

## **Effect of Vesicular Arbuscular Mycorrhiza *Glomus fasciculatum* on the growth and Physiological response in *Sesamum indicum* L.**

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### **ABSTRACT**

Plant growth and physiological response of sesame (*Sesamum indicum* L.) were studied in controlled environment using normal soil and indigenous Vesicular-arbuscular mycorrhiza (VAM) fungi treated soil. The seedlings of *Zea mays* were inoculated with *Gigaspora* species of VAM (*Glomus fasciculatum*) and the inoculum was multiplied with help of Zeamays seed bed. Sesame seeds were then inoculated into the bed and it was found that the plant height, shoots lengths, roots, biomass of shoot and roots were considerably increased in the mycorrhizal plants. The effect of VAM infection was assessed in pot experiment. In this comparative study, specific mycorrhizal fungi had consistent effects on various growth parameters such as the number of leaves, number of roots, shoot length, biomass of shoot and roots and biochemical parameters were observed at various time intervals by statistical analysis using two way ANOVA, it was confined with mycorrhizal and non-mycorrhizal infected plants. It was found that the ability of isolates to maintain the plant growth effectively in the case of mycorrhizal seedlings shows a maximum absorption of  $0.77 \pm 0.2$ , shoot length is about  $8.34 \pm 0.2$ , count of root and leaves are about  $8.10 \pm 0.3$ ,  $5.6 \pm 0.3$  respectively under mycorrhizal infection in 30 days of analysis and had a positive effect on the growth at all intervals. Biochemical analysis were carried out to estimate the total chlorophyll, chlorophyll A, chlorophyll B and Carotenoids contents and it was analyzed to be  $9 \pm 0.5$  mg/g,  $8.3 \pm 0.5$  mg/g,  $3.6 \pm 0.5$  mg/g,  $4 \pm 0.3$  mg/g respectively. At the 30<sup>th</sup> day of analysis for the mycorrhizal plants, it was found to be high in mycorrhizal seedlings which shows the symbiosis had improved the nutrient uptake of cultivated plants. Nevertheless *G. fasciculatum* was found to be the most efficient fungus and exhibited the highest levels of mycorrhizal colonization, as well as the greatest stimulation of physiological parameters.

**Keywords:** VAM; Sesamum; Zeamays; Mycorrhiza; Symbiosis

### **1. INTRODUCTION**

Vesicular Arbuscular mycorrhizal fungi are important in sustainable agriculture because they improve plant water relations and have an impact on environment based on

agrochemicals and high intensity farming, together with examining cost-effective crop production strategies that relegate less financial reliance on expensive artificial inputs, have stimulated the interest in the practical application of mycorrhizae and legumes in agriculture. 'Mycorrhiza' is the symbiotic association between soil born fungi and the roots of higher plants (Rebeca et al., 2013). A vesicular Arbuscular mycorrhizal fungus improves disease control and they increase mineral uptake, which reduces the use of fertilizers. Improved plant water status and changes in water relations have been attributed to a wide variety of mechanisms, including some mechanisms not directly related to phosphorous nutrition or water uptake (Davies et al., 1996). In fact, the inconclusive information that has been obtained suggests that more studies will be required to determine the direct or indirect mechanisms which control plant water relations in AM fungus plant symbioses. The abilities of specific fungus plant associations to tolerate drought are of great interest.

Mycorrhizal and Non-Mycorrhizal Plants of sesamum and Vesicular Arbuscular Mycorrhiza (VAM) is the most abundant kind of mycorrhiza described as 'a universal plant symbioses'. Lack of host specificity is even more characteristic of this symbiosis than other types known. Vesicular Arbuscular Mycorrhiza (VAM) is a potential biofertilizer (Sullia, 1991). Arbuscular Mycorrhiza Fungi is a type of mycorrhiza in which the fungus penetrates the cortical cells of the roots of a vascular plant. Mycorrhizal fungi that grow into the root cortex of the host plant and penetrate root cells to form two kinds of specialized structures, arbuscules and vesicles. Mycorrhizal Fungi is specifically designed to reduce transplant stress while improving soil hydration and fertility. Mycorrhizal association can also enable the plant host to access nutrients in an organic form which would be unavailable otherwise. Compare to normal plant roots Mycorrhizal structures can take up Phosphorus from lower concentration effectively (Howeler et al., 1981). Mycorrhizal association is responsible for up to 80% of the total Phosphorus uptake by plants (Marschner and Dell, 1994). One of the strongest effects of Arbuscular Mycorrhizal Fungi inoculation is an increase in the development of the host plant, which is attributed to an increase in nutrient uptake, particularly those that have low soil mobility and low concentration in the soil solution. A number of interacting factors affecting the successful combination of VAM fungi are pH, soil nutrients, Organic matter, moisture and temperature (Malakooti, 2000). Mycorrhiza fungi increase growth, photosynthetic pigments and photosynthesis of host plants by better mineral nutrition.

