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Studies on mass multiplication of *Glomus moseae* [Arbuscular Mycorrizhal Fungus] for "Phosphofert " bio-fertilizer production, it's efficacy on phosphatic fertilizer savings and productivity in high yielding mulberry garden under West Bengal conditions

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Abstract:*Mulberry is cultivated by farmers for its leaves, the sole food for silkworm (Bombyx mori L.) for commercial production of raw silk in Sericulture Industry. As mulberry is a perennial crop can be maintained for several years in the field, selection of suitable land and follow-up of recommended package of practices are inevitable for maintenance of potential productivity of the variety selected for cultivation. As the quality of mulberry leaves alone contributes about 38.2% for the success of silkworm cocoon crop, quality linked leaf productivity of mulberry leaves can be achieved through adequate supply of all required input into soil. Phosphorus is one of the important macronutrient required in larger quantity next to Nitrogen for mulberry. In order to reduce the high cost involved towards nitrogenous chemical fertilizers and to maintain the soil health in an eco-friendly way Integrated Nutrient Management (INM) approach in agriculture sector became popular and the same has been followed in mulberry cultivation as well in recent years. Use of different kinds of microbial inoculants as bio-fertilizer to fix atmospheric nitrogen in mulberry garden brought improvements in soil health maintenance and helps to reduce nitrogenous chemical fertilizer requirements and expenditure to farmers considerably without affecting the quality linked productivity.*

Keeping in view of the above a study was conducted to ascertain the consistent efficacy of Glomus moseae (Arbuscular Micorrhizal Fungus) culture as "Phosphofert" bio-fertilizer application in mulberry garden during July to September 2013 crop. S1635 mulberry variety in Paired Row System [PRS] of plantation with (150+90) x 60 cm spacing under irrigated condition with five treatments i.e., T1 : 20 tons FYM + (Recommended Dose of Fertilizer) NPK 336:180:112 (5 split doses) ha^{-1} yr^{-1} [Control]; T2 : 20 tons FYM + NPK 336:135:112 (5 split doses) ha^{-1} yr^{-1} + 75 kg Phosphofert ha^{-1} once in 4 years; T3 : 20 tons FYM + NPK 336:90:112 (5 split doses) ha^{-1} yr^{-1} + 75 kg Phosphofert ha^{-1} once in 4 years;T4 : 20 tons FYM + NPK 336:45:112 (5 split doses) ha^{-1} yr^{-1} + 75 kg Phosphofert ha^{-1} once in 4 years and T5 : 20 tons FYM + NPK 336:0:112 (5 split doses) ha^{-1} yr^{-1} + 75 kg Phosphofert ha^{-1} once in 4 years with all other package of practices recommended for mulberry cultivation were followed uniformly in all treatments with 4 replications in CRD. Average leaf yield of 9.55; 9.75; 9.38; 9.54 & 8.33 and total biomass of 16.42; 16.22; 15.66; 16.04; 13.79 tons ha^{-1} $crop^{-1}$ produced under T1; T2; T3; T4 & T5 respectively and quality of leaves on economic characters without significant difference between the treatments revealed the role of AMF in making available phosphorus to mulberry crop at reduced rate of phosphorus chemical fertilizer up to 75% (T4) and expenditure without affecting the quality linked leaf productivity. Mass culture of the AMF for preparation of "Phosphofert" bio-fertilizer, its application techniques are discussed in the paper.

KEY WORDS: *Mulberry leaf, AMF; bio-fertilizer, eco-friendly soil health, potential productivity.*

I. INTRODUCTION

Mulberry is cultivated by farmers for its leaves, the sole food for silkworm (*Bombyx mori* L) for commercial production of raw silk in Sericulture Industry. As mulberry is a perennial crop, can be maintained for several years, selection of suitable land and follow-up of recommended package of practices are inevitable in maintaining the potential productivity of the concerned variety selected for the establishment of mulberry garden. Quality of mulberry leaves contribute to a level of 38.2% for the success of silkworm cocoon crop (Miyashita, 1986), the maintenance of mulberry garden plays a vital role in sericulture. The quality linked leaf productivity of mulberry garden can be achieved through adequate supply of all required input into soil.

In India, during the Green Revolution period more emphasis was given for increasing the productivity of crops which facilitated in indiscriminate application of inorganic chemical fertilizers, chemicals to control various pests & diseases without considering the soil health maintenance for long-term use for agriculture purposes resulted in considerable damage to the soils in agriculture land. It was reported that out of 235 mha (million hectares) of cultivable area, almost 166 mha area soil has been damaged (Swaminathan, 1994) in the country necessitates to find alternate methods to improve the soil health in agriculture sector.

