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Research Article

INFLUENCE OF ARBUSCULAR MYCORRHIZAL FUNGUS ON GROWTH AND NUTRIENT STATUS IN CHICKPEA PLANT

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ABSTRACT

The greatest challenge of today's agriculture is to feed the growing population and restore the natural resources. World over demand of crops in market is more than production. Indian crop production needs to be doubled and just to maintain the present precipitate consumption. Excessive use of chemical fertilizers causes environmental pollution both at the manufacturing and application sites. It is therefore most necessary to reduce the dependence on chemical inputs in agriculture. This is possible only through eco friendly approaches of farming system. Besides other biotechnological interventions, the arbuscular mycorrhizal fungi could be used as bio inoculants for promotion of growth, development, quality and yield of vegetables that too under an integrated Plant growth substances management system. Mycorrhizal fungi are used in conventional agriculture to improve crop production and productivity. A pot culture was performed at botanical garden of School of Studies in Botany, Jiwaji University Gwalior to examine the effect of *Acaulospora kentinensis* on growth and certain biochemical parameters in chickpea. Plants were raised in triplicates through the pot culture, containing sterile soil. Plastic pots of 18" × 12"size were used for this purpose. Pots were placed at a sunny place after the seed sowing. And after the seed germination, plants were irrigated as when required. After germination the inoculated plants along with their controls was sampled at 30, 60 and 90 days. During sampling it was found that due to AMF symbiosis all growth and biochemical parameters were increased.

Keywords: *Acaulospora kentinensis*, Growth, Arbuscular Mycorrhizal fungi, Agriculture.

1. INTRODUCTION

Availability of nutrients such as P, Zn, Fe, Manganese etc. in the agricultural land is necessary for plants to grow. But these nutrients are not always available everywhere so different chemical based fertilizers are used which are harmful to soil health and could be harmful for human health. To overcome this problem use of environment friendly biofertilizers are being used now a day. Among several biofertilizers mycorrhizal fungi plays a vital role in sustainable agriculture. Application of mycorrhizal fungi to agricultural fields helps in obtaining equivalent or sometimes higher crop yield [1]. The plants which shows symbiotic association with mycorrhizal fungi possess higher amount of amino acids and proteins [2] and higher concentration of esterase and pectin activities [3]. AMF are a group of beneficial soil symbionts which establishes mutualistic association with the roots of about 80% of plant species and the large majority of food crops including cereals, Legumes vegetables and fruits [4]. Arbuscular mycorrhizal fungi colonize root system of plant and are directly involved in plant nutrition increment [5]. AMF can also help plants in stimulation of growth regulating substances, increase in the photosynthesis under different types of stresses [6]. A large number of agricultural plants establish symbiotic association with mycorrhizal fungi to improve the production [7]. Biotechnologically arbuscular mycorrhizal fungi (AMF) are considered as a potential tool for improving phytostabilization efficiency and plant tolerance to heavy metal-contaminated soils and phytostabilization [8]. AMF also helps to increase biomass of the host plant [9]. It was observed that AMF enhances the production of some secondary metabolites like alkaloids and terpenoids [10]. Arbuscular mycorrhiza helps in increasing the dry weight of above ground parts of its host plant [11]. Chickpea (*Cicer arietinum*) is an annual crop which ranks third in the world for legume production [12]. It is a rich source of dietary proteins, calories and minerals. In India, it is mostly consumed as cooked whole seeds and immature seeds. The nutritional quality of cowpea is poor due to the presence

of several anti nutritional factors such as trypsin inhibitors, flatulence causing oligosaccharides and polyphenols; low protein digestibility and deficiency of sulfur amino acids [13]. In the present work we investigated the effect of Arbuscular mycorrhizal fungus *Acaulospora kinentensis* on different growth, developmental and biochemical parameters of *Cicer arietinum* plant in comparison to control plants in pot culture

