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Effect of AM Fungi on Black Gram [*Vigna mungo* (L.) Hepper] Productivity from the Rhizosphere Soil of Dibru-Saikhowa Biosphere Reserve (DSBR) Forest of Assam, India

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A B S T R A C T

An experiment was conducted with efficient *AM* fungal isolates from the rhizosphere soil of Dibru-Saikhowa Biosphere Reserve (DSBR) forest of Assam, India in both laboratory and field condition with maize and black gram. Application of efficient isolates viz. AMF_3, AMF_7, AMF_{10} and AMF_{17} with host plant (*Zea mays* L.) had shown highest root colonization (75.86, 44.52, 52.90 and 73.11% respectively) and number of spores (17.67, 6.67, 7.33 and 12.67 g^{-1}, soil respectively) out of total isolates *in vitro* experiment. Lone inoculation of these four isolates in black gram with prominent parameters like dry weight (g, $plant^{-1}$) of shoot and root, chlorophyll content (mg, g^{-1} fresh wt.), N and P content (g/plant) and yield (kg, ha^{-1}) was done in field condition. Dry weight of nodule (0.99 g, $plant^{-1}$), shoot (8.15 g, $plant^{-1}$) and root (3.32 g, $plant^{-1}$) was high in AMF_3 inoculation while low (nodule= 0.54, shoot= 7.00 and root= 1.55 g, $plant^{-1}$) in AMF_{17}. The N and P content showed high (in shoot 3.33 mg/plant and in root 0.88 mg/plant) in AMF_3 in comparison to others. Subsequently, the highest productivity (450.65 kg, ha^{-1}) of the test crop observed in due application of AMF_3 while lowest (371.67 kg, ha^{-1}) in AMF_5. This study thus underlines the potentials of the *AM* fungi correspondence to the growth and development of black gram and submit the work in original form for greater benefit of mankind in future.

***Key words*:** Dibru-Saikhowa biosphere reserve, AM Fungi, Root colonization, Maize, Black gram

A study was carried out on soil microflora of Dibru-Saikhowa Biosphere Reserve (DSBR) forest of Assam, India and isolated, characterized, identified and preserved 17 numbers of *AM* fungal isolates [1]. In present work, screening, efficiency and evaluation of the isolates on maize plant (*Zea mays* L.) was done *in vitro* condition. Simultaneously, the efficient isolates viz. AMF_3, AMF_7, AMF_{10} and AMF_{17} were applied in the productivity of black gram. Soil is a rich micro habitat, dynamic biological system and difficult to determine the composition of microbial communities [2]. Microbial diversity comprises mostly bacteria, fungi, algae, actinomycetes etc. their ecological niches and nutritional habits vary a great deal. India has a rich biological heritage that qualifies it as one of the twelve-mega diversity nation of the world [3]. Soil fungi make a very important part of the ecosystem along with other microbes in turnover of the biomass [4]. There is an urgent need to conserve biodiversity at global level to preserve the endemic and endangered species, both microscopic and macroscopic ones which plays vital role for the maintenance of sustainable environment, agriculture and forestry [5]. Microbes as a whole make a living by scavenging on organic matter in the soil, by decomposing the plant or animal bodies and thereby releasing all the nutrients contained within the materials, to be used by plants for growth, but unfortunately, they have received very little attention and many of them are either extinct or in the verse of extinction before they were known to us for proper use.

Vesicular-arbuscular mycorrhizal (*VAM*) fungi are a group of obligate plant symbionts that influence plant growth and development. Arbuscular mycorrhizal fungal species and isolates are known to differ in their ability to increase plant growth [6-9]. These differences may be related to environmental conditions or the inherent characteristics of a specific *AM* fungi isolate. Therefore, the isolation and identification of effective mycorrhizal strains from a particular region may be important in the application of arbuscular mycorrhizal fungi to crop production [10].

