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Evaluating the Effect of AM (Arbuscular Mycorrhizal) Fungi in Morphogenesis and Longevity of Shoot and Root of Asparagus Racemosus

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Abstract – Asparagus racemosus is a Plant used in traditional Indian medicine (Ayurveda) and common throughout India and Himalayas. Asparagus racemosos, traditionally known as Shatavari, means "who possesses a hundred husband or acceptable to many. Because of its multiple uses, the demand for Asparagus racemosus is constantly on the rise. Arbuscular mycorrhizal (AM) fungi are ancient, widespread and form association with many plant species. The objective of this study was to evaluate the potential roles of AM fungi in the growth and P nutrition of Asparagus racemosus. The effects of AM colonization on test plant were investigated at different soil P supplies. This study was designed to provide a much better understanding of roles of AM fungi in Asparagus plant, particularly with respect to P nutrition growth.

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INTRODUCATION

Asparagus racemosus, traditionally known as Shatavari is found at low altitude throughout India. It is used as medicine like in Ayurveda, Unani & Siddha. In Ayurveda it is considered as a female tonic and beneficial in female infertility to increases libido and cures inflammation of sexual organs, enhances folliculogenesis and ovulation, prepares the womb for conception, prevents miscarriages, acts as post-partum tonic by increasing lactation and normalizing the uterus and the changing hormones. It also widely used in diseases including dysentery, in diabetic retinopathy, inflammations, tumor, bronchitis, nervous disorder, hyperacidity, certain infectious diseases, neuropathy, conjunctivitis, spasm, chronic fevers, and rheumatism.

Mycorrhizal fungi are species of fungi that associate with plant roots forming a symbiotic relationship with the plant providing sugars for the fungi and the fungi providing nutrients such as phosphorus to the plants. Arbuscular mycorrhizal (AM) fungi are major component of rhizosphere and play an important role in recycling the nutrients. Mycorrhizal fungi can absorb, accumulate and transport large quantities of phosphate within their hyphae and release to plant cells in root tissue. Endomycorrhiza (vesiclular arbuscular mycorrhiza; VA mycorrhiza; now known as arbuscular mycorrhiza, AM) play a very important role on enhancing the plant growth and yield due to an increased supply of phosphorus to the host plant. Plants inoculated with endomycorrhiza have been shown to be more resistant to some root diseases.

MATERIAL AND METHOD:

First of all the seed of *Asparagus, racemosus* are obtained from a reliable source. All seeds of test plant *Asparagus, racemosus* is surface sterilized after that germinated the seed between moistened paper towels on a plate enclosed in a loosely sealed plastic bags.

Pot and potting mixture:

Pots were filled with 6 kg sterlized soil and 2 kg sterlized sand / substrate after mixing thoroughly. Twenty percent of inoculums 1.6 kg was added to the mixture in the upper part. Before showing seed confirm the inoculums are well placed i.e. planting hole in every pot and the sowing the seed in every pot.

Water daily supply

Until water begins to flow out of the bottom of the pot. Apply a proper phosphorus and organic fertilizer. The amount of fertilizer applied depends on plant and size of the pot. Determine the proper amount of these through careful observation of plant growth. After six

weeks, studied the presence of AM fungi and after sixteen week studied the effect of AM fungi which was discussed in observation.

Phosphorus:

The major role of AM fungi is supply infected plant roots with phosphorus, because phosphorus is an extremely immobile element in soils. Even if phosphorus was added to soil in solution form soon, it becomes immobilized as organic Phosphorus, Calcium Phosphates or other fixed forms (Jackson and Moson, 1984; Wetterauter and Killorn, 1996) AM fungi are known to be effective in increasing nutrient uptake, particularly phosphorus and biomass accumulation of many crops in low phosphate soil (Osonubi *et. al.* 1991).

Fifty days old seedlings with uniform height were used in the experiment. The prepared Mycorrhizal Inoculums containing AM colonized roots, rhizospheric soil having extra material mycelium and spores were used as a source of inoculums for each seedling. The inoculums were directly attached to the roots of the seedlings. The experiment consists of eight treatments of each plant species and nine replicates of each treatment were grown, a total of 40 pots are arranged.