2. MATERIAL AND METHODS

At botanical garden of School of Studies in Botany, Jiwaji University Gwalior, the symbiotic relationship between AM fungus *Acaulospora kinentensis* and chickpea plant were studied under pot culture. Plastic pots of 18" × 12"size were used for this purpose. A total number of 40 pots were placed. 20 pots were inoculated with *Acaulospora kinentensis and* 20 were kept as control. A total number of 6 seeds were sown in each pot and after germination seed thinning were done manually and in each pot only 3 plants were kept. 10 to 15 mycorrhizal spores were given to a group of two seeds which were later thinned to 1 manually. The chick pea seeds (JG-620) were collected from agriculture college Gwalior and AMF spore was collected from The Energy and Resource Institute New Delhi under material transfer agreement. For procuring AMF free tissues Seeds were firstly washed with tape water and then surface sterilized with 4% sodium hypochlorite solution before sowing. The plastic pots were also washed with tape water and then were sterilized with 100% ethanol. The pots were placed at sunny surface in an open field. The plants were watched keenly and were watered after every second day. After germination the plants was analyzed for certain growth and biochemical parameters at 30, 60 and 90 days respectively.

2.1. Root clearing and staining technique

Roots were thoroughly washed with the tap water, cleared in 10% KOH by autoclaving for 20-25 minutes at 121°C and 15lbs pressure. Cleared roots were rinsed with water 4- 5 times then kept in HCl for 5 min. The HCl solution was decanted and left overnight in 0.05% tryphan blue stain as per the method of Philip and Hayman [14].

2.2. Estimation of root colonization

Above treated roots were cut in 1 cm long segments, mounted on microscopic slides in Lacto glycerol solution. 10 such root segments were arranged properly on a glass slide and then covered with another glass slide. Slides were observed under compound microscope for mycorrhizal structures *viz.* hyphae, vesicles and arbuscules as per the method of Bierman and Linderman [15].

2.3. Estimation of growth and development parameters

Plant height, Plant width, Number of branches, Total number of leaves, Root length, Number of pods, were measured with the scale. Fresh and dry weights of root and shoot components were measured with the help of laboratory balance.

2.4. Estimation of Chlorophyll Content

For chlorophyll a, chlorophyll b and total chlorophyll the method of Arnon [16] and Withman *et al* [17] were employed. The fully expanded fresh plant leaves from all the pots trials were collected in the polythene bags and transported to the laboratory. Weighted (0.5gm) fresh leaf material was homogenized and extracted thrice in chilled 80% acetone. The volume of the acetone extract was made up (10 ml) to a known one and the optical density was read at 645nm and 663nm wavelengths on a spectrophotometer.

2.5. Calculation

The concentration of the chlorophyll pigments was calculated using the following formula:

Chlorophyll a = [(12.7 X OD at 663) - (2.69XOD at 645)] X dilution factor

Chlorophyll b = [(22.9 X OD at 645) - (4.68XOD at 663)] X dilution factor

Total chlorophyll = [(20.2 X OD at 645) + (8.02 X OD at 663)] X dilution factor.

2.6. Estimation of biochemical parameters

The following methods were employed for certain biochemical analysis of the stem modified tissue.

- Protein estimation by Lowry's *et al.*, method [18].
- Estimation of non reducing sugar by Nelson-Somogyi [19].
- Estimation of reducing sugars by Nelson-Somogyi [19].
- For ascertaining total carbohydrates by Anthrone method of Hedge and Hofreiter [20] will be used.

Estimation of total phenol by the method of Mallick and Singh [21].

2.7. Statistical Analysis

The statistical analysis was done with three replicates of each treatment and experimental results were presented as the arithmetic mean ± SE (Standard Error) ($P \leq 0.05$) (Statistics software version 10.0) each experimental value was com-pared to its corresponding control.

3. RESULTS

3.1. Soil

The physical properties of soil used for pot culture of the respective plant shows dark grayish brown color and the texture of soil was sandy. Soil taken initially and finally shows some variation in some characters. Soil taken is generally neutral inclined towards the alkaline having a pH of about 7.98±0.32, the electric conductivity shown by the soil is 0.23±0.02 dsm-1. The organic carbon and moisture content are 0.42±0.02 and 6.45±0.24 percent respectively. Water holding capacity and soil density according to texture are 1.23±0.10 percent and 0.98±0.04 mg. m-3 respectively (Table 1). Among some macro and micro elements of soil, concentration of Nitrogen is 168.00±12.06 mg/Kg, Phosphorous and Potassium is 18.56±1.32 and 291.24±14.32 kg. ha^{-1} respectively; and those of the other micronutrients and their concentrations in the soil are iron (Fe), zinc (Zn), copper (Cu) & manganese (Mn) at 3.26±0.20, 7.14±0.14, 21.04±0.14 & 4.74±0.16 ppm respectively (Table 2).