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Researchers in the last few decades have established that mycorrhizal fungi may improve plant growth through increased uptake of phosphorus, especially in soils of low P-content [11-13]. According to Mosse [14] 75% of the phosphorus applied to the crops is not utilized by them but gets converted to forms unavailable to plants. It has been estimated that in soils of high pH, a major portion of the fertilizer applied becomes non-usable via chemical reaction. *AM* fungi have been found to be very effective under such conditions, because of their ability to utilize extremely small quantities of fertilizer [15]. The mycorrhizae have the beneficial effect on the phosphate nutrition of crop plants in soil low in phosphorous [16]. The synchronization between the two symbiotic systems needs an optimal P level in the nutrient medium to stimulate the nodulation and nitrogen fixation [17].

Cicer arietinum was the best host followed by *Zea mays* for *Glomus fasciculatum* where 76.50 and 70.60 percent of root colonization was observed respectively at 120 days of growth [18]. They also reported that only mycelial structures were observed in host roots after 15 days of sowing the inoculated seeds. Maximum root colonization 86.38, 77.1, 76.4 and 74.15% in *Glomus constrictum, Glomus fasciculatum, Glomus epigaeum* and *Acaulospora morroweae* respectively. *Glomus epigaeum* was found to be more efficient than rest of the three species in respect of dry shoot (202.3 g) and root weight (295.8 g). They also reported that black gram plants when inoculated with above four species of endomycorrhizal fungi produced more dry weight of root and shoot, chlorophyll content and chemical constituents [19]. The influence of *AM* fungi on growth and nutrient uptake in chickpea with five *AM* fungi viz. *Glomus mosseae, Glomus fasciculatum, Glomus constrictum, Acaulospora laevis* and *Gigaspora gilmorei*. They reported that the proper selection of efficient *AM* fungi for the right crop and environment was the key to their successful application in agriculture [20]. 62.1% root colonization and 200 spores, 100 g^{-1} soil when black gram was inoculated with *Glomus caledonicum*, where as co-inoculated plants with *Glomus caledonicum* and *Rhizobium* showed 76.1% root colonization and 214.6 spores, 100 gm^{-1} soil [21]. Single inoculation of the mycorrhizal fungus increased growth nutrient uptake and growth of cowpea. The shoot dry weight and phosphorus (P), nitrogen (N) contents of shoot and root, chlorophyll content, pod yield per plant and root colonization were significantly higher than the uninoculated plants [22].

Moreover, 20 million hectares of geographical area of Assam and a total of 25 million hectares of geographical area of the North Eastern states are acidic in nature. High soil acidity has practically discouraged the use of phosphate fertilizers which instantly get fixed into insoluble forms. To remove this constraint the use of phosphate solubilizing microorganisms is indispensable. Most bacteria and fungi particularly the *AM* fungi are of highest importance in this regard [23]. The effects of *AM* fungi on plant growth are generally positive, many researches are directed towards its advancements (as per above literature). Thus, it requires isolation, identification, characterization, screening and application for enhancement of pulse production in NE region where this technology has, hitherto, not been used. Over the years, the state of Assam has been facing acute shortage in pulse production. Hence, to become self-sufficient there is a need to develop the symbiotic fungi technology to enhance the pulse production. Since no substantial research work has been carried out in Assam (Dibru-Saikhowa Biosphere Reserve) so far as the efficiency of symbiotic fungi are concerned and keeping the above facts in view, the proposed investigation has, therefore, been undertaken.

MATERIALS AND METHODS

Screening of AM fungi (*Mycorrhization in maize plant*)

Healthy and uniform sized seeds of maize were selected and surface sterilized by dipping the seeds with 0.01% $HgCl_2$ for 4 minutes, washed 5 times with sterile distilled water and dried in hot air oven at ± 26°C in sterile condition. Sterilization of the oven was done with overnight fumigation of formaldehyde and potassium dichromate ($K_2Cr_2O_7$). A spore suspension of isolated and preserved *AM* fungi was made with a spore concentration of 100 spores, ml^{-1}.