They cause chlorophyll organs of plant to grow by absorbing required carbon, giving nutriment to plant and increasing efficiency of photosynthesis, showed that mycorrhiza – inoculated maize plants have more dry matter than non – inoculated plants due to salinity. Also inoculation of salt stressed tomatoes with mycorrhiza meaningfully increased their dry weight of root and shoots compared to non– mycorrhiza–inoculated plants (Al-Karaki, 2000). Many studies have been reported on the use of growth regulators or mycorrhizal fungi in decreasing harmful effects of environmental stress. Mycorrhizal fungi, vesicular arbuscular, are unique microorganisms residing in rhizosphere. These fungi form symbiotic colonies with most plants and in addition to increasing inorganic nutrients in plant, they can increase the resistance of plants to environmental stresses by stimulating growth regulators, increasing photosynthesis, and improving regulation of osmotic adjustment (Rabie and Almadani, 2005). The VAM fungus, *Glomus fasciculatum* (Gigaspora Species) is known to symbiotically associate with Plants and enhance the nutrient content in the plant (Allen, 1991). Studies on VAM fungi conducted during last few decades envisaged their occurrence in a wide variety of hosts, different habitats and variability in quality and quantity. Sesame is a flowering plant in the genus *Sesamum* belongs to the family of pedaliaceae. Numerous wild relatives occur in Africa and a smaller number in India.

It is widely naturalized in tropical regions around the world and is cultivated for its edible oil seeds (Ray, 2011). Sesame is drought-tolerant and is able to grow where other crops fail (Ram et al., 1990). The major contributing countries (68 %) are the world productions of Sesame are India, Sudan, Myanmar and China. Sesame seed is highly nutritive (50 % oil and 25 % protein) which are traditionally used for direct consumption and as a source of oil of excellent quality because of the presence of natural antioxidants such as sesamin and sesamol (Yamashita et al., 2003). The increasing economic importance for food, oil and medicine the yield potential of sesame is not impressive due to its cultivation in sub marginal lands and unavailability of high yielding varieties and inbuilt resistance to biotic and abiotic stress. Cephalin, a phospholipid from sesame seed has been reported to possess hemostatic activity. The oil has wide medical and pharmaceutical applications (Kandangath et al., 2010). Sesame seeds were used for treating wounds particularly burn wounds (Kiran and Asad, 2008). Mycorrhizal plant growth parameters such as plant length, number of roots and number of leaves were observed and statistically analysed with non mycorrhizal plant (Plenchette et al., 1983).

The AM fungi used in this study was *Glomus fasciculatum* species. In this study we compared *Glomus* species with a non mycorrhizal control. And determined the effects of fungal isolates on plant growth, biomass of shoot and root and the biochemical constituents like chlorophyll, carotenoids contents were analyzed.

## 2. MATERIALS AND METHODS

In the present investigation sesame CO<sub>2</sub> variety were used. The experiment was conducted in green house garden at Department of Biotechnology, K.S. Rangasamy College of Technology, Thiruchengode, Tamil Nadu, India.

### 2. 1. Preparation of innoculum

The pure culture of *Glomus fasciculatum* was inoculated in the mixture of sterile sand and soil in the ratio 1:1. *Zea mays* was chosen as host plant for this study and to transfer *Glomus fasciculatum* into the root of sesamum plant, *Zea mays* was grown in the mixture sand for of three months, after that period the aerial part of the plant was cut off from the soil and discarded. The root portion of *Zea mays* which was colonized with *Glomus fasciculatum*, was cut down into small pieces and mixed into the soil and shade dried it for growing of sesamum plant. Plant samples randomly from a total of 10 patches, which were chosen to cover a range of shoot densities from 100-200 shoots m<sup>-2</sup>. Plants were dug up with a spade carefully washed free from sediment using a sieve. Plants with undamaged roots were transferred to plastic bags and transported to the laboratory, were they kept in cold storage until the next morning when the roots were examined for VAM. Ten plants were sampled randomly from each of the 10 patches.

