crop. It not only helps in the rising of the economic status of the farmer but also saves the extra expenditure on the fertilizer that is used by the farmer.

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