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BENEFICIAL ASPECTS OF ARBUSCULAR MYCORRHIZAL FUNGUS *Rhizophagus irregularis* ON PLANT GROWTH AND VIGOUR OF *Arachis hypogaea* L.

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ABSTRACT

Biofertilizers are the organisms which are responsible for the enrichment of soil nutrients quantitatively and qualitatively. One of the most commonly used and ubiquitously distributed biofertilizer is AM fungi. In the present study our main motto was to produce ground nut by using mycorrhizal biofertilizer in low nutrient soil of the Birbhum district, West Bengal. In this study, we selected the plant Lathyrus sativus as a host for the production of AMF inoculum in pot culture. We have isolated the spores of Rhizophagus irregularis (previously known as Glomus intraradices) from the rhizospheric soil of the host plants like Zea mays, Aloe vera and Lathyrus sativus. Pot culture of the selected host plant L. sativus was done and the young seedlings as well as the soil in pot culture were inoculated with the spores of R. irregularis and the mycorrhizal inocula of R. irregularis have been produced in the pot culture as biofertilizer. The ground nut plants were then cultivated in red laterite soil and maintained in pot culture where mycorrhizal inocula of R. irregularis were applied. Field trial was also done by the use of AMF inoculum. The percentage of mycorrhizal root colonization, soil nutrients parameters like nitrogen, phosphorus, potassium, calcium etc. and physiochemical properties of soil like bulk density, specific gravity, percentage of clay, slit and sand had also been studied. It was evidenced that the percent of root colonization by R. irregularis was increased with concomitant increase in soil nutrients parameter like nitrogen, phosphorus, potassium and other microelements.

KEY WORDS

Arbuscular-mycorrhizal fungi, Biofertilizer, Nitrogen, Soil phosphorus.

INTRODUCTION

Mycorrhiza is a type of endophytic, biotrophic, mutualistic obligate symbiont prevalent in many cultivated and as well as in natural ecosystems throughout the world. Mycorrhizal fungi have greater applicability in enhancing plant growth under stressful environmental conditions. The feeder roots of plant which are being infected by mycorrhizal fungi become modified and transformed into unique morphological structures called mycorrhizal roots which can absorb nitrogen, phosphorus, potassium, calcium, sulphur, zinc, manganese etc from the soil and translocate these mineral nutrients to plants with those roots they are associated (Li et al., 1991; Champawat and Pathak, 1993; Abdul Malik, 2000). Improvement in plant growth and biomass, production of healthy plants, soil stabilization and development of resistance against soil-borne plant pathogens due to AM infection reveal that these fungi play an important role in the ecosystem (Abdul Khaliq et al. 1994-2000) and enhance the returns to the farmers. It leads to the improvement in plant photosynthesis, nutrients translocation, plant metabolism processes and reduce the use of chemical fertilizers that is why they are referred to as biofertlizers

(Abbott and Robson, 1999). Mycorrhizal biofertilizer increases establishment, nodulation and atmospheric N_2 fixation capacity of leguminous plants (Sylvia and Williams 1992).

Leguminous vegetable crops play an important role in improving livelihood, nutritional security of farmers and populations in less developed countries as well as sustainability of agriculture in dry areas worldwide. Beneficial effects, in terms of positive plant growth responses and enhanced uptake to AM inoculation obtained in groundnut (Rao and Parvathi 1982; Krishna and Bagyaraj 1984).

Fabaceae is one of the most important family of VAM association in dicots (Ghosh and Dutta 2016). Ground nut (*Arachis hypogaea*) belongs to the family Fabaceae (Sub family-Papilionaceae) is one of the most important oilseed crop grown throughout the tropics and subtropics. Its nutritional value and food value is very high. The seeds of *A.hypogaea* contain crude fibre, protein, lipid, unsaturated fatty acids, carbohydrates, calcium, phosphorus, and vitamins (E, K and B group) (Savage and Keenan, 1994).

In this present study, we evaluate the beneficial use of arbuscular-mycorrhizal fungi on the growth, development and production of ground nut plants.