Like other agricultural crops, mulberry requires the all sixteen nutrients. Off which phosphorous is an important macronutrient required in large quantity next to nitrogen for mulberry for quality linked productivity maintenance of leaf for silkworm rearing for production of silk cocoons. Based on the high cost involved in application of nitrogenous and phosphorous chemical fertilizers and to maintain the soil health in an eco-friendly manner, Integrated Nutrient Management (INM) approach in agriculture sector became popular. The same has

been followed in mulberry cultivation also. Introduction of bio-fertilizers application in mulberry garden brought improvements in maintaining the soil health and curtailment of expenditure towards the cost of chemical fertilizer and its requirements considerably. "Phosphofert" a bio-fertilizer contains *Glomus mosae* [Arbuscular Mycorrhizal Fungus] multiplied in soil, its application in mulberry garden proved to reduce about 70% requirement of phosphorous fertilizer and expenditure to that effect without affecting the quality linked leaf productivity in mulberry garden.

In the present work, under **Part-I** studies on the procedure of mass multiplication of *Glomus moseae* [Arbuscular Mycorrhizal Fungus] for Phosphofert bio-fertilizer production and in **Part-II** the efficacy of Phosphofert on phosphorous fertilizer saving in mulberry garden was planned with two objectives namely Mass multiplication of *Glomus mosae [Arbuscular Mycorrhizal Fungus]* for production of "Phosphofert" a Bio-fertilizer production and to find out the efficacy of Phosphofert on phosphorus fertilizer saving and productivity in mulberry garden.

To complete life cycle normally, living organism requires a large numbers of substances from outside are called nutrition. Green plants being autotrophic, requires only inorganic substances from outside (Pandey and Sinha, 1972). Only certain elements have been determined to be essential for plant growth. An essential element is defined as one whose absence prevents plants from completing its life cycle or one that has clear physiological role (Arnon and Stout, 1939). The inorganic substances are absorbed by plants in ionic forms from the soil by roots. Other mycorrhizal fungi and nitrogen fixing bacteria, often participate with roots in the acquisition of nutrients. In addition to nutrients being added to the soil as fertilizers, some mineral nutrients can be applied on leaves as sprays, known as foliar application (Taiz and Zeiger, 2003). Nutrients uptake by plants through leaves is most effective when the nutrients solution remains on the leaf as a thin film (Mengel and Kirkby, 1987).

High agricultural yields depend strongly on fertilization with mineral nutrients (Taiz and Zeiger 2003). In fact, yield of most crop plants increase linearly with the amount of fertilizer they absorb and crop plants, however, typically use less than half of the fertilizer applied (Loomis and Conner, 1992).Phosphorous is a mineral element required in larger amounts by plants. It serves as a constituent of nucleotides particularly in Adenosine Tri Phosphate synthesis and cell division and development of meristematic tissues in plants. Deficiency of phosphorous rapidly inhibits plant growth; stunted root, shoot growth, leaves turn dark green in color and turn reddish purple with necrosis at later stage.

Powdery mildew prominent in phosphorous deficient leaves. Under severe condition, the leaves become completely yellow and fall off the plant. Phosphorous deficient plants may have dark green upper leaves and reddish purple lower leaves (Taiz and Zeiger 2003). Excessive uses of nitrate and phosphatic fertilizers have led to extensive contamination of surface and ground waters were reported by Dahama (2003). The fate of nitrogen fertilizers in the soil environment is controlled by several physical, chemical and biological factors that interact with each other. The percentage of recovery of nutrients varies between the different types of fertilizers were reported as 50-60; 5-15; and 75% of N P K respectively and nitrogen deficiency is observed in plants grown on soils with low organic matter (< 0.4 % organic carbon) (Anonymous, 2011).

Mycorrhizal fungi can increase the yield of a plot of land by 30%-40%. It can absorb phosphorus from the soil and pass it on to the plant. Mycorrhizal plants show higher tolerance to high soil temperatures, various soil- and root-borne pathogens, and heavy metal toxicity. The use of biofertilizers is currently gaining interest as a cheap, safe alternative to conventional chemical fertilizers (Sharma, 2002). Biofertilizers can make significant contribution towards the development of strategies for productivity improvement which do not lead to an exponential rise in the consumption of non- renewable forms of energy (Subba Rao, 1982). The germination and hyphal growth from asexual spores in the soil is stimulated by signaling compounds released by roots (top right). These hyphae form infection structures (appresoria) on the surface of host roots the fungus grows into the root forming hyphae between cells and arbuscles that penetrate cell walls without killing the plant cells. Hyphae also grow out into the soil forming a branched mycelium that functions to explore the soil and take up mineral nutrients. Spores are formed by this external mycelium, completing the life cycle. Movement of nitrogen from soil to plants via AMF through the symbiosis was reprted by Jin *et al.,* (2005).

