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The Influence Of Host Plant Species And Valeriana Wallichi Residue As Substrate For Inoculum Production Of Arbuscular Mycorrhizal Fungi For Sustainable Agriculture

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Abstract:
In the present world, human fraternity looking for the production of quality food in a very sustainable way. The arbuscular mycorrhizal fungi (AMF), the nature's own biofertilizer, are one of the sustainable tools that aids in growth and development of plants. However, there are several limitations in obtaining large amount of inoculums. Therefore, a pot experiment was carried out under polyhouse pot conditions, to evaluate the efficacy of Valeriana wallichi residue as substrate with three host plant species viz. Sesbania aculeata, Sorghum bicolor and Zea mays on mass production of Glomus mosseae. The results in the present investigation showed that AM fungal endophyte was significantly influenced using different concentration of substrate with various host plant species utilized. Comparatively, maize was proved to be most suitable host as it increased mycorrhizal root colonization and spore population to a maximum level with 180gm. concentration of substrate. These findings present AM fungi as one of the most promising tools capable of reducing usage of hazard causing chemicals thereby playing an utterly important role in the direction of sustainable agriculute. The present study was done with an aim to enhance the production of AM fungi.
Key Words: *AM fungi, G.mosseae, inoculum production, V. wallichi*

INTRODUCTION:

Among the symbiotic microorganisms, the AM fungi have a great ability for use as biofertilizers in agriculture, floriculture, horticulture and forestry (Singh, 2002). AM fungi show strong impact on root morphogenesis and induced changes in root architecture (Berta *et al.,* 2002). They derive most of their essential organic matter from their symbiotic niche in roots, which in turn help better growth of their host plant due to enhanced phytochrome levels, the uptake of nutrients from the soil, imparted resistance or tolerance to stresses and afford protection against pathogens, salinity and drought extremes (Kapoor *et al.,* 2004, Turnau and Haselwandter, 2002, Azcon- Anguilar *et al.,* 2002, Giri *et al.,* 2005, 2007). The arbuscular mycorrhizal (AM) symbiosis are typically mutualistic as obligate symbionts as the significant development to complete a life cycle is only achieved in the presence of a host plant (Sahay *et al.,* 1998, Tahat *et al.,* 2008).

The inherent capability of AM fungi in abundance depends upon the method of growth of AM fungi and the production of a qualitative and huge volume of AM inoculum in a more economic way. However, the utilization of plant having efficacious affinity with the variable AM fungal species can be favourable for attaining the desired production of AMF inoculums ensuring potential benefits of symbiosis for crop improvement.

The most important step to commence the inoculums production is selection of suitable host and fungal species keeping a check on the specificity of host towards a particular AMF species. In current times, numerous techniques have been designed giving desirable results for the mass scale commercial production of AM fungal inoculums. Although, the traditional pot culture method of multiplying AM

fungi in the rhizosphere of suitable host is still followed widely. Still the rapid production and maintenance of viable AMF inoculums is still on uphill task from commercial point of view. Variety of wastes that can be used as substrate to promote the mycorrhization in the host rhizosphere for mass production of AM fungi. Trap plants are usually propagated vegetatively through cuttings to minimize chances of disinfection.

Keeping in view the above information, the present investigation was aimed to carry out mass production of AM fungi. Therefore, pot experiment inoculum production was undertaken in the present study with an ultimate objective for evaluation of the effect of various host plants (*Sesbania aculeata*, *Sorghum bicolor*, *Zea mays*) and waste substrates (*Valerianaa wallichi* residue) on the development of AM fungi i.e. *G. mosseae*.

MATERIAL AND METHODS:

Source of AM spores/AM endophytes:

The soil samples taken from the rhizospere of four medicinal plants and dominant AM fungus i.e. *G.mosseae* was selected for mass multiplication. These AM fungal endophytes was identified by using the keys of Walker (1986), Schenck and Perez (1990), Mukerji (1996) and Kumar *et al.* (2009a).

Selection of Substrates:

The substrate, *Valerianaa wallichi* residue, a wastes of medicinal plant was selected for the mass production of *G.mosseae*. The substrate was first sterilized by autoclaving. The *Valeriana* residue was obtained from Sant Bhama Enterprises, Mandi, Himachal Pradesh.