The plants which are naturally growing having the higher percentage of colonization are usually considered to use as a stock plant (host) and the stock plants are used for the culture of AM inoculum intended for utilization as biofertilizer to enhance the production of agricultural crops and to reduce the use of chemical fertilizers also to protect environment free from pollution (Parmita, 2005). In present study we have used the plant *Lathyrus sativus* as stock plant (host) for the production of AM inoculum in pot culture and application of this biofertilizer for enhanced production of ground nut in low nutrient, less fertile red laterite soil of sonajhuri area of Bolpur, Birbhum district.

MATERIALS AND METHODS:

Study location: Present study was carried out in the field area of the mycology and plant pathology laboratory of the department of Botany of The University of Burdwan, West Bengal. Field trial was also done for this experiment to measure the production of ground nut in nutrient deficient soil.

Selection of host: The plant *Arachis hypogaea* was selected as a host plant for the mass multiplication of AM fungal spore and the application of AM fungal inoculum for increase in the growth and production of ground nut (*A.hypogaea*). The plant *A.hypogea* selected as a host plant on the basis of their thick root system, sporulation and higher colonization of the plant, annual growth habit and suitable environmental conditions.

Production of starter culture (Arbuscular-mycorrhizal inoculum):

Rhizophagus irregularis (previously known as *Glomus intraradices*) (N.C. Schenck and G.S. Sm. C.Walker and Schuessler, 2010) was propagated in soil-sand (3:1) pot cultures with *Lathyrus sativus* as a host plant. After 90 days the plants were cut down at the base of the stem and the roots were chopped roughly about 1 cm. Then chopped roots were mixed back into the soil and air-dried soil containing *R. irregularis* spores stored as a crude inoculum. This mixture is known as root-soil inoculum and it can be stored up to one year.

Rhizophagus irregularis spores were isolated from rhizospheric soil of *Alium cepa* and *Lathyrus sativus* by wet sieving and decanting technique (Gerdemann and Nicolson, 1963) method followed by sucrose centrifugation technique (Daniel and Skipper, 1982). After sterilization spores were immediately used.

Collection of soil sample: Present experiment was conducted in red laterite soil collected from the Birbhum district, West Bengal. Such soils are not suitable for agriculture because of aberrant weather, drought, crusting, acidity, water erosion, mass wasting, water-logging, N, P,K deficiency, low water holding capacity and wide range of land use conversion. By the use of AMF inoculum as a biofertilizer such degraded land can also capable of produce different types of fruits and vegetables.

Pot and Potting mixture: Open pot culture was used for the mass growth of AM fungi. About 3 kg capability pots were taken and 3 kg sterilized soil-sand (3:1) mixtures were taken in each pot. 100 gm AMF inoculum thoroughly mixed with the soil. 4 replicates of pot culture were maintained for the culture of ground nut plant.

Surface sterilization of seeds: For the decrease of microbial contamination and to achieve the healthy seedlings, seeds of *Arachis hypogaea* were surface disinfected with 0.01% (w/v) Hg_2Cl_2 for about 2-3 minutes and then washed with distilled water.

Quantification of AM Fungi: Roots samples were collected 15 days interval. Minimum 50 plant root

segments were considered for the estimate of mycorrhizal colonization. The roots were macerated in 10% KOH solution, acidified with 1(N) HCl and was stained with 0.05% Trypan blue. The excess stain was removed by washing with lactophenol root segments were provisionally mounted on the slide by using lactophenol and the edges of the cover slips were sealed with DPX and observed under the microscope (Leica DMLB 3000). Percent of root colonization was calculated by using the following formula (Gupta and Mukherjee 1999).

$$\text{Colonization (\%)} = \frac{\text{No. of root segments colonized with VAM}}{\text{Total no. of root segments observed}} \times 100$$

Quantification of AM Fungal spore: Rhizospheric soil samples were collected after 30 days and 60 days of inoculation. AM spores were determined per 100 gm of soil. Spores were isolated from the rhizospheric soil by wet sieve and decanting method (Gerdemann and Nicolson, 1963) followed by sucrose centrifugation technique (Daniel and Skipper, 1982).

