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ISOLATION OF VAM FROM DEHRADUN AND A STUDY FOR DETERMINING BIOSYNERGISTIC EFFECTS OF VAM STRENGTHENING POSSIBLE FUTURE STRATEGY DESIGN FOR ERADICATING SOIL POLLUTION

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Abstract

Vesicular arbuscular mycorrhiza (VAM) possesses many properties for enhancing plant growth when used in synergy with other microbes and replenish soil. The collected samples contained species like *Glomus, Acaulospora, Gigaspora* were isolated and were found to colonize with Fenugreek (*Trigonella foenum-graecum*) in fertilizer free soil. The growth parameters of plants like root, shoot and leaf etc. were examined and were compared with control which represented plants growing in VAM free soil. It was deduced that the plants growing without VAM presence in soil depicted lower growth potentials. This strengthens the benefit and possibilities of VAM usage as biofertilizers or in tandem with some other organic fertilizers.

Key words: Vesicular-arbuscular mycorrhiza (VAM), microorganisms, fungi, fertilizers

Introduction

Improvement in the field of agricultural development by accelerating certain activities has been achieved by a large group of microorganisms especially in fungi and arbuscular mycorrhiza representimg one of the current research target for attaining sustainability. The association between soil borne fungi and roots of the higher plants is able to depict symbiosis and corresponds to the healthy interaction without harming the plant. The association between fungi and plant roots is not new but can be traced back to about 450 million years which was revealed by DNA sequences analysis and fossilized evidences (Redecker, 2013; Delaux, 2017). Arbuscular mycorrhiza fungi can be present in about 80-90% of the plant species but the fruitful association development between the roots and fungi is largely different but still capable of providing protection against pathogens and diseases (Kabdwal *et al.*, 2019). Apart from protection from disease these VAMs are capable of changing the fertility of soil by altering the physical and biological properties of soil. Mycorrhizal association also helps in enhancement of nutrient uptake, formation of growth assisting components, promoting drought and salt tolerance, regulating plant defense mechanism which has been deciphered by molecular and taxonomy related tools (Spatafora *et al.*, 2016; Ghosh *et al.*, 2020; Nurbaity *et al.*, 2019; Singh *et al.*, 2020). The utilization of modern molecular tools has elucidated that VAMs offer many other attributes which may also be looked upon in future (Lee *et al.*, 2013).

Thus, it can be debated that VAM fungi forms an important part of these agro systems and play an important role in directly benefiting crops by enhancing disease resistance, drought tolerance and adverse soil conditions, competitive ability over non-mycorrhizal plants, indirectly via improving structure of soil and increasing retention of

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soil nutrients. (Cavagnaro *et al.*, 2015; Köhl and van der Heijden, 2016; Thirkell *et al.*, 2017; Goomaral *et al.*, 2019). Despite these advantages, there are various factors limiting the mycorrhizas and their utilization in modern agricultural systems which include physical interference of hyphal networks by tillage, higher utilization of inorganic fertilizers and fungicides and selective breeding of modern crop varieties which might accidently decrease the ability of plants for the formation of a successful symbiotic association of AM fungi (Pérez-Jaramillo *et al.*, 2016; Bowles *et al.*, 2017). The objective of the present study was to isolate different VAM studies from adjoining regions of Dehradun, Uttarakhand, India and then their cytological identification for wider application on study related to plant growth parameters.

Materials and Methods

The soil samples were collected from different adjoining regions of Dehradun (Raipur and Mussoorie), Uttarakhand around an altitude ranging from 600-2000 asl. The physicochemical analysis of soil was performed to ensure differentiation in distribution of species. Apart from soil, some plant root samples were also collected from the target site.

Fungi spores present in soil (5g of soil) was mixed with 50 ml of distilled water and agitated for AMF spores separation from the soil. The soil was allowed to settle down and the supernatant was collected by pouring. The supernatant was further subjected to pass through standard sieves of different sizes and then studied using microscope. The spores present in the samples were picked with the help of needle and mounted in lactophenol on glass slide. This helped in specific genera and species identification of VAMs present in the sample.

The VAM spores from the slides were observed carefully and photographed. The morphological characters of VAM photographed VAM spores were compared with the standard monographs proposed by Malik *et al.*, 2016; Ajaz *et al.*, 2017; Bindu *et al.*, 2018 and with the help of INVAM.