### 2. 2. Staining for colonization

For staining the roots were cleared in 10 % KOH at 90 °C for 15 min. (Philips and Haymen, 1970). After the cleaning, the root samples were stained in 0.05 % typan blue in lactoglycerol at 90 °C for 5 min. from each of sesame 1 cm segments was taken for the staining. All the segments were examined for the presence of fungal structures (eg. Vesicles,

arbuscles and hyphae) at 200-400 x magnification using Phase contrast microscope (Carl Zeiss, Germany).

### **2. 3. Measuring length of plant root**

The root lengths were measured by a ruler and recorded in centimeter. For each treatment group, three repetitions were recorded and the mean was reported in cm.

### **2. 4. Measuring dry weight of root and of shoot**

After separating roots from shoots, each of them was separately placed in aluminum sheet and then put in oven at 80°C for 10 days until their weight was fixed and their dry weight was measured in terms of grams.

### **2. 5. Measuring fresh weight of root and of shoot**

After separating roots from shoots, their fresh weights were measured in terms of gram. For each treatment, three replications were measured and mean was recorded in terms of gram.

### **2. 6. Measuring the number of roots and leaves**

Number of roots and leaves were counted and recorded in numbers for all time periods. For each treatment, three replications were measured and mean was recorded in terms of numbers.

## **3. BIOCHEMICAL ANALYSIS**

### **3. 1. Measuring chlorophyll and carotenoid content**

Chlorophyll and carotenoid contents of the plants were measured according to the method suggested by Lichtenthaler (1987). In this method, 0.2 g fresh texture of leaf was weighed and then ground in Chinese mortar containing 80 % acetone. Then 5 ml Acetone was added to it and solution volume was reached to 15 ml. Three ml of this solution was poured in a cuvette and its absorption intensity was read in 470, 663, 647 nm by Spectrophotometer. For regulating spectrophotometer, 80 % Acetone was used as witness. Pigment density was determined in terms of mg/g fresh weight of the plant essence.

### **3. 2. Estimation of growth rate**

Growth rate was estimated through the length of the plant as it grows with varying time intervals and percentage had been calculated from it. For each treatment, three replications were measured and mean was recorded.

$$\text{Growth rate} = \frac{\text{Final length of the plant} - \text{Initial length of the plant}}{\text{Time interval}}$$

$$\text{Growth [\%]} = \frac{\text{Final length of the plant} - \text{Initial length of the plant}}{\text{Time interval}} \cdot 100\%$$

### 3. 3. Data analysis and statistical studies

This experiment was performed with three replications based on a completely randomized design. Data analysis was performed by two way ANOVA. The Figures were drawn by GRAPHPADPRISM soft ware.

**Table 1.** Influence of VAM on vegetative characteristics, biochemical constituents and growth rate in Sesame.

PARAMETERS	TIME INTERVALS IN DAYS					
	10		20		30	
	VAM	CONTROL	VAM	CONTROL	VAM	CONTROL
<b>Length of Plant</b>	2.78±0.2	2.1±0.2	4.88±0.2	3.31±0.2	8.34±0.2	5.54±0.3
<b>Number of Leaves</b>	2.60±0.5	2.02±0.23	6.08±0.3	4.08±0.3	8.10±0.3	5.94±0.5
<b>Number of roots</b>	1.47±0.3	1.68±0.3	3.60±0.3	3.30±0.3	5.60±0.3	4.40±0.3
<b>Dry weight of root</b>	0.27 ±0.3	0.07±0.01	1.20±0.2	0.10±0.01	1.98±0.1	1.15±0.1
<b>Fresh weight of root</b>	0.67±0.1	0.25±0.1	1.31±0.3	0.65±0.1	1.95±0.1	1.01±0.04
<b>Dry weight of stem</b>	0.29±0.3	0.07±0.01	1.34±0.2	0.14±0.01	2.21±0.3	1.34±0.3
<b>Fresh weight of stem</b>	1.02±0.4	0.45±0.1	1.57±0.2	0.65±0.15	2.17±0.3	1.01±0.04
<b>Chlorophyll A (mg/g)</b>	13±0.5	9±0.4	15±0.5	8±0.3	15.6±0.5	8.3±0.5
<b>Chlorophyll B (mg/g)</b>	6±0.5	3.6±0.5	6.6±0.4	3.3±0.5	7±0.5	3.6±0.5
<b>Total Chlorophyll (mg/g)</b>	17.3±0.5	9±0.3	17.3±0.5	8.6±0.3	18±0.7	9±0.5
<b>Carotenoids (mg/g)</b>	5±0.4	3.3±0.5	6±0.5	4±0.4	6.3±0.2	4±0.3
<b>Growth Rate</b>	0.77±0.2	0.32±0.3	0.75±0.4	0.58±0.3	0.72±0.3	0.67±0.2
<b>Growth Percentage</b>	77	32	75	58	72	67