Spore count: VAM fungal spores were extracted from three replicates of 100 gm rhizospheric soil by following different spore isolation methods. The decandant were filtered through a filter paper (Whatman filter paper No.1) with grid lines. The filter paper was then spread on a petri dish under a stereo zoom dissecting microscope and counted and expressed as spores per 100 gm of dry rhizospheric soil.

Identification of AM Fungal Spore: The arbuscular mycorrhizal fungi were determined by using manuals and the synoptic key of Schencz and Perez (1990), Walker and Trappe (1982), Morton and Benny (1990).

Data analysis: All the data were taken in four replicates and the standard error of mean (SEM) value (±) was calculated. Each of the data was checked for interpretation whether they were statistically significant or not. The data were analyzed by using the statistical method like, analysis of variance (ANOVA), and critical difference (CD) at 5% level was calculated.

Estimation of total chlorophyll (Arnon, 1949): 1 gm. finely cut fresh leaves of *A. hypogaea* were taken and ground with 20 ml of 80% acetone. It was then centrifuged at 5000-10000 rpm for 5 minutes. The supernatant was decanted, and procedure was repeated until the residues were completely becomes colourless. The absorbance of the solution was red at 645 nm against the solvent (acetone) blank. The chlorophyll content was expressed as milligram per gram dry tissue (mg g-1) dry tissue.

Estimation of total protein (Lowery, et al., 1951):

Materials:

1. Sodium carbonate solution – 2% in 0.1 N sodium hydroxide
2. Copper sulphate solution – 0.5%
3. Sodium potassium tartrate solution – 1%
4. Alkaline copper reagent – a mixture of 50 ml sodium carbonate solution and
5. 0.5 ml of each of copper sulphate solution and Sodium potassium tartrate solution.
6. Folin's phenol reagent –1:1 dilution with distilled water.
7. Standard protein solution –100 mg % in 0.1 NaOH.

Procedure: 0.2 ml protein solution was taken in test tube and adds 2 ml. of alkaline copper sulphate reagent and mixed the solution well. The solution was incubated at room temperature for 10 minutes. Then added 0.2 ml of reagent Folin Ciocalteau solution and mixed it well. The mixture was kept for another 30 minutes. The absorbance was measured at 675 nm in Beckman DU-64 spectrophotometer. The system devoid of sample was used as blank. The concentration of protein was determined by comparing the absorbance got with a standard curve prepared.

RESULT AND DISCUSSION:

Table-1: Percentage and intensity of mycorrhizal root colonization in *A.hypogaea*

Mycorrhizal colonization (%) of *A.hypogaea*				
Treatment	**Period (Days)**			
	15	**30**	**45**	**60**
Control	5.3±0.31	12.05±0.44	18.3±0.61	21.82±0.62
With AMF inoculum	21±1.2	41.75±0.85	70.825±0.6	96.5±0.64

Table-2: Shoot dry weights and the percent of RFMD for the plant *A.hypogaea* (after 60 days)

Soil treatments	Shoot height (cm)	Shoot fresh weight (g)	Shoot dry weight (g)	** Percent RFMD
Control	28.5 ± 0.72	30.02± 0.12	2.764± 0.25	
Inoculated with AMF	55.15± 1.6	62.78± 0.76	9.594± 0.8	71.19

Table-3: Root dry weights and the percent of RFMD for the plant *A.hypogaea* (after 60 days)

Soil treatments	Root length (cm)	Root fresh weight (g)	Root dry weight (g)	** Percent RFMD
Control	7.7± 1.2	9.530	2.964	-
Inoculated with AMF	13.6±1.0	16.630	6.263	52.674

*** * % RFMD (Relative field mycorrhizal dependency) = (Dry weight of mycorrhizal plant) – (Dry weight of non-mycorrhizal plant)/ (Dry weight of mycorrhizal plant) X10**

Table-4: Spore population of AM fungi per 25 gm. soil from the rhizosphere soil of the plant *A.hypogaea* (after 30 days and 60 days)

Treatments	Number of spores/25 gm soil	
	30 Days	**60 Days**
Control	32	52
With AMF inoculum	88	232

Table-5: Total Chlorophyll content in the leaf of *A.hypogaea* plants at different stages of growth