Fenugreek (*Trigonella foenum-graecum*) seeds were grown in sterile soil. There were two sets of plants grown, one without VAM (control) and another with VAM for specific comparison purpose. After growing the plants, the data was gathered for three weeks. The data was gathered mainly for shoot length, root length and leaf area.

Results and Discussion

The soil samples were collected from four different locations (site 1, site 2, site 3 and site 4) in Dehradun, Uttarakhand. The VAM spores were determined by microscopc observation and its genus was determined as *Gigaspora, Glomus* and *Acaulospora*. The genus isolated from different location exhibited specific characteristics viz *Glomus* showed subtending hyphae, Acaulospora had ornaments while Gigaspora showed bulbous suspensor at the base of its hyphae. Depending upon the number of spores of VAM, sieves of pore sizes 44 BSS (355µ), 60 BSS (250µ), 120 BSS (120µ) and 150 BSS (105µ) were used for the study. It was found that in sieve size 40 BSS and 60 BSS, VAM remained absent but VAM passed through the larger pore size. Interestingly, 120 BSS sieve size represented a total of 516 spores followed by 150 BSS having 303 spores table 1.

The collected soil samples were tested at Soil Testing Laboratory, Haridwar. The results of the chemical properties of soil samples have been represented in table 2. These samples showed the presence of VAM fungal spores. VAM fungi were well distributed throughout Dehradun.

Species of *Acaulospora* was found to be dominant at site 1 with a total 95 spores. *Acaulospora* was found dominant at site 2 with 70 spores followed by *Glomus* (67 spores). The site 3 represented 123 spores of *Glomus* and site 4 revealed a total of 142 *Glomus* spores table 2 and 3. The most abundant VAM species recorded was

Table 1: Species of VAM spores isolated from Different sieve size.

Species	Parameter	Site 1		Site 2		Site 3		Site 4	
		120 BSS	150 BSS	120 BSS	150 BSS	120 BSS	150 BSS	120 BSS	150 BSS
Glomus	Average spores	27.00±2.517	31.00±1.528	35.00±2	32.00±2.517	79.00±2.517	44.00±2.517	82.00±2.517	60.00±1.00
	% of spores	28.72%	40.26%	36.25%	50%	53.02%	49.44%	47.95%	82.19%
Acaulo spora	Average spores	50.00±1	45.00±3.512	39.00±2	31.00±1.00	50.00±3.512	42.00±2.517	55.00±0.577	10.00±0.578
	% of spores	53.19%	58.44%	38.24%	48.44%	33.56%	47.19%	32.16%	13.70%
Gigas pora	Average spores	13.00±1.528	-	21.00±2.517	-	14.00±2.517	-	24.00±2.517	-
	% of spores	13.83%	-	20.59%	-	9.40%	-	14.04%	-
Other	Average spores	4.00±0.577	1.00±0.512	5.00±0.577	1.00±0.577	6.00±1.528	3.00±0.578	10.00±2.517	3.00±0.577
	% of spores	4.26%	1.30%	4.90%	1.56%	4.03%	3.37%	5.85%	4.11%

Glomus, followed by species of *Acaulospora* and *Sclerocystis*. The *Glomus* species observed were *Glomus fasciculatum, Glomus aggregatum, Glomus mosseae, Glomus etunicatum, Glomus intraradices, Glomus constructum, Glomus destericola, Glomus cerebriforme, Glomus clavidporum*. The observed species of *Acaulaspora* included *Acaulospora laevis, Acaulospora mellea, Acaulospora delicata* and other