Arbuscular mycorrhizal fungi are considered as obligate symbiotic biotrophs, in that they can't grow without a host plant supplying them with carbohydrates (Muchovej, 2001; Harrison, 2005; Martin *et al.,* 2007; Hamel and Plenchette, 2007). Arbuscular mycorrhizal associations are reported to occur in about 80% of terrestrial plants (Gregory, 2006). Inorganic N (NO_3^- and NH_4^+) is taken up by the external mycelium, assimilated and converted to arginine, which is transported (probably in association with Polyphosphate) within

the fungus to the fungal mycelium inside plant roots. George (2005) reported that *Azotobacter* use a variety of carbohydrates, alcohols and salts of organic acids as sources of carbon and can grow in the pH range from 4.8 to 8.5. However the growth favored at a temperature of 20- 30 °C (Tepper *et al.,* 1979).AMF colonization was positively correlated with soil total nitrogen in spring in *Pyrus glabra.* factors like temperature, luminosity, dynamics of plant species, rainfall, soil fertility, root exudations and competition with other microorganisms as well as possible interactions influence the AMF life cycle was studied by Touran Feyzi Kamareh *et al.,* 2011.

Suitable combinations of AM fungi (by contact or by inoculation) and rhizobia increase the plant growth and the P use efficiency, enhancing N2 fixation under limited P supply conditions in common bean (*Phaseolus vulgaris* L.) was reported by Fatma Tajini, Mustapha Trabelsi and Jean-Jacques Drevon. (2012). A few studies have shown that some bacterial species respond to the presence of certain AM fungi (Andrade *et al.,* 1997; Artursson *et al.,* 2006), suggesting a high degree of specificity between bacteria associated with AM fungi. Thus, the specific bacteria together with AM fungi may create a more indirect synergism for plant growth (Barea, 1997) including nutrient acquisition (Barea *et al.,* 2002) and enhancement of root branching (Gamalero *et al.,* 2004).

In addition, the AM fungi themselves have also been shown to have an impact on the composition of bacterial communities in their mycelium environment (Artursson *et al.,* 2006). The rhizobia - bean symbiosis when in association with arbuscular mycorrhizal fungi (AMF) is known to benefit from a better supply of phosphorus (Sanginga *et al.,* 2000). The AMF is also able to acquire phosphorus in organic form that is not directly assimilated by plants (Bucher *et al.,* 2001). The mechanisms affecting the efficiency of absorption and utilization of phosphorus in plants are related to colonization by mycorrhizae (Jia *et al.,* 2004).

Furthermore, Jin et al. (2010) found that dual inoculation with AMF and rhizobia decreased the harmful influence of sulphate salinity on plant growth and nutrient accumulation (P, N and Proline) in *Lathyrus sativus*, compared with the control treatments. Both symbioses share parts of signalling pathways, indicating intimate interactions between all three partners during co-evolution (Demir and Akköpru, 2007; Stancheva *et al.,* 2006; Xiao *et al.,* 2010). Xiaomei Cheng *et al.,* (2008) suggest that even low rates of fertilizer, which are typical of wine grape production, may be incompatible with legume crop incorporation with respect to both AMF-mediated N capture and root uptake of N. If organic matter is used as a primary means of modifying soil fertility, as in organic vineyards where mineral fertilizers are forbidden, it may be important to incorporate the material into vineyard soil with the highest root densities and roots had a dominant role over hyphae in N uptake.

II. MATERIALS AND METHODS

Part-I : Mass multiplication of *Glomus moseae* [Arbuscular Mycorrhizal Fungus] for Phosphofert bio-fertilizer production.

About 100 kg of garden soil collected from the field of CSR&TI., Berhampore loaded in horizontal autoclave and sterilized at 15 lb pressure per cm^2 for one hour, after cooling the same process was repeated for double sterilization of the soil. After cooling of the soil transfer it to commercial AMF growth chamber and equal quantum of *Glomus moseae* (AMF) mother culture soil maintained 100 numbers of spores (Annexure : 1 Plate : 1) per 5 gram of soil in mother culture growth chambers was thoroughly mixed to it and spread. Maize seed at a distance of 10 cm sown at a depth of 3 cm and apply water at frequent interval to maintain available soil moisture around 50% (ASM) from its total capacity for better germination and growth of the maize plants.