Plant Host's Selection:

In order to select most suitable host, three host plant species viz. *Sesbania aculeata*, *Sorghum bicolor* and *Zea mays* were tried for mass production of *G.mosseae*. All the host selected for experiment are known for fast growing capability, producing hairy and fine roots which could help the fungi to produce more spores and root colonization.

Starter inoculum production:

Initially, the pure culture for *G.mosseae* was prepared by 'Funnel Technique' method of Menge and Timmer (1982) using wheat as host (Plate-1). Here, the sterilized soil and sand mixture (3:1) was used as a substrate and filled in funnel. Spores were isolated from rhizosphere of selected plants and ten to twelve spores of *G.mosseae* were introduced in the funnel mixture. Then the seeds of wheat were sown in the funnel and watered regularly. After 75 days, seedling roots were processed to study AM colonization (Philips and Hayman, 1970) and soil sample was studied for AM spore quantification. The seedlings having AM colonized root and soil samples containing the AM spores were transferred to bigger earthen pots for further multiplication.

Pot and Potting Mixture:

The earthern pots (25×25cm.) were used for the experimentation. They were filled with sterilized soil:sand (3:1) mixture and different concentrations of substrate (*Valeriana* residue) were added and mix thoroughly so as to make a final volume of 700 gm. In control earthern pots only 700 gm. of soil:sand (3:1) mixture was raised. A layer of ten percent (70gm.) of inoculum was spread over the pot mixture (sterilized soil:sand + waste substrate) in earthern pots. The inoculum consisted of AM colonized root pieces and soil containing AM spores. Different treatments made in the experiment were as under:

***Valeriana* residue:**

Sr.No.	Treatment	Soil (gm.)	Sand (gm.)	*Valeriana* residue (Substrate)
1.	Control	525.00	175.00	00.00
2.	T_1	480.00	160.00	60.00
3.	T_2	435.00	145.00	120.00
4.	T_3	390.00	130.00	180.00

Surface disinfection and sowing of seeds:

Healthy seeds of *Sesbania aculeata*, *Sorghum bicolor* and *Zea mays* were surface sterilized with 10% solution of sodium hypochlorite for 1-2 minutes and washed several times with sterilized distilled water before sowing them. Twenty seeds were directly planted in the pots under polyhouse conditions.

Multiplication and Maintenance of fungi:

The pure culture of *G.mosseae* was used for pot culture inoculations of *Sesbania aculeata*, *Sorghum bicolor* and *Zea mays* as the host for their multiplication. Each treatment with different hosts and substrates was

replicated thrice. The plants were watered regularly and nourished by Hoagland solution (100ml/pot) after regular interval of 15 days during the experiment.

Mycorrhizal Root Colonization:

Root samples were procured from the different hosts after 75 days of growth. The percentage of adventitious and lateral root colonization was evaluated microscopically after following 'Rapid Cleaning and Staining Technique' (Philips and Hayman, 1970). The following equation was used to calculate the percentage of root infection (Giovannetti and Mosse, 1980).

AM root colonization(%)= (Number of colonized segments / Total number of segments examined) × 100

Spore Population:

Soil samples were collected after 75 days from all plant hosts for spore population determination. The AMF propagules were obtained from the soil by 'Wet Sieving and Decanting Method' (Gerdemann and Nicolson, 1963) and quantification was done by 'Grid Line Intersect Method' (Adholeya and Gaur, 1994) under stereo-binocular microscope.

Effectiveness of AM fungi:

Effectiveness of AM fungi by using different concentrations of substrates was determined on different hosts by taking plant height, shoot weight (fresh & dry) and root weight (fresh & dry) after 75 days of growth period.

Data analysis:

Data were statistically subjected to interpret by an analysis of variance (ANOVA) followed by post hoc test using SPSS 16.0 software. Means were then ranked at P=0.05 level of significance using Duncan's Multiple Range Test for comparison.

RESULTS AND DISCUSSION:

Seventy five days after inoculation, the results envisaged that the mass production of selected AM fungi i.e. *G.mosseae* showed a variablility in results with all the different hosts as well as with different substrates.