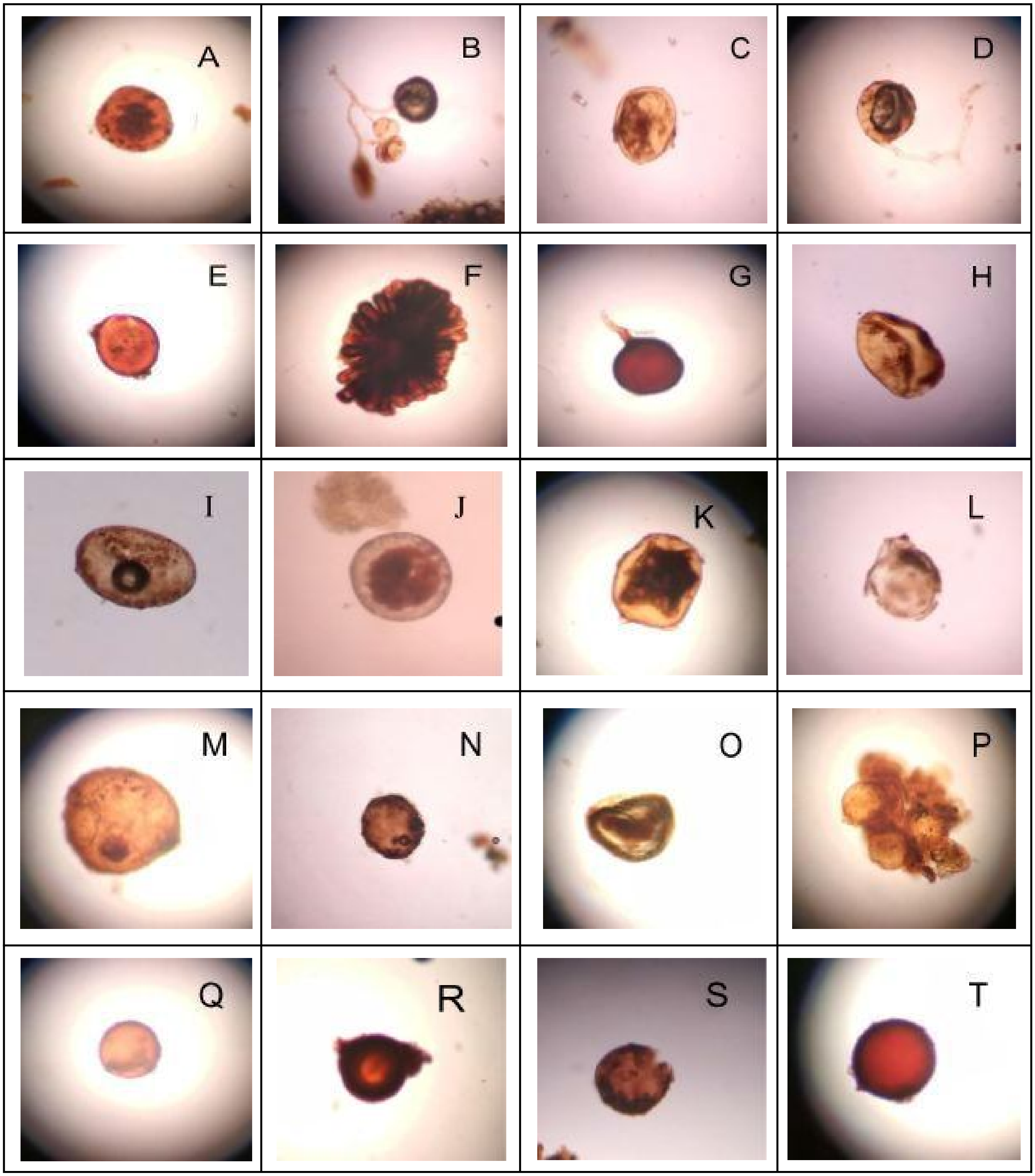

Fig. 1: Spores of various species of VAM ((A-E) *Glomus* species, (F) *Glomus clavisporum*, (G) *Glomus deserticola*, (H) *Glomus intraradices*, (I-J) *Acaulospora species*, (K) *Acaulospora mellea*, (L) *Acaulospora delicata*, (M-N) *Gigaspora species*, (O) Ruptured spore of *Gigaspora* species, (P) *Sclerocystis coremoides*, (Q-R) *Scutellospora species*, (S) *Scutellospora callospora*, (T) *Glomus* species).

unidentified species of *Acaulospora*. The *Gigaspora* species of VAM fungi was not completely identified due to low frequency of distribution at the collection sites Fig. 1.