3.2. Mycorrhizal plant root colonization

The percentage of root mycorrhizal colonization was found to be going on increasing towards the growth and development of plant which was 21.46 after 30 days, 42.32% at 60 days and 66.22% at 90 days of interval (Fig.1)

Table 1: Some physical properties of soil which were used for the experiment

Colour	Texture	pH	Electric conductivity (dsm-1)	Organic carbon (percent)	Moisture content (percent)	Water holding capacity (percent)	Density (mg. m-3)
Dark grayish brown	sandy	7.98±0.32	0.23±0.02	0.42±0.02	6.45±0.24	1.23±0.10	0.98±0.04

Table 2: Macro and micronutrient analysis of soil which was used for practical.

Nitrogen (kg. ha-1)	Phosphorous (kgs. ha-1)	Potassium (kg. ha-1)	Iron (ppm)	Zinc (ppm)	Manganese (ppm)	Copper (ppm)
168.00±12.06	18.56±1.32	291.24±14.32	3.26±0.20	7.14±0.14	21.04±0.14	4.74±0.16

Average ± standard error

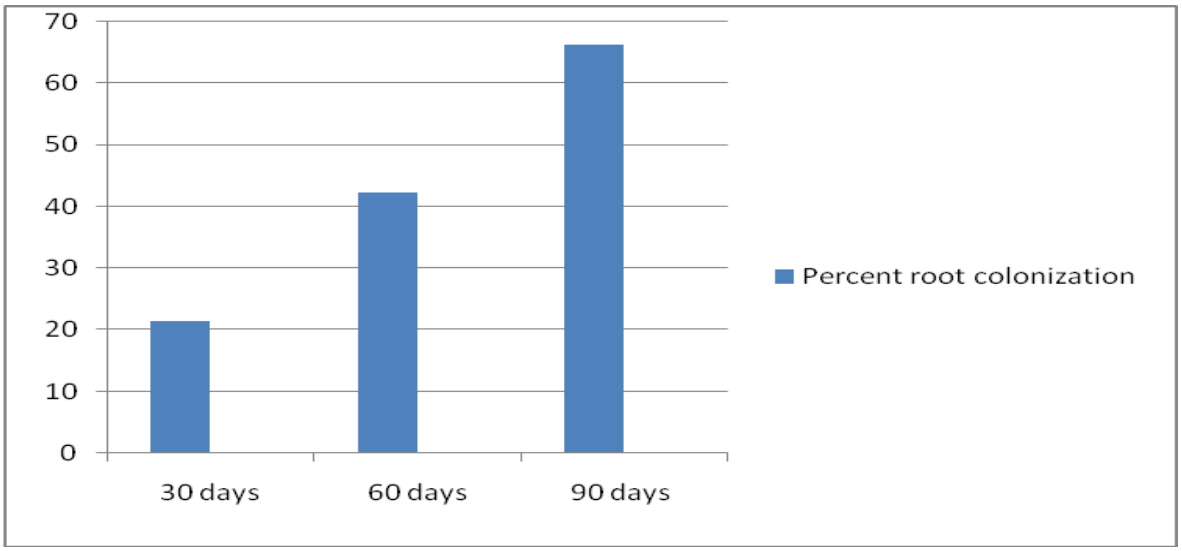

Fig. 1: percent root colonization

3.3. Growth and Biomass

The growth and plant biomass of *Cicer arietinum* were assessed at 30, 60 and 90 days of germination for both inoculated and non inoculated plants. Mycorrhizal treatment showed significant effect on the growth parameters like plant height, plant width, root length, total number of leaves, total number of flowers and total number of pods. Analysis was 61.25±1.76 (average ± standard error) and 52.13±0.54 (average ± standard error) in inoculated and non inoculated plants respectively. Similarly the root length was 19.16±0.59 and 14.8±0.49 in inoculated and non inoculated plants respectively. AMF inoculation shows much increase in number of leaves also, after 90 days of germination in AMF inoculated plants the total number of leaves were 67.15±0.36 and in control plants it was 43±2.22. The flowering starts at 59 days after germination in both inoculated and control plants and the first pod in inoculated plants were seen at 47 days after germination and in non inoculated plants it was seen several days later (Table 4 and 5).