Root colonization

Root colonization of *AM* fungi was estimated by grid–line intersects method [24]. Roots were cleaned in 10% (w/v) KOH by boiling for 15-20 minutes and washed off excess KOH with alkaline H_2O_2. The roots were then dipped in 0.01 N HCl for 15-20 minutes and excess HCL solution was decanted. The roots were then stained with 0.01% acid fuchin-lactic acid staining solution. For distaining and for mycorrhizal assay lacto glycerol solution was used. Stained root segments (1cm in length) were cross sectioned/ crushed, mounted and presence or absence of colonization was examined under microscope (40x). The percentages of root colonization of *AM* fungi were calculated with the following formula and presented in (Table 1).

$$\text{Percentage of } AM \text{ fungi colonization} = \frac{\text{No. of positive segments}}{\text{Total No. of root segments studied}} \times 100$$

Evaluation of AM fungi

This practice was carried out according to the method described by Talukdar and Germida [25]. The surface soil was collected from the experimental field and soil was air dried and ground to pass through a 2mm sieve and mixed with sand (1:1). The soil mixture was packed in polypropylene bags (4kg, bag^{-1}) and sterilized in autoclave with 15 psi at 120°C for 20 minutes and kept overnight and autoclaved again in the next day as earlier. The autoclaved soil mixture was transferred to earthen pots (4kg, pot^{-1}).

Mycorrhization of the sterile soils in the sterile pots (4kg, pot^{-1}) were done with soil drenched/ soil mixture method [26-27]. Two holes (2 cm diameter and 1cm depth) were made in the sterile soils of each pot and 1ml of spore suspension was placed in each hole followed by sowing of 1-3 sterilized maize seed. The hole was covered evenly with nearby soil. In control pot, holes were served with sterile water without *AM* fungi. Each treatment was replicated six times of which three were harvested at 90 days. The pot culture experiment was set during January to April so that plants received an average 8-hour day light with a mean temperature 28°C. The plants were irrigated with Jensen's nitrogen (+) medium solution as proposed by Somasegaran and Hoben [28]. The pots were arrangement with randomized block design and sufficient gap was maintained between the pots to avoid contamination. The shoot and root dry weight of the maize plants were recorded by

conventional method. The observations were recorded and presented in (Table 2, Fig 1a-e).

Physico-chemical properties of field soil

Soil texture, pH, soil moisture content (SMC) and water holding capacity (WHC) of field soil was determined by standard method as proposed by Trivedi *et al*. [29]. Organic carbon [30] and total nitrogen by Kjeldahl method. Dilute acid extraction method was used for measuring available soil phosphorous as proposed [31].

RESULTS AND DISCUSSION

Screening of efficient strains of AM fungi [*Root colonization in host plant* (*Maize*)]

From (Table 1), it was observed that among the 17 *AM fungal* species, four species viz. *Glomus mosseae* (AMF_3), *Acaulospora laevis* (AMF_5), *Glomus fasciculatum* (AMF_{10}) and *Gigaspora gilmorie* (AMF_{17}) showed the maximum percentage of root colonization and number of spores per gram of soil at 90 days of maturity of maize plant [32]. The experiment was conducted with maize plant because it was a good host [33]. The maximum percentage of root colonization and number of spores (75.86% and 17.67 spores, gm^{-1} soil) were recorded in *Glomus mosseae* followed by *Gigaspora gilmorie* (73.11% and 12.67 spores, gm^{-1} soil), *Glomus fasciculatum* (52.90% and 7.33 spores, gm^{-1} soil) and *Acaulospora laevis* (44.52% and 6.67 spores, gm^{-1} soil) after 90 days of maturity of the host plant. In general, the percent of root colonization and number of spores of *AM* fungi increased with the increasing age of the host plant and decreased thereafter [34-35].