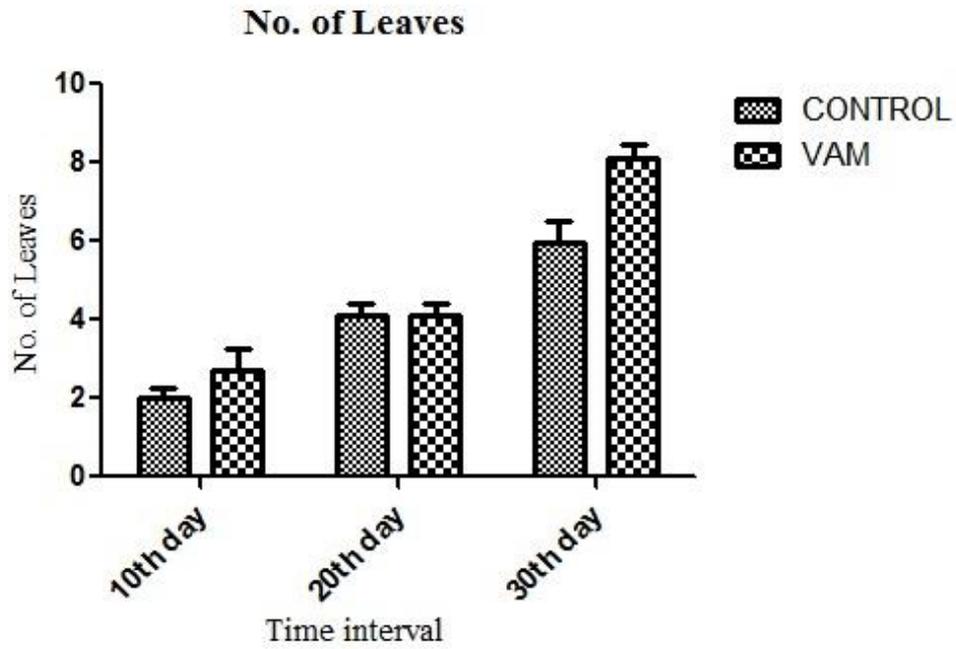


Fig. I. Interaction of mycorrhizal fungus on the number of leaves at varying time intervals.