The AMF spore germinate, hyphae grow profusely in the soil (Annexure : 1 Plate: 2) and multiply on establishment of maize plants due to symbiotic relationship with the roots of the maize plants. The maize plantation was maintained for about 60 days and just before initiation of flowering in maize plants, soil sample was collected to check the spore population per 5 gram soil.

On ascertaining the multiplication of AMF spore population @ 100 numbers per 5 gram soil, maize shoots at ground level were harvested leaving its roots intake in the soil. The soil contains 100 AMF spore population per 5 gram soil packed in polythene bag as 1 kg packet as "Phosphofert" as per standard procedure followed in AMF culture (Annexure : 1 Plates :3 - 7) Sudhakar *et al.,* 1999). Maintaining the required soil moisture of 50% available soil moisture of field capacity of the soil and temperature around 20^o C facilitate better growth and multiplication of AMF in the soil.

Part-II Efficacy of Phosphofert on phosphorous fertilizer saving in mulberry garden in the field:

The experiment was carried out in the mulberry garden (Plot No. A9) of Agronomy section at Central Sericultural Research and Training Institute, Central Silk Board, Berhampore, Murshidabad, West Bengal during July to September 2013. Well established irrigated mulberry garden raised in alluvial soil with S1635 improved high yielding variety under Paired Row System with plant spacing (150+90) x 60 cms. The plants were pruned at a height of ¾ foot, removed weeds and inter-cultivation operation carried out by using Power Tiller & Power Weeder (Annexure : 1 Plate : 8**)** and 20 plots were demarked each with two paired rows with 20 plants each and thus a total 40 number of plants (Chaturvedi and Sarkar, 2000) in Completely Randomized Design [CRD] (Annexure : 2 Plate : 9) as described by Sukhatme and Amble (1985).

The experiment consists of 5 treatments and 4 replications each. In T1 recommended FYM manure @ 20 ton FYM ha^{-1} $year^{-1}$ in two equal split doses and chemical fertilizers NPK @ 336 : 180 : 112 kg. ha^{-1} $year^{-1}$ in 5 equal split dose (Ray *et al.,* 1973) as control and in T2 - T5 except for 25; 50; 75 & 100% reduced dose of phosphorous fertilizer respectively with additional 75 kg of Phosphofert bio-fertilizer (Annexure : 2 Plates : 10 - 12) as below

T1: 20 tons FYM + (RDF) NPK 336:180:112 (5 split doses) ha^{-1} yr^{-1} [Control].

T2: 20 tons FYM + NPK 336:135:112 (5 split doses) ha^{-1} yr^{-1} + 75 kg Phosphofert ha^{-1} once in 4 years.

T3: 20 tons FYM + NPK 336:90:112 (5 split doses) ha^{-1} yr^{-1} + 75 kg Phosphofert ha^{-1} once in 4 years.

T4: 20 tons FYM + NPK 336:45:112 (5 split doses) ha^{-1} yr^{-1} + 75 kg Phosphofert ha^{-1} once in 4 years.

T5: 20 tons FYM + NPK 336:0:112 (5 split doses) ha^{-1} yr^{-1} + 75 kg Phosphofert ha^{-1} once in 4 years.

70^{th} day after pruning observations were made in all plots in two treatments from 5 randomly selected plants on the following parameters and yield was estimated (Annexure : 2 Plates : 13 - 16) as suggested by Sreenivasa Shetty *et al.,* (1990).

a) Growth Parameters :

i. Average number of branches / plant,
ii. Average height of branch (cm),
iii. Average total shoot length / plant (m),
iv. Average number of leaves / branch,
v. Average number of leaves / plant,
vi. Average leaf weight / plant (g) [Green weight],
vii. Average shoot weight / plant (g) [Green weight],
viii. Average leaf weight ha^{-1} $crop^{-1}$ (ton),
ix. Average shoot weight ha^{-1} $crop^{-1}$ (ton),
x. Average green biomass weight ha^{-1} $crop^{-1}$ (ton),
xi. Average dry biomass weight ha^{-1} $crop^{-1}$ (ton),

b) Leaf quality Parameters :

i) Moisture Content of leaf (MC (%) :

50 leaves between 5^{th} to 9^{th} position from the top were randomly collected from 10 different branches at 8.30 a.m. from each treatment plot in each crop, after recording the initial weight, the leaves were allowed to release moisture at room temperature for 48 hours and then dried in an oven at 80°C for 48 hours and the MC were calculated using the following formula (Vijayan *et al.,* 1997).