Evaluation of screened AM fungi

From (Table 2), it was observed that the results of shoot and root dry weight of maize plant were almost similar. The maximum (78.12 gm) shoot dry weight was recorded with inoculation of *Glomus mosseae* and followed by *Gigaspora gilmorie* (64.27 gm), *Glomus fasciculatum* (58.09 gm) and *Acaulospora laevis* (46.32 gm) while the maximum (24.02 gm) root dry weight was recorded with inoculation of *Glomus mosseae* and followed by *Glomus fasciculatum* (19.03 gm), *Gigaspora gilmorie* (18.42 gm) and *Acaulospora laevis* (13.26 gm) after the maturity of 90 days of maize plant. The highest and the lowest (4.01 and 2.12 mg, $plant^{-1}$) nitrogen content was recorded in *Gigaspora gilmorie* (AMF_{17}) and *Acaulospora laevis* (AMF_5) whereas, the highest and the lowest (2.02 and 0.81 mg, plant-1) phosphorus content were in *Gigaspora gilmorie* (AMF_{17}) and *Acaulospora laevis* (AMF_5) respectively [33].

Table 1 Screening of *AM* fungi on host plant, maize (*Zea mays* L.) in laboratory conditioned

Isolated AM fungi	Host Plant (Maize plant)	
	Root colonization (%)*	AM fungal spore, g^{-1} soil*
AMF_1	38.10 ± 0.23	5.33 ± 0.13
AMF_2	25.26 ± 0.03	4.00 ± 0.11
AMF_3	75.86 ± 0.05	17.67 ± 0.05
AMF_4	19.33 ± 0.01	2.33 ± 0.01
AMF_5	44.52 ± 0.01	6.67 ± 0.02
AMF_6	17.67 ± 0.05	2.33 ± 0.02
AMF_7	24.31 ± 0.23	4.00 ± 0.06
AMF_8	27.00 ± 0.06	3.33 ± 0.12
AMF_9	24.04 ± 0.07	1.67 ± 0.02
AMF_{10}	52.90 ± 0.06	7.33 ± 0.07
AMF_{11}	27.00 ± 0.05	1.67 ± 0.01
AMF_{12}	32.33 ± 0.12	4.67 ± 0.03
AMF_{13}	22.67 ± 0.07	2.33 ± 0.02
AMF_{14}	28.90 ± 0.01	5.33 ± 0.03
AMF_{15}	31.66 ± 0.13	4.33 ± 0.03
AMF_{16}	31.00 ± 0.08	3.00 ± 0.06
AMF_{17}	73.11 ± 0.06	12.67 ± 0.01

*Mean of 3 replications and SE
AMF= Arbuscular Mycorrhizal Fungi

Table 2 Evaluation of screened arbuscular mycorrhizal fungi on host plant (maize) *in vitro* condition

Screened AM fungi	Plant growth parameters* (Dry Weight)			Measure of shoot nutrient (mg, $plant^{-1}$)*	
	Shoot dry weight (g)	Root dry weight (g)	Root to shoot ratio	N-content	P-content
AMF_3	78.12±1.05	24.02±0.16	0.31±0.04	3.47±0.13	1.67±0.02
AMF_5	46.32±0.56	13.26±0.17	0.29±0.01	2.12±0.03	0.81±0.01
AMF_{10}	58.09±0.54	19.03±0.06	0.33±0.02	3.66±0.12	1.24±0.01
AMF_{17}	64.27±0.61	18.42±0.29	0.29±0.01	4.01±0.01	2.02±0.08

*Mean of 3 replications and SE
AMF_3= *Glomus mossea*
AMF_5= *Acaulospora laevis*
AMF_{10}= *Glomus fasciculatum*
AMF_{17}= *Gigaspora gilmorie*
N= Nitrogen; P= Phosphorus

Relationship between % of root colonization and growth parameters of maize

The relationship amongst mycorrhizal root colonization and number of spores (Table 1), shoot and root dry weight, N and P content (Table 2) of maize plants were observed and presented in (Fig 1a-e). The correlation is expressed by regression equation where it showed positive correlation in all variations. In dry weight of shoot it is nearby 95 % of the variability (R^2=0.946) [19].

Physico-chemical parameters of field soil

The physico-chemical properties viz. soil texture, pH, soil moisture content, water holding capacity, organic carbon, available P and available N of the field soil was analyzed to ascertain the status of the experimental field before cultivation.