The treatments consisted of non AM control i.e. Zero Phosphate level and three different levels of 50 mg, 100 mg and 150 mg respectively and AM inoculated Plant with Zero Phosphate level and three different Phosphate levels respectively. Each plant species seedlings maintained in each pot containing 8 kg. of autoclaved soil and seedlings were watered daily according to their needs. K_2HPO_4, dipotassium phosphate was used as a source of phosphate. Phosphate treatment was given after 30 days (or after six weeks) of AM inoculation and after that Phosphate was given weekly until the last observation was recorded. Observation was recorded after 60, 90 and 120 days AM inoculation.

Growth Measurement which shows Morphogenesis

Plants were harvested after 60, 90 and 120 days of AM inoculation and were than analysed for morphological parametes such as shoot length and root length and leaf number.

Pot cultured experiment were conducted using control measures discussed in observation. The plants of the lime of flowering stage (150 days) were harvested along with their root system and roots were cleared by dipping them in R.O. water 2 to 3 times till the adhering soil particles were removed.

Assessment of AM association and its biomass estimation

Few root samples of *Asparagus recemosus* plant both treated and untreated were cut in to 1 cm. bits and fixed in a standard FAA (formalin aceto alcohol) and processed further for the assessment of AM colonization (Percentage mycorrhizal association) by staining Philip and Heyman.

The plants were oven dried for 72 b at 70°C root and shoot systems were than weighted.

a) For calculating the percentage of vesicle colonization use this formula –

$$\frac{Number\ of\ root\ pieces\ contain\ vesicles}{Total\ number\ of\ root\ pieces}\ x\ 100$$

b) For calculating the percentage of arbuscule colonization use this formula –

$$\frac{Number\ of\ root\ pieces\ contain\ arbuscules}{Total\ number\ of\ root\ pieces}\ x\ 100$$

Estimation of Protein content

Protein content of leaf samples was determined by the method developed by Bradford.

Bradford reagent

One hundred mg Coomassio Brilliant blue G 250 was dissolved in 50 ml of 95% ethanol and mixed with 100 ml of concentrated phosphoric acid. The volume was made up to 200 ml with distilled water prior to use.

One-gram fresh leaf sample was extracted with 2 ml of 0.1 M Phosphate buffer (pH 7,0). The extract was centrifuged at 5000 rpm for 10 min at room temperature.

0.1 of clear supernatant, 5.0 ml of Bradford's reagent was added. The blue color developed was red at- 595 m in Spectrophotometer. From the standard graph prepared using Bovine serum albumin over a concentration ranging from 1 to 100 My / ml the protein concentration of loof sample was estimated and expressed as My/g of fresh weight of sample.

Observation

In the present investigation AM fungi was found to have a significant effect on the growth and development Asparagus recemosus. The difference between the micorrhizal and non-micorrhizal in test plant was visible after 30 days of AM inoculation before phosphate was being applied. AM inoculated plants flowered two week & before the control. As shown in the plate significant increase was observed in various morphological parameter, like shoot length and root length in the test plant inoculated with AM fungi and different dose of phosphate 60, 90 and 120 days as compared to non-mycorrhizol plants at all level of phosphate.