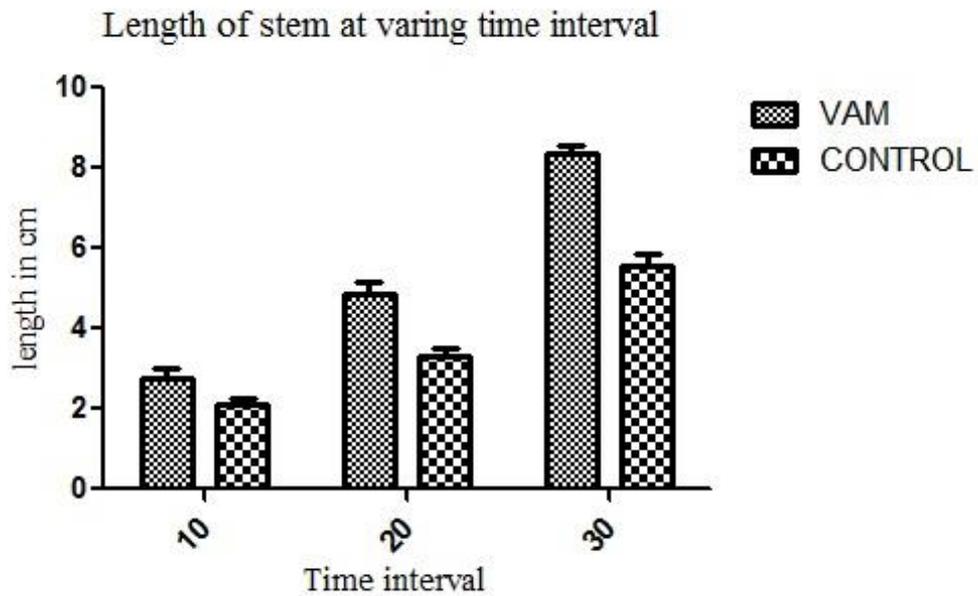
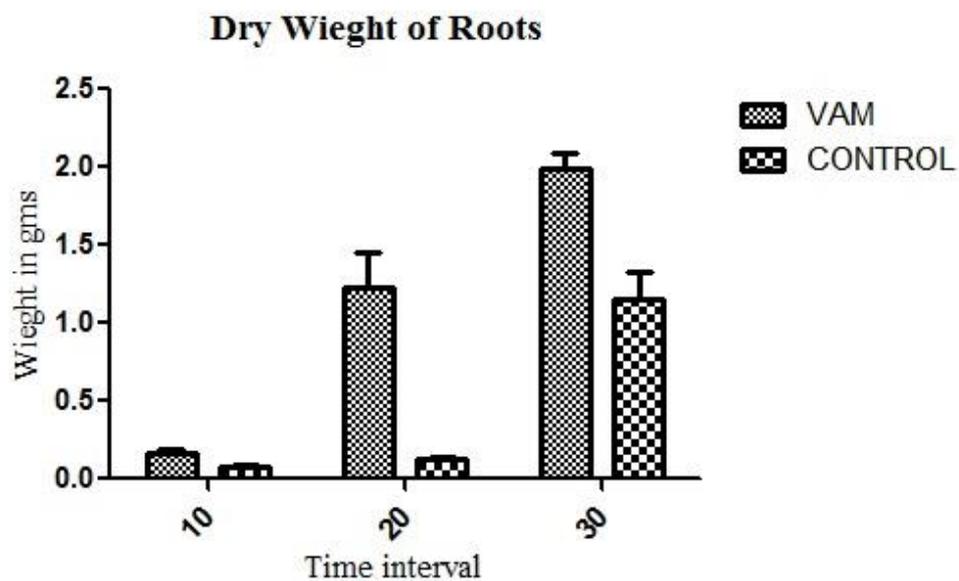
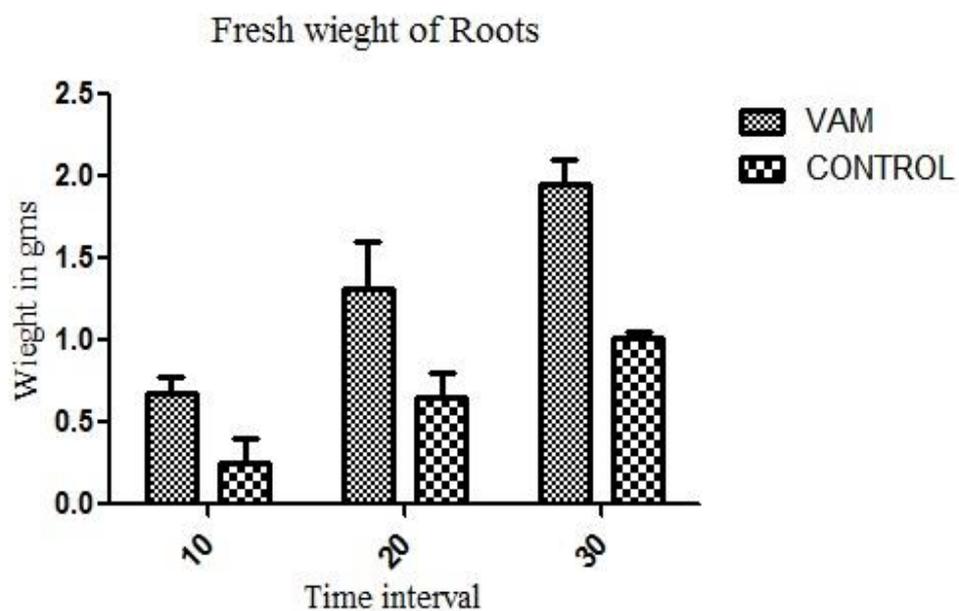