Regarding the mass production of *G.mosseae*, when *Sesbania* was used as host and *Valeriana* residue as a substrate, the highest spore count (25.6±2.5) was registered at 180gm. amount of *Valeriana* residue followed by 120gm. and 60gm. Similarly, the maximum mycorrhizal root colonization (57.920±2.24) was observed at 180gm. of substrate followed by 60gm. and 120gm. The various growth parameters i.e. plant height (98.06±1.24), shoot weight (13.116±4.36, 1.684±0.71: fresh & dry) and root weight (0.883±0.47, 0.168±0.10: fresh & dry) also showed their maximity at 180gm. of substrate concentration. When *Sorghum* was used as a trap plant with *Valeriana* residue as a substrate, the maximum number of AM spores (18.66±2.51) was recorded at 120gm. of substrate followed by 60 and 180gm. Similarly, the highest mycorrhizal root colonization (63.64±7.08) was observed at 120gm. amount of substrate followed by 180 and 60gm. The plant height (77.06±1.10), shoot biomass (0.566±0.33, 0.073±0.03: fresh & dry) and root biomass (0.091±0.01, 0.012±0.01: fresh & dry) were registered maximum at 180gm. concentration of substrate but followed the different trend. In similar way, when maize was used as host plant and *Valeriana* residue as substrate, all the growth parameters i.e. plant height (82.26±2.15), shoot weight (2.683±1.64, 0.363±0.16: fresh & dry) and root weight (1.092±1.02, 0.111±0.07: fresh & dry) were found maximum at 180gm. concentration of substrate. Maximum mycorrhizal root intensity (73.86±8.94) and sporulation (26.66±1.52) were recorded at 180 gm. followed by 120gm., 60gm. and 60gm., 120gm. respectively. On comparison of hosts with *Valeriana* residue as substrate, maize was proved to be most suitable host as it increased mycorrhizal root colonization and spore population to a maximum level with 180gm. concentration of substrate (Table 1, Fig, 1, Plate-2). Among the three host plants tested for mass multiplication of *G.mosseae*, *Zea mays* was found most appropriate host. *Valerianaa wallichi* residue was found more suitable for mycorrhizal root colonization.

The present study presents a method to multiply culture of *G.mosseae* using different substrates and hosts. A considerable difference has been reported in population of AM spores and percentage of colonization of infected roots grown in soil sand mixture supplemented with different hosts. The multiplication or production AM spores are not always correlated with percentage mycorrhizal root colonization. The existence of varied range of colonization might be attributed to the soil factor which affected the number of vesicles per root and spores in the rhizospheric soil. The application of different

substrates influenced the formation of vesicles and arbuscules in present work corroborated with the findings of Baby and Manibushanrao (1996). Soil texture also shows its impact on sporulation and mycorrhizal root colonization (Tacon *et al.,* 1979). In the present investigation, addition of substrates

enhanced the AM spore density as well as mycorrhizal root colonization with different host plants. Another biological consideration in the production of inoculum is the host plant upon which the fungus will grow. AM fungi respond to host exudates with extensive hyphal growth and branching (Giovanneti *et al.,* 1993). Hyphae elongates 20 times more slowly in the absence of host roots than host's presence (Be'card and Piche, 1989). In the present study the difference in spore population in the different plant species could be due to the characteristics of plant hosts which vary in their ability to adapt to the growth conditions like soil temperature, soil pH, soil moisture, soil fertility, soil microorganism interactions, light conditions and others (Mukerji *et al.,* 2002). For increasing the colonization percentage, the initial inoculums used is also very important as more inoculum resulted in more chance to produce high number of AM fungi. Kaushish (2008) while compared three different hosts, *Sesbania aculeata* was proved to be best host for AM sporulation with vermicompost as a substrate. Results of the growth performance of host after inoculation with AM fungi clearly indicated that AM inoculation increased the different growth parameters of all trap plants with different concentrations of substrates as compared to control plants. This may be due to the more uptakes of nutrients and water by AM fungi from the substrate. Increased plant growth and yield by consortium of AM fungi (*G.mosseae* and *A.laevis*) has been well documented by earlier workers (Kumar *et al.,* 2009b, Sharma *et al.,* 2007).

CONCLUSION: In developing a system for inoculum production of AM fungi, the work was carried out to make a potent and effective inoculums of *G.mosseae*. The present agriculture era needs the commercialization, rapid production and distribution of variety of AM fungal inoculums for the application in fields.

FUTURE SCOPE: The current findings showed that the AM fungi have a great future comparative to chemical applications for sustainable agriculture. The intervention of government is highly desirable to use these bioinoculants in different growing systems towards sustainable future in crop production.

CONFLICTS OF INTEREST: There is no conflict of interest.