3.4. Chlorophyll content

Under normal conditions (control plants) at 30 days after germination the chl a, chl-b and total chl of Cicer arietinum were 0.059±0.002, 0.120±0.026 and 0.188 ±0.005 respectively. However there was a significant increase in mycorrhizal treated plants. The chl-a, chl-b and total chl content at 30 days after germination were 0.068±0.001, 0.120±0.026 and 0.188±0.005, respectively. At every stage of growth *i.e.* at 30, 60 and 90 days after germination the chlorophyll content was slightly increased in mycorrhizal inoculated plants.(Table 6)

Table 4: Various growth and developmental parameters of plants with and without AMF

Days	Plant height (cm)		Plant width(cm)		Root length original to last (cm)		Total number of leaves per plant		Total number of flowers per plant		Total number of pods per plant	
	+AMF	-AMF	+AMF	-AMF	+AMF	-AMF	+AMF	-AMF	+AMF	-AMF	+AMF	-AMF
30	28.4. ± 0.01	24.9 ± 0.01	1.2 ± 0.03	1± 0.04	14± 0.03	11.17± 0.03	21 ±0.02	14±0.01	-	-	-	-
60	48.36 ±1.42	42.96 ±1.20	1.8 ±0.04	1.19 ±0.14	16 ±0.04	13.9 ±1.5	62.56 ±2.22	39.19 ±3.55	17.9 ±0.47	11 ±1.69	-	-
90	61.25 ±1.76	52.13 ±0.54	2.9 ±0.07	2.1 ±0.07	19.16 ±0.59	14.8 ±0.49	67.15 ±0.36	43±2.22	-	-	7.3±0.002	2.3 ±0.001

Table 5: Fresh and dry matter content with and without AMF in the plant (Weight gm. Plant-1)

Days	Root Fresh		Shoot Fresh		Total fresh		Root dry		Shoot dry		Total dry	
	+AMF	-AMF	+AMF	-AMF	+AMF	-AMF	+AMF	-AMF	+AMF	-AMF	+AMF	-AMF
30	0.38 ±0.21	0.12 ±0.20	2.3 ±0.03	1.19 ±0.01	2.68 ±0.13	1.31 ±0.12	0.09 ±0.02	0.07 ±0.01	0.45 ±0.01	0.32 ±0.03	0.54 ±0.58	0.39 ±0.02
60	0.47 ±0.01	0.15 ±0.02	2.87 ±0.01	1.48 ±0.02	3.35 ±0.02	1.63 ±0.04	0.11 ±0.22	0.08 ±0.14	0.56 ±0.01	0.4 ±0.01	0.67 ±0.02	0.48 ±0.04
90	0.55 ±0.03	0.17 ±0.01	3.36 ±0.03	1.74 ±0.01	3.91 ±0.03	1.91 ±0.01	0.13 ±0.13	0.1 ±0.02	0.65 ±0.02	0.47 ±0.12	0.78 ±0.03	0.57 ±0.02

Table 6: Chlorophyll fractions and their content in the Cicer arietinum with and without the presence of AMF (mg. gm.-1 leaves)

Days	Chl-a		Chl-b		Total chl	
	+AMF	-AMF	+AMF	-AMF	+AMF	-AMF
30	0.068±0.001	0.059±0.002	0.120±0.026	0.013±0.00	0.188±0.005	0.072±0.00
60	0.129±0.02	0.081±0.04	0.138±0.00	0.162±0.02	0.267±0.003	0.243±0.02
90	0.220±0.01	0.18±0.01	0.249±0.01	0.199±0.02	0.469±0.02	0.379±0.001

3.5. Nutrient Status/ Biochemical parameters

All the biochemical parameters like reducing sugars, non-reducing sugars, total sugars, total protein, and total phenol was gradually increased in those plants which were inoculated with AMF spores at every stage of growth and development. The protein content in AMF treated plants at 90 days after germination was 62.33±0.47 and in non inoculated plants it was 44.0±0.72. Our results find that at arbuscular mycorrhizal fungi helps the plant in increasing all the biochemical parameters (Table 7).