	Total chlorophyll content (mg/g dry tissue)			
Treatments	**15 days**	**30 days**	**45 days**	**60 days**
Control	0.66± 0.084	0.78 ± 0.05	1.18± 0.059	2.52 ± 0.075
With AMF inoculum	0.68± 0.084	2.52± 0.011	4.16 ± 0.015	6.76 ± 0.90

Table-6: Total protein content of the seeds of non-mycorrhizal and mycorrhizal plant of *A. hypogaea* (after 60 days)

Treatments	Total protein (mg/g fresh tissue)
Control	5.104± 0.051
With AMF inoculum	14.536±0.075

Table-7: Physicochemical properties of rhizospheric soil of *Arachis hypogaea*

Serial no.	Physiochemical Properties	Observation	
		Control	With AMF
1.	Moisture content (%)	8.77±0.001	8.98 ±0.001
2.	Temperature (^{0}C)	36.5±0.5	33.6 ±0.56
3.	pH	5.84±0.01	6.01 ±0.05
4.	Electrical conductivity(ms/cm)	0.030±0.03	0.036±0.002
5.	Cation exchange capacity (Cmol/kg)	17.06±0.001	11.67 ±0.023
6.	Bulk density (g c.c^{-1})	1.035±0.003	1.327±0.003
7.	Specific gravity	1.045±0.001	1.167±0.001
8.	Porosity (%):	52.30±0.05	76.23±0.05
9.	Particle density (g/cc)	2.17±0.01	4.71±0.01
10.	Clay (<0.002 mm) %	12.38±0.53	4.34±0.050
11.	Slit (0.002-0.02 mm) %	48.41±0.015	36.62±0.05
12.	Sand (0.02-0.2mm) %	39.21±0.002	59.04±0.06
13.	Organic carbon (%)	3.19±0.013	2.22 ±0.02
14.	Organic matter (%)	3.73±0.017	2.67 ±0.03
15.	Total Nitrogen (%)	0.042±0.001	0.511 ±0.001
16.	Available Phosphorus (%)	8.486±0.041	67.13 ±0.07
17.	Available Potassium (%)	1.430±0.0320	26.78 ±0.1
18.	Available Calcium (meq 100g-1)	0.016±0.001	0.030 ±0.001
19.	Available Magnesium(meq100g-1)	0.019±0.001	0.023 ±0.002
20.	Iron (%)	5.87±0.013	3.10 ±0.05
21.	Sodium (%)	0.012±0.001	0.0143±0.001

Table-8: Growth rate and yield in *A.hypogaea* in field condition after 120 days of inoculation.

Treatments	Shoot length (cm.)	Shoot weight (gm)		Root length (cm.)	Root weight (gm.)		Ratio of soot and root	No. of nodules	Weight of nodule (gm.)	Percentage (%) of mycorrhizal colonization	Yield (Kg/ Hector)
		Fresh	Dry		Fresh	Dry					
Control	28.05± 0.08	4.54±0 .18	1.08 ± 0.02	9.15± 0.22	2.07± 0.02	0.78± 0.01	2:1	-	-	25±0. 005	96.33±7. 31
Mixed inoculum	71.56± 0.34	18.28± 0.27	6.15 ± 0.02	16.41 ±0.28	6.31± 0.09	3.27± 0.04	3:1	128.6 ±2.02	1.12±0.0 1	90.33 ± 1.76	312± 9.07

The plant ground nut (*A.hypogaea*) is a commercially important vegetable crop of the world. The occurrence and intensity of root colonization of AM inoculated crop of groundnut plant were represented that in non-mycorrhizal plant the percentage of root colonization showed 22% after 60 days, whereas in the AMF inoculated plant showed 96% root colonization after 60 days of inoculation. *A. hypogaea* is a highly mycorrhizal-dependent plant. Mycorrhizal fungi help the plant as a biofertilizing agent increase the phosphorus uptake. Because laterite soil is high iron containing and low phosphorus soil. Marked difference observed in root-shoot dry weight as well as in length of the plant. Percent of RFMD (relative field mycorrhizal

dependency) in root was 52.674% and shoot was 71.19%.