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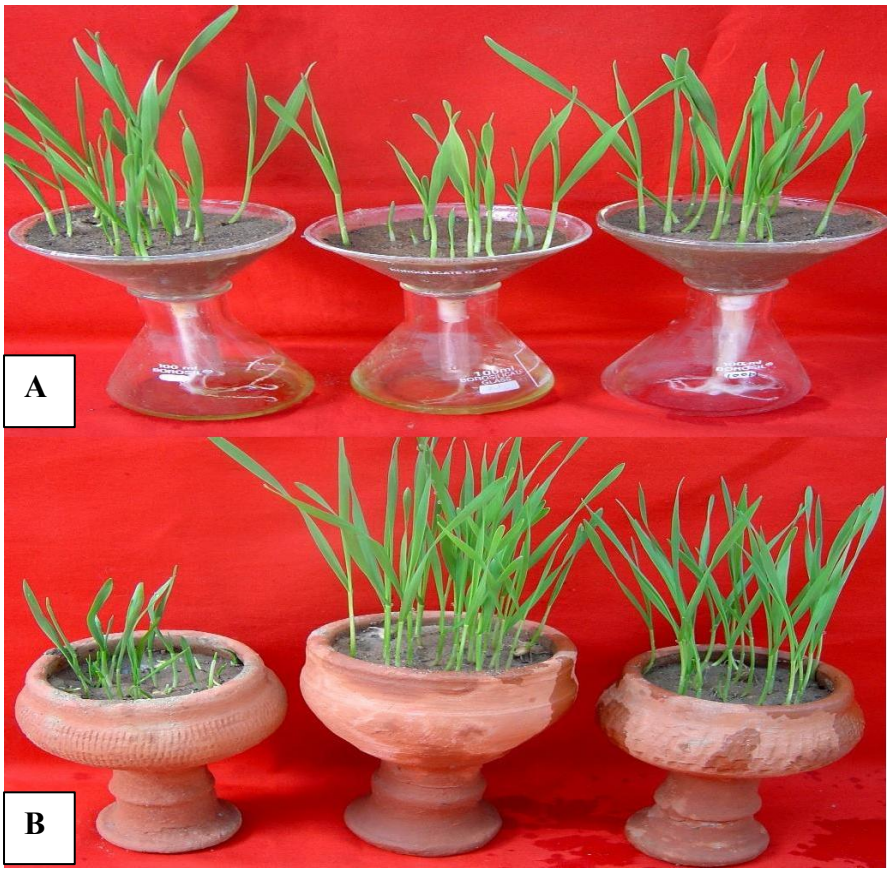
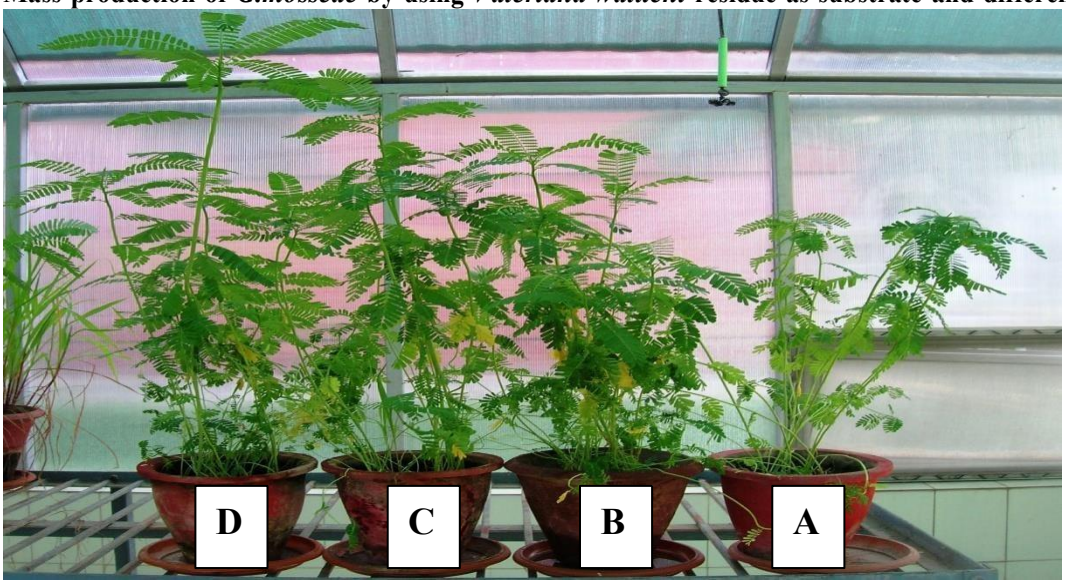
PLATE-1