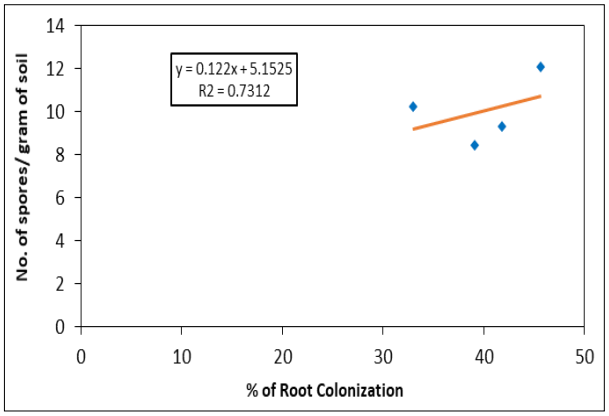

Fig 1a Correlation between % of root colonization vs number of spores per gram of soil of maize plant *in vitro* condition

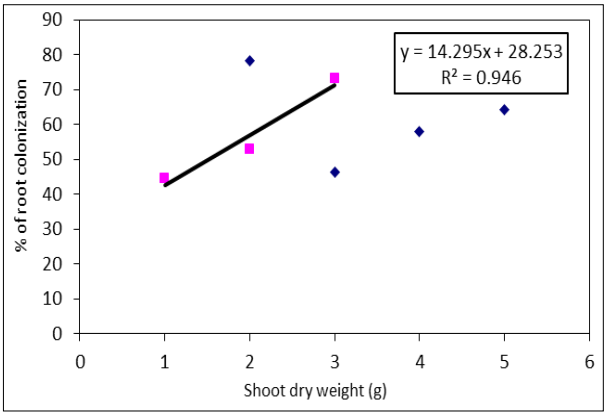

Fig 1b Correlation between mycorrhyzal root colonization vs shoot dry weight of maize plant *in vitro* condition

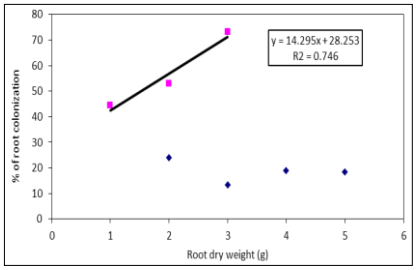

Fig 1c Correlation between mycorrhizal colonization vs root dry weight of maize plant *in vitro* condition

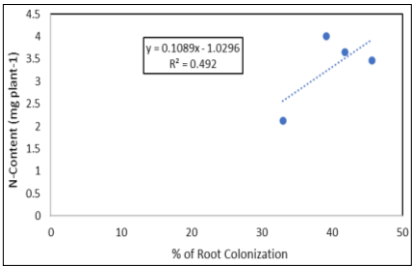

Fig 1d Correlation between % of root colonization vs N-content in maize plant *in vitro* condition

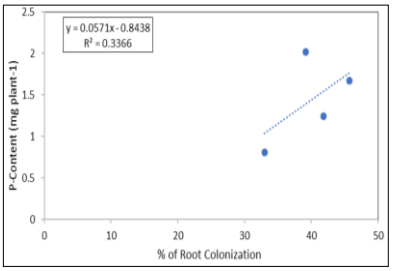

Fig 1e Correlation between % of root colonization vs P-content of maize plant in vitro condition

Table 3 Physico-chemical parameters of the field soil before cultivation of black gram

Physico-chemical parameters	Measure
Soil texture	Clay loam (CL)
pH	6.02
SMC	41%
WHC	65.03%
Organic C	0.60%
Available P	107.54 kg, ha^{-1}
Total N	189.67 kg, ha^{-1}