Table 7: Some metabolite fractions as affected by the presence of AMF during growth of Cicer arietinum after inoculation along with control plants (mg.gm-1 fresh weight)

Days	Reducing sugar		Non reducing sugar		Total sugar		Total protein		Total phenol	
	+AMF	-AMF	+AMF	-AMF	+AMF	-AMF	+AMF	-AMF	+AMF	-AMF
30	11 ±0.11	7.21 ±0.13	43.24 ±0.75	22.66 ±0.93	54.66 ±0.94	29.87 ±0.27	55.41 ±0.47	34.9 ±0.47	36 ±0.47	23.83 ±0.94
60	68 ±0.48	61.93 ±0.41	44 ±0.27	25 ±0.72	112.66 ±0.72	86.93 ±0.47	59.93 ±0.27	41.4 ±0.27	41 ±0.98	29.8 ±0.47
90	126±0.93	114 ±0.27	108 ±0.11	92 ±0.53	234.66 ±0.72	206.5 ±0.72	62.33 ±0.47	44.0 ±0.72	47.75 ±0.54	37.00 ±0.27

4. DISCUSSION

The research field of arbuscular mycorrhiza has been developing interest from past several years. The symbiotic relation between plants and mycorrhiza has seen increased interest for direct recycling of nutrients from organic matter to plants by mycorrhizal fungi [22]. The inoculations of arbuscular mycorrhizal (AM) fungi with plants are effective in enhancing plant development and soil properties [23]. The present investigation was undertaken to assess the effect of arbuscular mycorrhizal fungi (*Acaulospora kentinensis*) on the growth and nutrient status of *Cicer arietinum*. Recently it was observed that inoculation of AM fungi significantly increased growth parameters of wheat such as plant height as compared to non inoculated wheat plants. Similar observation was noted in mycorrhiza treated olive plants [24]. Recently it was observed that addition of AM fungi significantly increased growth parameters of wheat such as plant height as compared to non-mycorrhizal wheat plants [25]. Our result shows that AMF being in the symbiotic relationship with crop plant enhances growth and biochemical parameters of the plants. In our experiment inoculation of AMF spores increased growth and developmental characters, chlorophyll content, carbohydrate, protein and several other biochemical parameters in chick pea. Based on our results we suggest that AMF must be explored at all levels to further investigate their role in nature as a bio-fertilizer for sustainable agricultural production.

5. CONCLUSION

Mycorrhiza is a beneficial type of fungi that grows in association with most plant roots. Mycorrhizae increase the root's ability to absorb nutrients and water from the soil by increasing the surface absorbing area of roots from 100 to 1,000 times. Mycorrhizae also release powerful enzymes that help dissolve nutrients such as organic nitrogen, phosphorous, and iron. Mycorrhiza plays a vital role in plant growth, disease protection and overall soil quality. There are seven types of mycorrhiza viz: arbuscular, ecto, ectendo, Arbutoid, monotropoid, ericoid and orchidaceous mycorrhizae. Arbuscular Mycorrhizal Fungi (AMF) constitutes a group of root obligate biotrophs that exchange mutual benefits with about 80% of plants. They are considered natural bio fertilizers, since they aid in the acquisition of water and soil nutrients, also help host defense mechanism against pathogens in exchange for photosynthetic products. An increase in crop production is essential to meet the future food demand. Soil fertility and soil structure of agricultural systems is to be managed by effective use of fertilizers with increased profitability and reduced harm to the environment. Microbial inoculants, including arbuscular mycorrhizal (AM) fungi for increasing the efficient use of fertilizers are potential components of such management. The process of re-establishing the natural level of AMF richness can represent a valid alternative to conventional fertilization practices, with a view to sustainable agriculture. The need for AMF as a biofertilizer, with a view to sustainable agriculture, is becoming increasingly urgent since the appropriate management of these symbiotic fungi could potentially decrease the use of agro chemicals. The history of AMF applications in controlled and open-field conditions is now long. AMF exhibit multifunctional mutualistic symbiosis between plants and members of phylum Glomeromycota. AMF plays a vital role in reduction of plant pathogens. AMF shows the ability to reduce bacterial disease in different crops. Presence of AMF in soil regulates the biogeochemical cycling of organic and inorganic nutrients which maintains soil quality. AMF are also involved in the production of growth hormones such as IAA, Cytokinins, GA3 and vitamins like vitamin B. AMF is very important for sustainable agriculture.

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Author Contribution

SAT Designed the study, Carried out experiment and analyzed data; SP supervised the whole study.

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Conflict of Interest

The authors declare no conflict of interest.

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