$$MC\ (\%) = \frac{\text{Fresh weight - Oven dry weight}}{\text{Fresh weight}} \times 100$$

ii) Moisture Retention Capacity of leaf (MRC(%) :

50 leaves between 5^{th} to 9^{th} position from the top were randomly collected from 10 different branches at 8.30 a.m. from each treatment plot in each crop, after recording the initial weight, the leaves were allowed to release moisture at room temperature at 6^{th} hr. weight of leaves were recorded again and then at 48^{th} hr. and were dried in an oven at 80°C for 48 hrs. and the MRC in % after 6 hours of harvest were calculated using the following formula (Vijayan *et al.,* 1997).

$$\text{MRC (\%)} = \frac{\text{Weight after 6hrs - Oven dry weight}}{\text{Fresh weight - Oven dry weight}} \times 100$$

All data of the experiment are subjected to statistical analysis using AGRES Software and the results are tabulated and discussed separately.

III. RESULTS AND DISCUSSION

Part-I : Mass multiplication of *Glomus moseae* [Arbuscular Mycorrhizal Fungus] for Phosphofert bio-fertilizer production.

From 100 kg double sterilized garden soil mixed with equal quantity of AMF mother culture soil consists of 100 AMF spores per 5 gram of soil in 60 days culturing in AMF commercial growth chamber 200 kg of Phosphofert Bio-fertilizer was prepared and packed as one kg packet in polythene bags printed with all specifications for use in mulberry garden and preservation of the Phosphofert.

Part-II Efficacy of Phosphofert on phosphorous fertilizer saving in mulberry garden in the field :

Studies conducted by IARI, New Delhi, showed that inoculation with *Azotobacter* or *Azospirillum* and phosphate solubilising bacteria culture in the presence of 1% rock phosphate is a beneficial input to obtain good quality compost rich in Nitrogen (1.85%) and AMF inoculation saves as much as 70% phosphorous fertilizers (Sharma, 2002).

Microbial inoculants in carrier based preparations containing beneficial microorganisms in a viable state intended for seed or soil application and designed to improve soil fertility and help plant growth by increasing the population and biological activity of desired microorganism in the root environment (Subba Rao, 1982).

Inoculation of Azotobacter and VAM Fungus or co-inoculation of all these organisms in mulberry has proved beneficial in terms of economizing N and P fertilizer application by 50% without any adverse effect on leaf yield and quality (Das *et al*, 1994).

Further it has been observed from the field studies at farmers level that application of n-triacontanol (Vipul) as foliar spray and use of *Azotobacter* bio-fertilizer could increase the leaf yield by 15-20% besides, 50% reduction in nitrogenous fertilizer application (Rajanna *et al*, 2005) and Phosphofert bio-fertilizer application @ 75 kg ha^{-1} once in four years was able to curtail 70% phosphorous fertilizer requirement of mulberry without affecting yield and quality of leaves (Sudhakar *et al*, 2000). Similar results were obtained in the present study are discussed as below :

a) Growth Parameters:

i) **Average number of branches / plant :** An average of 12.95; 12.2; 10.75; 11.0 and 12.6 number of branches recorded per plant in T1; T2; T3; T4 and T5 respectively with over all grand mean average of 11.9 number of branches per plant and the difference in number of branches per plant between the treatments were statistically non-significant @ CD 5% level (Table : 1).

ii) **Average height of branch (cm) :** An average height of 121.49; 120.55; 127.48; 124.83 and 108.85 cm. per branch was recorded per plant in T1; T2; T3; T4 and T5 respectively with over all grand mean average of 120.6 cm. height per branch.

iii) **Average total shoot length / plant (m) :** An average total shoot length of 15.65; 14.73; 13.67; 13.63 and 13.63 m. per plant was recorded per plant in T1; T2; T3; T4 and T5 respectively with over all grand mean average of 14.26 m. length per plant.

iv) **Average number of leaves / branch :** An average of 30.38; 30.32; 33.49; 31.23 and 26.06 number of leaves per branch recorded in T1; T2; T3; T4 and T5 respectively with over all grand mean average of 30.29 number of leaves per branch.

v) **Average number of leaves / plant :** An average of 391.73; 370.30; 358.25; 340.83 and 325.13 number of leaves per plant recorded in T1; T2; T3; T4 and T5 respectively with over all grand mean average of 359.25 number of leaves per plant.