SMC=Soil moisture content
WHC= Water holding capacity

Lone inoculation of AM fungi

The *AM* fungal spores from the effective isolates viz. AMF_3, AMF_5, AMF_{10} and AMF_{17} as per % of root colonization and number of *AM* fungal spores, gm^{-1} soil (Table 1) and shoot and root dry weight, N and P content (Table 2) in maize plant were inoculated with black gram to study their impact on the growth and yield. Their impact on dry weight of shoot and root, chlorophyll content, nitrogen and phosphorus content of shoot and root and yield at maturity (90 days) were recorded and data presented in (Table 4). It was revealed that the maximum dry weight of shoot (8.15 gm, $plant^{-1}$) and root (3.32 gm, $plant^{-1}$), chlorophyll content (3.10 mg, gm^{-1}), N and P content of shoot (3.33 and 0.91 mg/plant) and root (0.88 and 0.75 mg/plant) and yield (450.65 kg, ha^{-1}) in black gram were observed in inoculation with AMF_3 (*Glomus mosseae*), while the minimum dry weight of shoot (6.36 gm, $plant^{-1}$) and root (1.25 gm, $plant^{-1}$), chlorophyll content (2.13 mg, gm^{-1}), N and P content of shoot (2.15 and 0.51 mg/plant) and root (0.49 and 0.43 mg/plant) and yield (371.67 kg, ha^{-1}) in black gram were in AMF_5 (*Acaulospora laevis*) [21].

Table 4 Effect of *AM* fungi on black gram to see the parameters of dry weight of (shoot and root), chlorophyll content, N and P content and yield

AM Fungal Isolates	Dry weight (g, $plant^{-1}$)*		Chlorophyll content (mg, g^{-1} fresh wt.)*	N-content (mg/plant)*		P-content (mg/plant)*		Yield (kg, ha^{-1})*
	Shoot	Root		Shoot	Root	Shoot	Root	
Control	5.22±0.05	1.10±0.03	1.25±0.06	1.19±0.02	0.36±0.01	0.43±0.04	0.30±0.01	286.56±1.53
AMF_3	8.15±0.04	3.32±0.05	3.10±0.01	3.33±0.04	0.88±0.02	0.91±0.02	0.75±0.01	450.65±3.06
AMF_5	6.36±0.04	1.25±0.03	2.13±0.10	2.15±0.04	0.49±0.04	0.51±0.01	0.43±0.03	371.67±3.79
AMF_{10}	7.64±0.02	3.10±0.02	2.85±0.06	2.28±0.04	0.63±0.01	0.60±0.04	0.59±0.03	424.67±3.21
AMF_{17}	7.00±0.06	1.55±0.06	2.06±0.04	3.23±0.07	0.72±0.02	0.76±0.05	0.47±0.03	412.33±4.16

*Mean of 3 replications and SE
AMF_3= *Glomus mosseae*, AMF_5= *Acaulospora laevis*, AMF_{10}= *Glomus fasciculatum*, AMF_{17}= *Gigaspora gilmori*
N= Nitrogen, P= Phosphorus

ANOVA analysis

Over all, the calculated P-value for all the parameters viz. shoot and root dry, N and P-content of shoot and root and yield is less than < 0.05, it exhibited significant positive variation in all *arbuscular mycorrhiza* (*AM*) fungal isolates (Table 5).

Table 5 ANOVA for dry weight of shoot and root, N and P content of shoot and root and yield of the efficient isolates (AM fungi) in black gram

Parameters	Sum of square	Degree of freedom	Mean of square	f-crait value	P-value
Shoot dry weight (g)	15.716	4	3.929	7.858	< 0.05
	0.000	9	5.000		
	675.009	14			
Root dry weight (g)	13.510	4	3.378	1.013	<0.05
	0.000	10	3.333		
	77.124	15			
N-content of shoot (g)	9.283	4	2.321	3.552	< 0.05
	0.007	10	0.001		
	97.523	15			
N-content of root (g)	0.486	4	0.122	3.648	< 0.05
	0.001	10	3.333		
	6.117	15			
P-content of shoot (g)	0.454	4	0.113	3.402	< 0.05
	0.001	10	3.333		
	6.649	15			
P-content of root (g)	0.339	4	0.085	1.475	< 0.05
	0.001	9	5.745		
	3.940	14			
Yield (kg/ha)	50103.759	4	12525.940	2.659	< 0.05
	4.711	10	0.471		
	2320242.462	15			

CONCLUSION

The present study concludes that the productivity of black gram due to lone inoculation of AMF_3 (*Glomus mosseae*) in field condition is highly productive rather than other *AM* fungal isolates. However, the role of micro climate and soil nutrient status cann't be completely ruled out. For soil nutrient management rhizosphere microrgainisms may further be applied as alternative biofertilizer instead of chemicals.