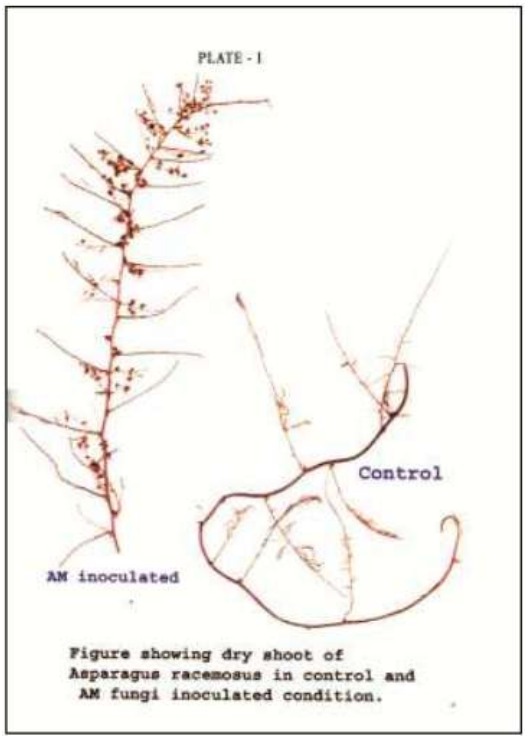
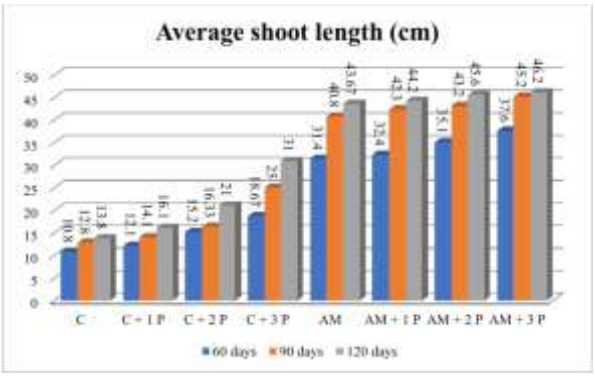


Figure showing dry shoot of Asparagus racemosus in control and AM fungi inoculated condition.

Table 01– Morphological Paremeters of Asparagus racemosus L. after 60, 90 and 120 days of AM inoculation under three phosphate condition

Treatments	Average shoot length (cm)			Average root length (cm)		
	60 days	90 days	120 days	60 days	90 days	120 days
C	10.8	12.8	13.8	9.6	11.2	13.00
C + 1 P	12.1	14.1	16.1	12.1	20.3	24.3
C + 2 P	15.2	16.33	21.00	14.2	22.2	27.2
C + 3 P	18.67	25.00	31.00	16.3	30.1	38.2
AM	31.4	40.8	43.67	13.7	24.2	29.3
AM + 1 P	32.4	42.3	44.2	14.8	27.2	31.3
AM + 2 P	35.1	43.2	45.6	16.3	28.1	32.1
AM + 3 P	37.6	45.2	46.2	19.9	29.2	33.1



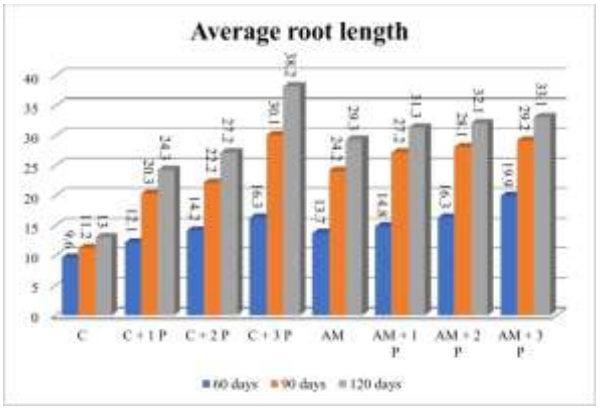


Figure showing comparative study of Phosphorus and AM fungi effect in *Asparagus recomposes* plant in control and AM inoculated condition after 60.90 and 120 days.

Table 02 – Average diameter of Asparagus racemosus storage root inoculated with AM fungi after 60, 90 and 120 days under three phosphate condition

Average Diameter of storage root			
Treatments	60 days	90 days	120 days
C	1.97	1.99	2.1
C + 1 P	1.99	2.1	2.4
C + 2 P	2.1	2.3	2.6
C + 3 P	2.4	2.6	2.8
AM	2.4	2.6	2.8
AM + 1 P	2.5	2.7	2.9
AM + 2 P	2.7	2.9	3.1
AM + 3 P	3.1	3.3	3.4

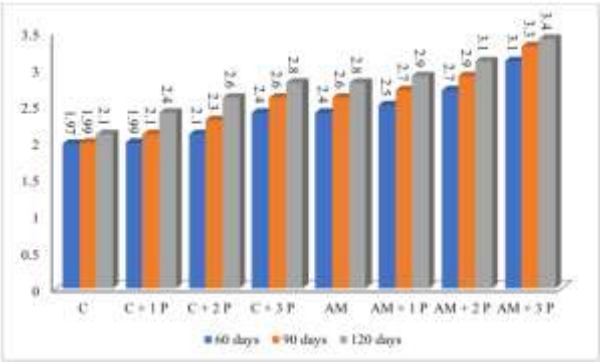


Figure showing effect of phosphorus and AM fungi in storage root diameter of *Asparagus recemosus* plant after 60.90 and 120 days