vi) **Average leaf weight / plant (g) :** An average weight of 687.66; 701.75; 675.03; 686.90 and 599.95 g leaves per plant recorded in T1; T2; T3; T4 and T5 respectively with over all grand mean average of 359.25 g weight of leaves per plant.

vii) **Average shoot weight / plant (g) :** An average weight of 494.79; 466.43; 452.66; 468.08 and 393.29 g shoot per plant recorded in T1; T2; T3; T4 and T5 respectively with over all grand mean average of 359.25 g shoot weight per plant.

viii) The above said growth parameters recorded under different treatments were statistically on par and without significant difference @ CD 5% level (Table : 1).

ix) **Average leaf weight ha^{-1} crop^{-1} (ton) :** An average estimated weight of 9.55; 9.75; 9.38; 9.54 and 8.33 ton leaves ha^{-1} crop^{-1} recorded in T1; T2; T3; T4 and T5 respectively with over all grand mean average of 9.31 ton weight of leaves ha^{-1} crop^{-1}.

x) **Average shoot weight ha^{-1} crop^{-1} (ton) :** An average estimated weight of 6.87; 6.48; 6.29; 6.50 and 5.46 ton shoots ha^{-1} crop^{-1} recorded in T1; T2; T3; T4 and T5 respectively with over all grand mean average of 9.31 ton weight of shoots ha^{-1} crop^{-1}.

xi) **Average green biomass weight ha^{-1} crop^{-1} (ton) :** An average estimated weight of 16.42; 16.22; 15.66; 16.04 and 13.79 ton green biomass ha^{-1} crop^{-1} recorded in T1; T2; T3; T4 and T5 respectively with over all grand mean average of 15.63 ton biomass ha^{-1} crop^{-1}.

xii) **Average dry biomass weight ha^{-1} crop^{-1} (ton) :** An average estimated weight of 5.90; 5.79; 5.59; 5.74 and 4.92 ton dry biomass ha^{-1} crop^{-1} recorded in T1; T2; T3; T4 and T5 respectively with over all grand mean average of 15.63 ton biomass ha^{-1} crop^{-1}.

The above said economic growth parameters recorded under different treatments were statistically on par and without significant difference @ CD 5% level (Table : 2 and Figs. 1).

b) Leaf quality Parameters :

i) **Moisture Content of leaf (%) :** An average moisture content of 81.37; 81.16; 81.96; 81.35 and 81.80 % of leaf recorded in T1; T2; T3; T4 and T5 respectively with over all grand mean average of 81.53 % of moisture content of leaf.

ii) **Moisture Retention Capacity of leaf (%) :** An average moisture retention capacity of 95.07; 95.19; 94.98; 95.22 and 94.87 % of leaf recorded in T1; T2; T3; T4 and T5 respectively with over all grand mean average of 95.06 % of moisture retention capacity of leaf.

The above said quality parameters recorded under different treatments were statistically on par and without significant difference @ CD 5% level (Table : 2 and Figs. 2).

Table: 1 Growth parameters (Average) recorded during the experiment season (Aug-Oct.2013)

Treatment	Number of branches / plant	Height of branch (cm)	Total shoot length/plant (m)	Number of leaves / branch	Number of leaves / plant	Leaf yield / plant (g)	Shoot weight / plant (g)
T1	12.95	121.49	15.65	30.38	391.73	687.66	494.79
T2	12.20	120.55	14.73	30.32	370.30	701.75	466.43
T3	10.75	127.48	13.67	33.49	358.25	675.03	452.66
T4	11.00	124.85	13.63	31.23	340.83	686.90	468.08
T5	12.60	108.85	13.63	26.06	325.13	599.95	393.29
Gr. mean	***11.9000***	***120.6445***	***14.2615***	***30.2940***	***357.2500***	***670.2570***	***455.0490***
SEd	*0.9602 (NS)*	*7.6268 (NS)*	*0.9912 (NS)*	*2.5497 (NS)*	*28.8053 (NS)*	*66.6450 (NS)*	*63.0570 (NS)*
CD@5%	*2.0467*	*16.2564*	*2.1128*	*5.4346*	*61.3975*	*142.0518*	*134.4040*
CV %	*11.41*	*8.94*	*9.83*	*11.90*	*11.40*	*14.06*	*19.60*

Table: 2 Average yield & quality parameters recorded during the experiment season (Aug-Oct.2013)