LITERATURE CITED

1. Das K. 2012. Study on PGPR and Symbiotic fungi from Dibru-Saikhowa Biosphere Reserve Forest and field application of a few isolates with reference to pulse production. *Ph. D. Thesis*, Gauhati University, Guwahati, Assam (India). pp 21-147.
2. Nannipieri P, Ascher J, Ceccherini MT, Landi, L, Pietrnellara G, Renella G. 2003. Microbial diversity and soil functions. *European Journal of Soil Science* 54: 655-670.
3. Yasmeen. 2002. *Diversity of Soil Fungi in Bansar Wildlife Sanctuary. Microbial Diversity: Status and Potential Applications*. Scientific Book Centre, Guwahati. pp 10-16.
4. James EBG, Hyde KD. 1998. Methods for the study of Mangrove Fungi. *In*: Mangrove Microbiology. Role of Microorganisms in Nutrient Cycling of Mangrove Soils and Waters. (Eds) by Agate AD, Subramanian CV and Vannuccie H. UNDP. pp 9-27.
5. Jha DK, Ahanthem S, Singh KN. 2002. Structural and Functional Diversity of Bacteria. *Microbial Diversity: Status and Potential Applications*. Scientific Book Centre. Guwahati, India. pp 54-67.
6. Mosse B. 1972. Effect of different Endogone strains on growth of *Paspalum notatum*. *Nature* 239: 221-223.
7. Abbott LK, Robson AD. 1978. Growth of subterranean clover in relation to the formation of endomycorrhizas by introduced and indigenous fungi in a field soil. *New Phytology* 81: 575-585.
8. Powell CL. 1982. Selection of efficient VA mycorrhizal fungi. *Plant Soil* 68: 3-9.
9. Sylvia DM, Burks JN. 1988. Selection of *vesicular- arbuscular mycorrhizal* fungus for practical inoculation of *Uniola paniculata*. *Mycologia.* 80: 565-568.
10. Talukdar NC, Germida JJ. 1993. Propagation and storage *of vesicular-arbuscular mycorrhizal* fungi isolated from Saskatchewan agricultural soils. *Canadian Jr. of Botany* 71(10): 1328-1335.
11. Gianinazzi S, Trouvelet A, Gianinazzi-Pearson V. 1989. Conceptual approaches in agriculture for the rational use of VA-endomycorrhizae in agriculture: Possibilities and limitations. *Agriculture, Ecosystems and Environments* 29: 153-161.
12. Santhi R, Kothandaraman GV. 1995. Effect of endomycorrhizal colonization and phosphorus sources on the phosphorus recovery, nutrient uptake and growth of green gram. *Indian Jr. Agric. Research* 29: 209-214.
13. Kumar R, Gupta PP, Jalali BL. 2001. Impact of *VA-mycorrhiza*, *Azotobacter* and *Rhizobium* on growth and nutrition of cowpea. *Jr. Mycol. Pl. Pathology* 31(1): 38-41.
14. Mosse B. 1973. Advances in the study of vesicular-arbuscular mycorrhiza. *Annu. Rev. Phytopathology* 11: 171-196.
15. Chandra S. 1992. VA Mycorrhiza- Dimensions of its application. *Indian Phytopathology* 45(4): 391-406.