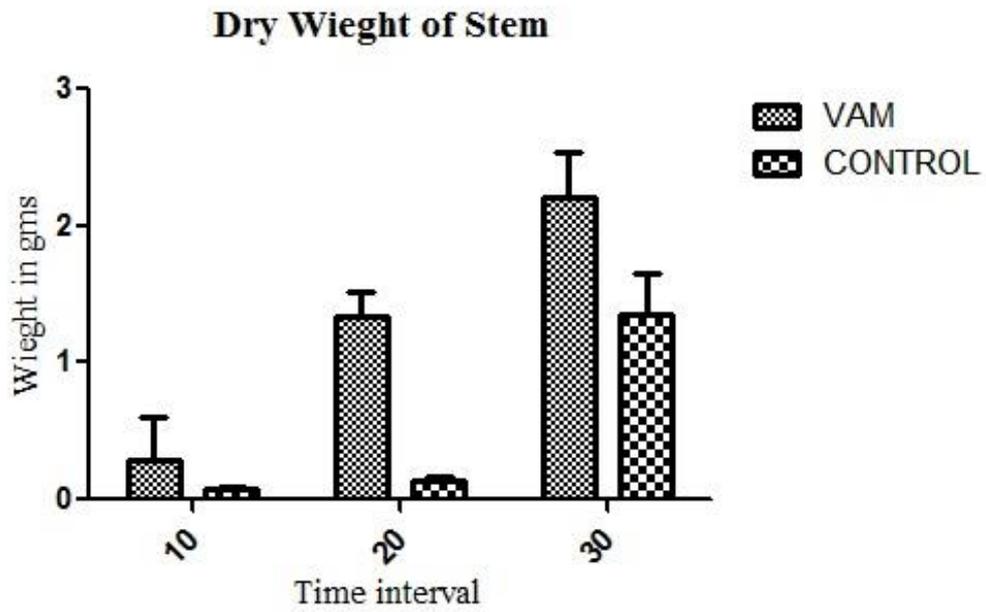
Fig. II. Interaction of mycorrhizal fungus on the shoot length at varying time intervals.



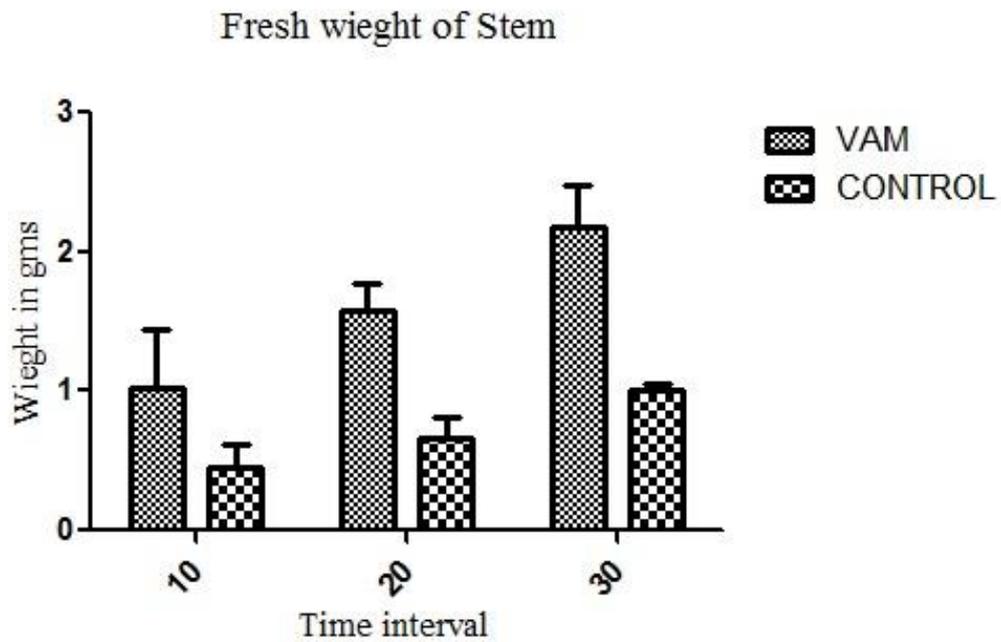
**Fig. III.** Interaction of mycorrhizal fungus on the dry weights of the roots at varying time intervals.