Treatment	Leaf yield ha^{-1} crop^{-1} (t)	Shoot weight ha^{-1} crop^{-1} (t)	Total green biomass ha^{-1} crop^{-1} (t)	Total dry biomass ha^{-1} crop^{-1} (t)	Leaf moisture content (%)	Leaf moisture retention capacity (%)
T1	9.55	6.87	16.42	5.90	81.37	95.07
T2	9.75	6.48	16.22	5.79	81.16	95.19
T3	9.38	6.29	15.66	5.59	81.96	94.98
T4	9.54	6.50	16.04	5.74	81.35	95.22
T5	8.33	5.46	13.79	4.92	81.80	94.87
Gr. mean	***9.3080***	***6.3215***	***15.6290***	***5.5880***	***81.5280***	***95.0630***
SEd	*0.9260 (NS)*	*0.8752 (NS)*	*1.7736 (NS)*	*0.6550 (NS)*	*0.3313 (NS)*	*0.1886 (NS)*
CD@5%	*1.9737*	*1.8655*	*3.7804*	*1.3961*	*0.7063*	*0.4021*
CV %	*14.07*	*19.58*	*16.05*	*16.58*	*0.57*	*0.28*

Gr. mean. : Grand mean; NS. : Non singnificant; CD.: Critical difference; CV.: Coefficient of variation

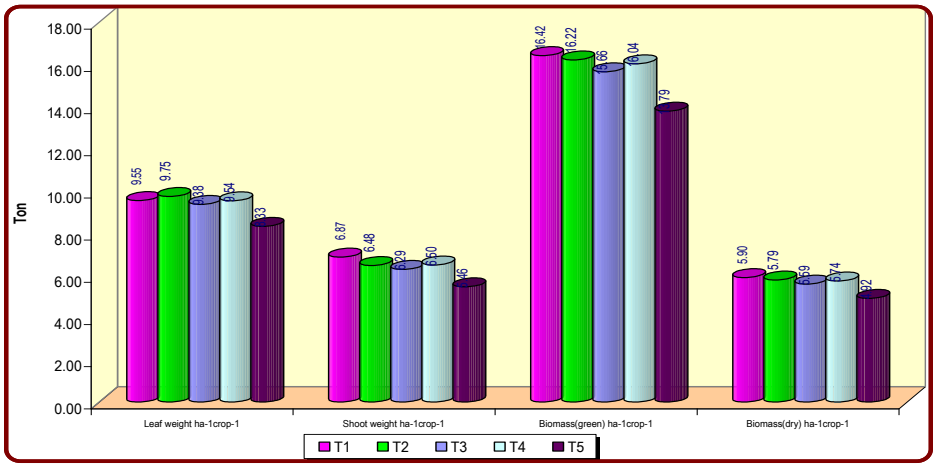

Fig : 1 Graph showing average yield details recorded during the experiment season (Aug-Oct.2013)

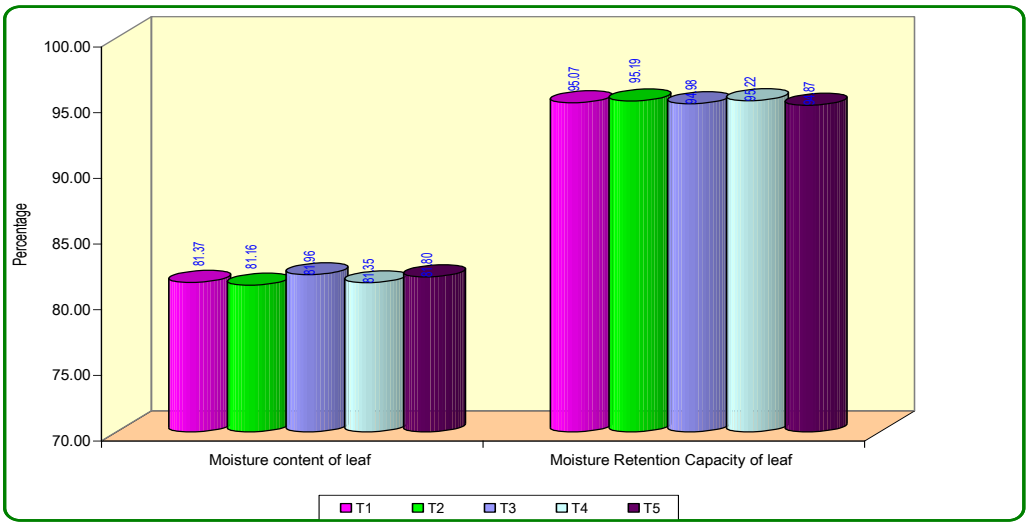

Fig : 2 Graph showing average yield details recorded during the experiment season (Aug-Oct.2013)

IV. CONCLUSION AND SUMMARY

It is summarized that the maintenance of *Glomus moseae* [Arbuscular Mycorrhizal Fungus] mother culture and mass multiplication of the same in sterilized garden soil with maize roots under controlled conditions for production **"Phosphofert"** a **Bio-fertilizer** [Microbial inoculants] is viable.