COMPARATIVE STUDY OF THE INOCULATED AND CONTROLLED PLANTS IN TERMS OF BIOMASS AND CHEMICAL CONSTITUENT

Inoculation of AM fungi during an early stage of acclimatization process has become, an alternative strategy for better establishment by improving the plant growth. The occurrence of micro-organisms

especially AM fungi on the medicinal plants have been reported earlier.

The AM fungi association had not only enhance the growth of medicinal plants but also improve the productivity of medicinal compounds.

Hence in this parameter of the experiment deals the plants improving the quality and quantity of changes produced from native medicinal plants in relatively shorter period at lower expose by using AM fungi.

Observation

Table 03 – Intensity of vesicle and arbuscule in Asparagus racemosus effect of AM fungi inoculums.

Vesicle – arbuscule status

Plants	Control Plant		AM fungi inoculated plants	
		Intensity % of formation		Intensity % of formation
	% of AM fungi association	arbuscules	% of AM fungi association	arbuscules
Asparagus racemosus	53.15	58±1.5	58.15	42±2.0

Table 04 – Effect of AM fungi inoculation on the shoot dry wt. root dry wt. and protein content in Asparagus racemosus

Plants	Control Plants			AM fungi inoculated plants		
	Shoot dry Wt. (g/plant)	Root dry Wt. (g/plant)	PC Mg/g of Plant extract	Shoot dry Wt. (g/plant)	Root dry Wt. (g/plant)	PC Mg/g of Plant extract
Asparagus racemosus	14	9	67.2	22.10	16.20	84.00

It is clearly evident from the data that the root system of the Asparagus racemosus plant after AM fungi inoculation showing percentage AM fungi association as well as intensity percentage of vesicle and arbuscule formation whereas in control plants there was evident of AM fungi association. In the inoculated plants the percent AM association is arbuscules 30 to 40% which was highly significant for plant growth and yield product. The plant shoot dry weight, root dry weight and the protein content of the AM fungi inoculated plant was higher in comparison to control plant table 02 to 04.

It is clearly evident from the present study on the effect of AM fungi inoculation in Asparagus racemosus plant that these plants have readily response to AM fungi inoculation in comparison to control plants.

RESULT AND DISCUSSION:

Asparagus (shatavari) is actually considered to be the most helpful herb for women as it helps in the balancing the female hormonal system. It nourishes the womb, ovum and almost prepare the female organs for pregnancy and prevents threatened miscarriage Ayurveda called shatavari as the queen of herbs and is the primary herb for female health.

For the study of role of AM fungi in nutrient uptake the pot culture method is applied. In recent years due to over exploitation of natural resources bio fertilizer have emerged as important component of integrated nutrient supply system and hold a premise for reducing the production costs, improving the crop yields, quality nutrient supplies and sustaining the productivity over a longer period (gill et. al., 2002). Beneficial effect of AM symbiosis on plant growth, nutrient uptake and tolerance to environmental stress has been extensively reported. Koide and Mosse 2004, Audet and Charest, 2009.

This work clearly indicates the beneficial effect of AM fungi on the growth and biomass of economically important parts (shoot, root), which are often the harvest products in medicinal plants specifically in the experimental plant i.e. A. racemosus. The study shows that the application of AM fungi controls the growth and development, inorganic fertilizer specially phosphorus above is suitable for experimental plant cultivation.

The result of the present study coincides with the reported findings of Jakobeen et. al. (1992) who have reported that the fungal hyphal growing beyond the rhizopheric soil increase the absorbtic surface area of the root, which result in or great efficiency of nutrient absorption, especially slowly diffusing mineral ions like phosphorus was observed in uninoculated control plants. The increased growth of treated plants and result shown in observation are inconformity with these of Gupta and Janarthenan and Earanna et. al. These results confirm the earlier reports by Bagyaraj and Manjunath (1980) and Rao et. al. (1989).

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