16. Chen X, Tang JJ, Zhi GY, Hu SJ. 2005. Arbuscular mycorrhizal colonization and phosphorus acquisition of plants: effects of coexisting plant species. *Appl. Soil Ecology* 28: 259-269.
17. Geneva M, Zehirovl G, Djonova E, Kaloyanova N, Georgiev1 G, Stancheva1 I. 2006. The effect of inoculation of pea plants with mycorrhizal fungi and *Rhizobium* on nitrogen and phosphorus assimilation. *Plant Soil Environment* 52(10): 435-440.
18. Gill TS, Singh RS. 2001. Effect of host and substrates on development of VA mycorrhizal colonization and sporulation of *Glomus fasciculatum. Indian Phytopathgology* 54(2): 261-263.
19. Devi GU, Sitaramaiah K. 2001. Response of endomycorrhizal fungi at different levels of inoculum in black gram. *Indian Phytopathology* 54(3): 377-379.
20. Kulshreshtha M, Khan WM. 2002. Effect of ozone on root colonization by VAM fungi and root nodulation on black gram. *Indian Phytopathology* 55(3): 264-268.
21. Boby VU, Balakrishna AN, Bagyaraj DJ. 2007. Effect of combined inoculation of an AM fungus with soil yeasts on growth and nutrition of cowpea in sterilized soil. *World Jr. Agril. Sciences* 3(4): 423-429.
22. Yaseen T, Burni T, Hussain F. 2011. Effect of arbuscular mycorrhizal inoculation on nutrient uptake, growth and productivity of cowpea (*Vigna unguiculata*) varieties. *African Journal of Biotechnology* 10(43): 8593-8598.
23. Subba Rao NS. 1999. *Biofertilizers in Agriculture and Forestry*. Oxford & IBH Publishing Co. Pvt. Jana path, New Delhi.
24. Giovannetti M, Mosse B. 1980. An evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. *New Phytology* 84: 489-500.
25. Talukdar NC, Germida JJ. 1993. Occurrence and isolation of vesicular-arbuscular mycorrhizae in cropped field soils of Sask. Canada. *Canadian Jr. Microbiology* 39: 567-575.
26. Menge JA, Timmer LW. 1984. Procedures for inoculation of plants with vesicular arbuscular mycorrhizae in the laboratory. In: Methods and principles of Mycorrhizal Research. (Eds) By Schenck, N. C. *American Phytopathol.* Soc. St. Paul, Minn. pp 59-68.
27. Bhowmik SN, Singh CS. 2004. Mass multiplication of AM inoculum: Effect of plant growth promoting rhizobacteria and yeast in rapid culturing of *Glomus mosseae*. *Current Science* 86(5): 705-709.
28. Somasegaran P, Hoben HJ. 1994. *Handbook for Rhizobia*. Springer-Verlag. New York. pp 1-438.
29. Trivedi RK, Goel PK, Trisal CL. 1987. *Practical Methods in Ecology and Environmental Science*. Enviro Media Publications, Karad, India.
30. Walkley A, Black IA. 1934. An examination of the method for determining soil organic matter and a proposed modification to the chronic acid titration method. *Soil Science* 37: 29-38.
31. Jackson ML. 1973. *Soil Chemical Analysis*. Prentice Hall of India Pvt. Ltd. New Delhi. pp 134.
32. Das S, Saikia P, Baruah PP, Chakraborty A. 2016. Isolation and Identification of Soil Bacteria Collected from Dibru-Saikhowa, the National Park and Biosphere Reserve Forest of Assam, India. *International Journal of Science and Research* 5(1): 1937-1940.
33. Wright SF, Morton JB, Sworobuck JE. 1987. Identification of a vesicular-arbuscular mycorrhizal fungus by using monoclonal antibodies in an enzyme-linked immunosorbent assay. *Appl. Environ. Microbiology* 53: 2222-2225.
34. Tiwari N, Chauhan UK, Tiwari D. 1995. Response of VAM colonization in the rhizosphere soil of *Cajanus cajan* L. and *Zea mays* L. at different growth stages. *In*: (Eds) Adholeya, A. and Singh, S. Proceedings of the third national conference on Mycorrhiza, 13-15 March, 1995. (*Mycorrhizae Biofertilizers for Future*), Tata Energy Research Institute, New Delhi. pp 352-355.
35. Al-Raddar AM. 1995. Mass production of *Glomus mosseae* spores. *Mycorrhiza* 5: 229-231.