The **"Phosphofert"** (with 20 AMF spores per gram soil inoculants) application in mulberry garden as recommended @ 75 kg ha^{-1} once in four years may help to sericulture farmers in curtailment of about 75% phosphorus chemical fertilizer requirement in high yielding mulberry garden maintenance without affecting the quality and productivity in mulberry sericulture and thus saves expenditure considerably to farmers.

In addition to the above, application of **"Phosphofert"** helps to improve the soil health in mulberry garden in eco-friendly manner through increasing the population of economically important microorganisms, availability of nitrogen and other micro nutrients in the soil.

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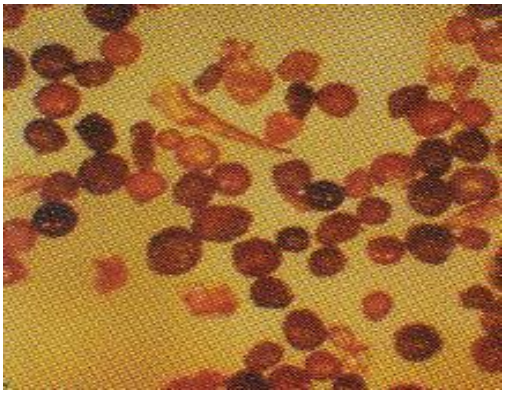
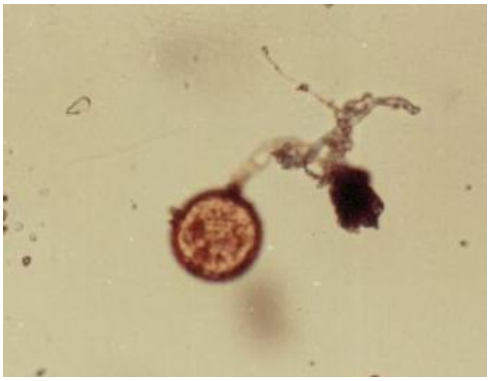
Annexure: 1 Plates showing different stages in mass multiplication of *Glomus moseae* [Arbuscular Mycorrhizal Fungus] & Phosphofert bio-fertilizer production





Plates : 1 & 2 AMF spores and germination

Plate: 3 & 4 Maize seed sowing & growth in AMF mass multiplication chamber

Plate : 5 AMF ready for harvest

Plate : 6 Packing of Phosphofert

Plate : 7 Phosphofert bio-fertilizer

Plate : 8 Experiment plot preparation

Annexure: 2

Plates showing different activities in the field during experiment

Plate : 9 AMF incorporated plot

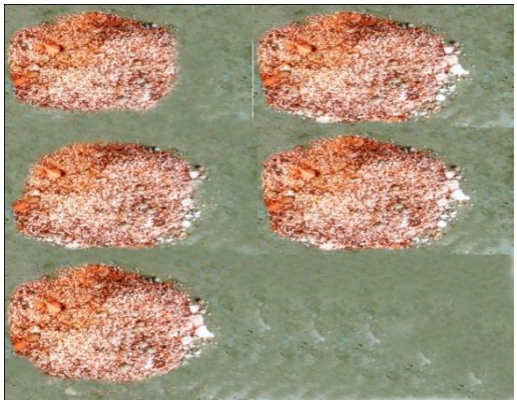


Plate : 10 NPK for application in plot

Plate : 11 Application of NPK

Plate : 12 Experiment plot

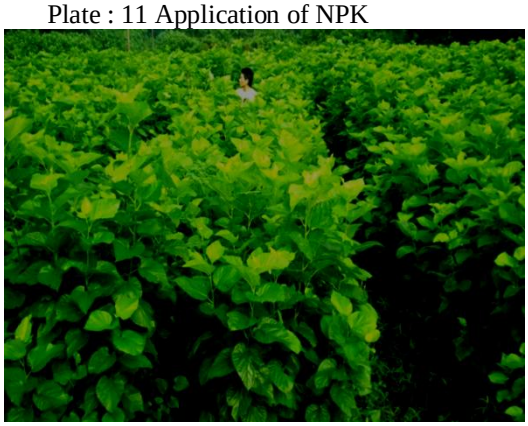


Plate : 13 Plot ready for harvest

Plate : 14 Harvesting for data record

Plates : 15 & 16 Recording of data