Colonization of isolated species of *Glomus*, *Acaulospora*, *Gigaspora* and a combination of these species were studied in Fenugreek. The number of plants germinated was recorded on the third day of sowing and root/shoot length were measured after the completion of 10 days table 3, 4. *Glomus* species had a positive effect on the leaf number of Fenugreek plants. Control plants and plants having other combinations rather than *Glomus* sp produced only two leaves while the plants having *Glomus* sp had the presence of three leaves along with the largest leaf area. Positive effect in shoot length was expressed by *Gigaspora* sp. In case of root length, *Glomus sp* produced longest roots. Mix culture showed the presence of branched roots Fig. 2.

The present work aimed at testing soil samples from four different sites for exploring distribution of VAM. On the basis of obtained result it could be stated that on an

Table 2: Physiochemical analysis of soil samples.

Site no.	pH	EC	OC	P	K	S	Zn	B	Fe	Mn	Cu
Site 1	6.8	0.66	0.55	13.5	181.34	11.80	0.490	0.44	7.663	3.763	0.354
Site 2	7	0.62	0.47	13.5	201.56	13.56	0.698	0.57	5.458	2.547	0.174
Site 3	6.9	0.59	0.53	18.0	198.95	17.50	0.588	0.70	4.743	2.236	0.311
Site 4	7.1	0.74	0.50	13.5	172.30	9.74	0.467	0.94	6.598	3.654	0.266

Table 3: Number of plant seed germinated on the third day of sowing.

Sample	Control	Glomus	Acaulospora	Gigaspora	Mix culture
Image					
germinated	13	11	11	9	7

Table 4: Length of root and shoot and diameter of leaves after 10 days.

Inoculum	Shoot length (cm)	Root length (cm)	Leaf length (cm)	Leaf breadth (cm)
Control	3.4±0.058	2.1±0.153	1.15±0.050	0.59±0.015
Glomus	5.23±0.493	7.07±0.152	1.3±0.035	0.6±0.006
Acaulospora	3.8±0.115	4.3±0.306	1.03±0.980	0.49±0.015
Gigaspora	5.77±0.153	2.5±0.4	1.23±0.058	0.39±0.021
Mix	4.36±0.416	4.43±0.379	1.16±0.057	0.46±0.059

Fig. 2: Comparative analysis of different species of VAM on the growth of the plant.

average the VAM is present in a total number of 171 to 244 per 100 g of soil. The numbers of spores were minimum in Raipur area and maximum in Old Mussoorie road while Rajpur had moderate number of VAM species. VAM spores helped in root colonization display variation in rhizosphere under bulbous plants in different ecological conditions **(**Karaarslan *et al.,* 2015).

The current study reflected the similar finding that VAM may enhance plant growth and related ecology (Nanjundappa *et al.,* 2019 and Mitra *et al.,* 2020). The maximum numbers of spores were isolated from old Mussoorie road followed by Rajpur road and two sites from Raipur area. As range difference in altitude did not differ drastically, there was no sudden drop or increase of VAM spores. The previous work of Malik *et al.,* 2016 and Ajaz *et al.,* 2017 suggested differential distribution and mycorrhizal colonizations effect over community and environmental gradients owing to species composition which held true in our study.

Glomus showed increasing trend with increase in altitude whereas a negative interaction was observed between the species of *Acaulospora* and *Gigaspora.* The total number of spores was found maximum in the altitude range 900-1000 m, moderate in Rajpur having range 700-800 m and lowest in Raipur area having range 600-700 m which showed that higher altitude suits the distribution and increase in number of VAM. The maximum number of spores were obtained in the sieve size 120 BSS (514 spores) and minimum in 150 BSS (303 spores) while no VAM was isolated in sieve size 60 BSS and 40 BSS due to the bigger pore size of sieves. This showed that lower sieve size hindered for the passage of VAMs. After inoculation of VAM with Fenugreek seeds, it was observed that VAM plants were significantly superior to VAM-free plants in shoot length, root length as well as leaf area. Further, extensive field experiments are needed for establishing and extending our results for utilizing VAMs for human welfare.

Conclusion

VAM are known to improve the soil quality and are very important to the plant in terms of ecosystem, as it helps in phosphorus uptake and other nutrients. VAM if grown with others as bio-fertilizer offered potentials of removing soil pollution and cleaning environment. This kind of strategy can help in reliance of human on inorganic fertilizers and chemical to sustain population in crop fields. Overall, this study concluded that use of VAM was useful and beneficial for both living and non-living factors of the ecosystems but its correct use is still a matter of debate.

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