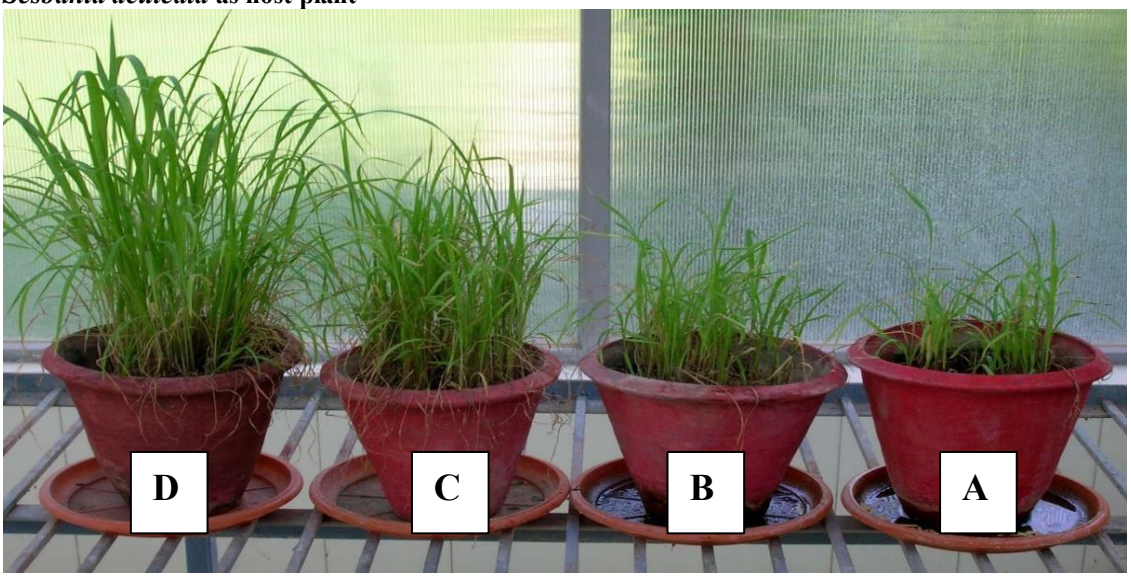
(A) Starter culture of *G.mosseae* by funnel technique
(B) Culture of *G.mosseae* in earthern pots
(C) Substrate Used: *Valeriana wallichi* residue

PLATE- 2

Mass production of *G.mosseae* by using *Valeriana wallichi* residue as substrate and different hosts



***Sesbania aculeata* as host plant**



***Sorghum bicolor* as host plant**

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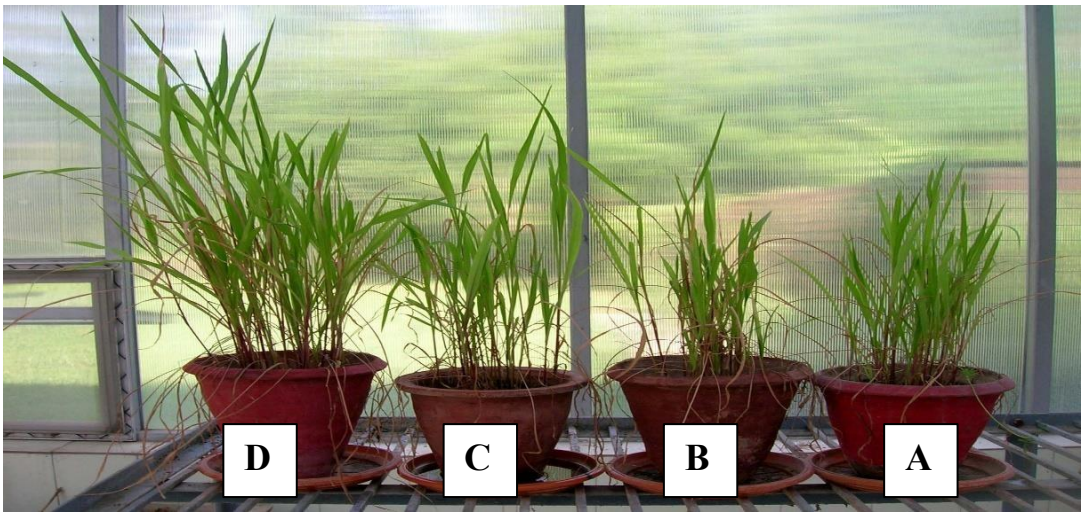