In fact, the nutritionally significant function of mycorrhiza depends on soil exploration by fungal hyphae. Mycorrhizal roots and are characterized by increased branching with enhanced efficiency of mineral nutrients uptake from the soil subsequently with increased growth and vigor of the crops. Physiochemical properties of soil showed that N, P,K value was more in mycorrhizal plant than non-mycorrhizal one.

The chlorophyll content of plant and total protein of seed was estimated because ground nuts are rich in protein. After 60 days of inoculation chlorophyll content was highest i.e. 7.76 and protein content was higher in mycorrhizal plant than the control. By the use of AMF inoculums in red laterite soil, protein content was 14.536 and in control one it showed 5.104.

The spore population of rhizosperic soil was estimated after 30 days and 60 days of interval. Number of AMF spores was the highest (232) in mycorrhiza inoculated plants over the control set of the plants were there was the 52 spores/100gm soil. The dominant species of AM fungi were *Glomus mossae, Glomus intraradices, Glomus aggregatum, Acaulospora scrobiculata*. *Arachis hypogaea* is an underground pod legume of the family Fabaceae. Total nut produced after 2 months by using mycorrhizal biofertilizer was 120.75 grams. On the other hand, non-mycorrhizal plants produce only 30.025 grams of groundnuts. Mycorrhizal fungus improved the colonization of plant roots of *A.hypogaea* by the fungus *R. irregularis* and augmented in the actual number short tertiary roots which they are associated. In the field condition monospecific inoculum of Rhizophagus *irregularis* had a consequence on the mycorrhization and growth of ground nut plant, observed as an increased in the percentage of mycorrhizal infection and as an enhanced fresh and dry weight of root and shoot. In the field testing, average 312 kg ground nut was produced per hector area by using AMF inoculum in contrast to non-inoculated plants they only produced 96.33 kg ground nut per area. Inoculation of the field and transplanting inoculated seedlings in the field markedly increase the yield of this crop.

Conclusion:

From the results it is established that mycorrhizal fungus *Rhizophagus irregularis* (*Glomus intraradices*) may serve as a very good biofertilizer for improvement of yield of *Arachis hypogaea* especially in the nutrient deficient and high iron containing red laterite soil. Considering the escalating environmental hazards caused by the indiscriminate use of chemical fertilizers, the present works may certainly have some significant input in basic and fundamental research on plant science.

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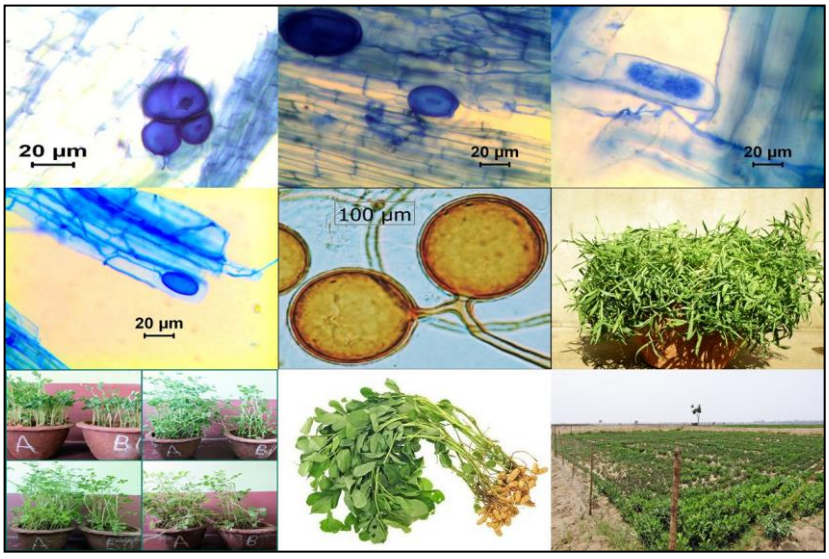

Figure showing vesicles, arbuscules of *R.irregularis*, monospecific culture of *Lathyrus sativus* and pot culture and field trial experiment of the plant *Arachis hypogaea*

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