***Zea mays* as host plant**

A- Control, B-60 gm., C-120 gm., D-180 gm. *Valeriana wallichi* residue

Table-1: Inoculum production of *G.mosseae* using different hosts and *Valeriana wallichi* residue as substrate

Sr. No.	Substrate (gm.)	Soil:Sand	*Sesbania aculeata* PH	FSW	DSW	FRW	DRW	AM SC	%MRC
1	Control	525:175	*72.43±1.72^{a}	5.215±1.7^{b}	0.811±0.04^{a}	0.641±0.19^{a}	0.101±0.02^{a}	9.0±1.0^{c}	33.855±5.71^{c}
2	60	480:160	87.10±1.21^{b}	8.908±3.94^{ab}	1.139±0.54^{a}	0.681±0.09^{a}	0.104±0.02^{a}	13.0±1.0^{b}	46.886±7.92^{ab}
3	120	435:145	84.90±2.22^{b}	9.221±2.59^{ab}	1.285±0.11^{a}	0.703±0.25^{a}	0.121±0.05^{a}	14.3±1.5^{b}	39.286±7.85^{bc}
4	180	390:130	98.06±1.24^{a}	13.116±4.36^{a}	1.684±0.71^{a}	0.883±0.47^{a}	0.168±0.10^{a}	25.6±2.5^{a}	57.920±2.24^{a}
	Sorghum bicolor								
5	Control	525:175	34.5±1.17^{d}	0.282±0.13^{a}	0.047±0.02^{a}	0.058±0.03^{a}	0.009±0.01^{a}	8.0±2.0^{c}	18.07±1.89^{c}
6	60	480:160	44.2±2.16^{c}	0.494±0.24^{a}	0.061±0.03^{a}	0.088±0.04^{a}	0.011±0.003^{a}	14.0±1.0^{b}	30.45±2.53^{b}
7	120	435:145	48.83±1.87^{b}	0.307±0.14^{a}	0.051±0.02^{a}	0.047±0.02^{a}	0.010±0.01^{a}	18.66±2.51^{a}	63.64±7.08^{a}
8	180	390:130	77.06±1.10^{a}	0.566±0.33^{a}	0.073±0.03^{a}	0.091±0.05^{a}	0.012±0.01^{a}	11.33±1.52^{bc}	40.10±7.25^{b}
	Zea mays								
9	Control	525:175	54.46±1.35^{d}	0.977±0.58^{a}	0.138±0.05^{b}	0.137±0.06^{c}	0.028±0.01^{b}	11.33±1.52^{d}	32.95±3.60^{c}
10	60	480:160	60.26±0.80^{c}	1.885±0.45^{a}	0.319±0.12^{ab}	0.336±0.14^{b}	0.048±0.02^{ab}	18.33±1.52^{b}	38.59±1.95^{c}
11	120	435:145	69.63±2.85^{b}	1.950±0.94^{a}	0.283±0.04^{ab}	0.462±0.08^{b}	0.072±0.01^{ab}	15.33±1.52^{c}	55.27±5.02^{b}
12	180	390:130	82.26±2.15^{a}	2.683±1.64^{a}	0.363±0.16^{a}	1.092±1.02^{a}	0.111±0.07^{a}	26.66±1.52^{a}	73.86±8.94^{a}

***** Each value is mean of three replicates

Means values followed by different alphalbet/s or letter/s are significant over one another at P= 0.05.

PH =Plant Height (cm.)
FSW =Fresh Shoot Weight (gm.)
DSW =Dry Shoot Weight (gm.)
FRW =Fresh Root Weight (gm.)
DRW =Dry Root Weight (gm.)
AM SC =Arbuscular mycorrhizal Spore Count
% MRC =Percent Mycorrhizal Root Colonization

Fig. 1 Efficacy of *Valeriana wallichi* residue on inoculum production of *G.mosseae* with different host plants

C- Control, T1- 60 gm; T2 – 120 gm; T3- 180 gm. of substrate (*Valeriana wallichi* residue) used