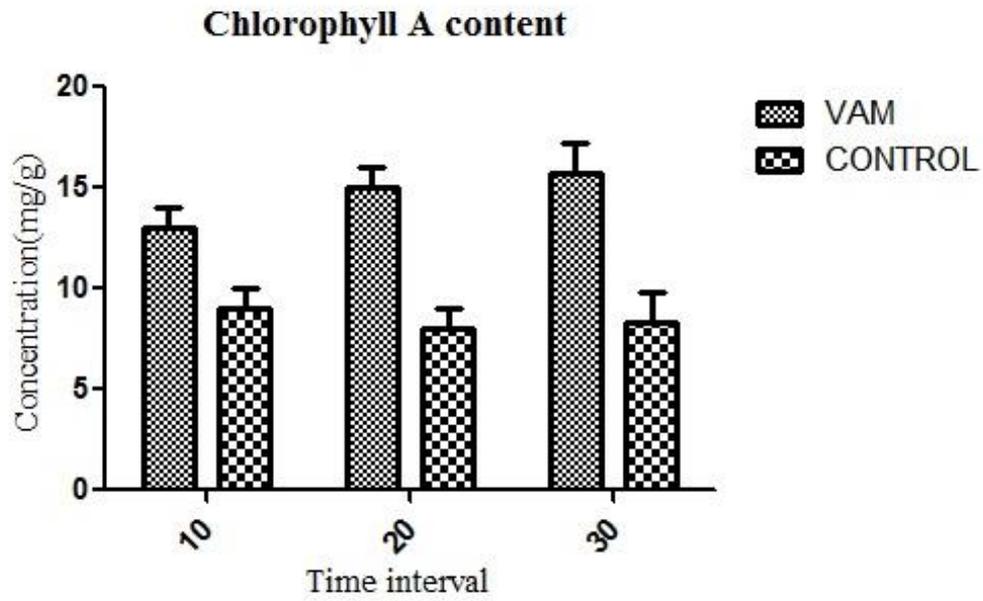
**Fig. IV.** Interaction of mycorrhizal fungus on the fresh weight of the roots at varying time intervals.



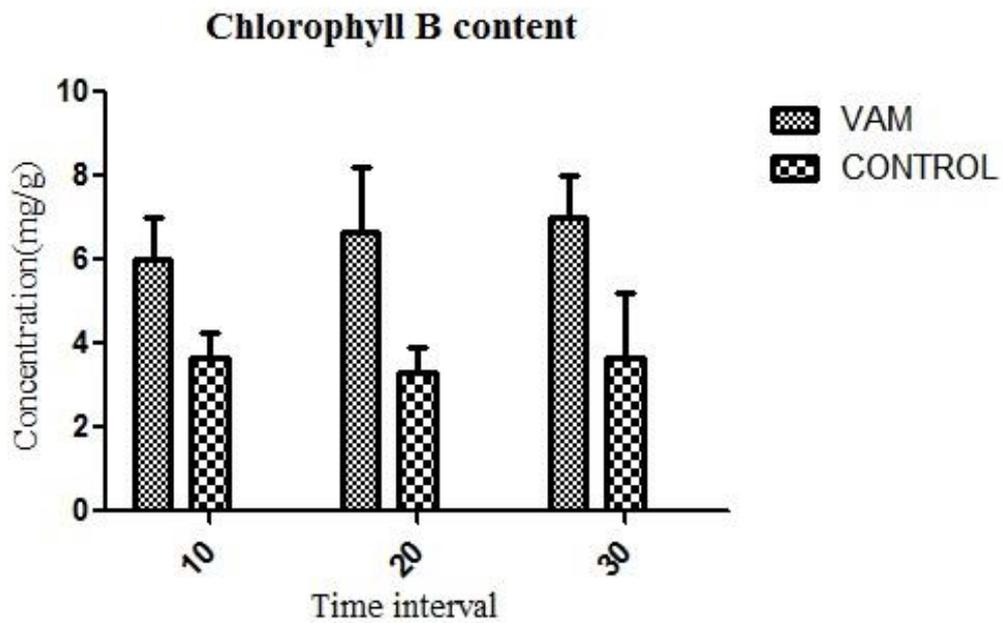
**Fig. V.** Interaction of mycorrhizal fungus on the dry weights of the shoots at varying time intervals.



**Fig. VI.** Interaction of mycorrhizal fungus on the fresh weight of the shoots at varying time intervals.



**Fig. VII.** Interaction of mycorrhizal fungus on the chlorophyll A content at varying time intervals.



**Fig. VIII.** Interaction of mycorrhizal fungus on the chlorophyll B content at varying time intervals.

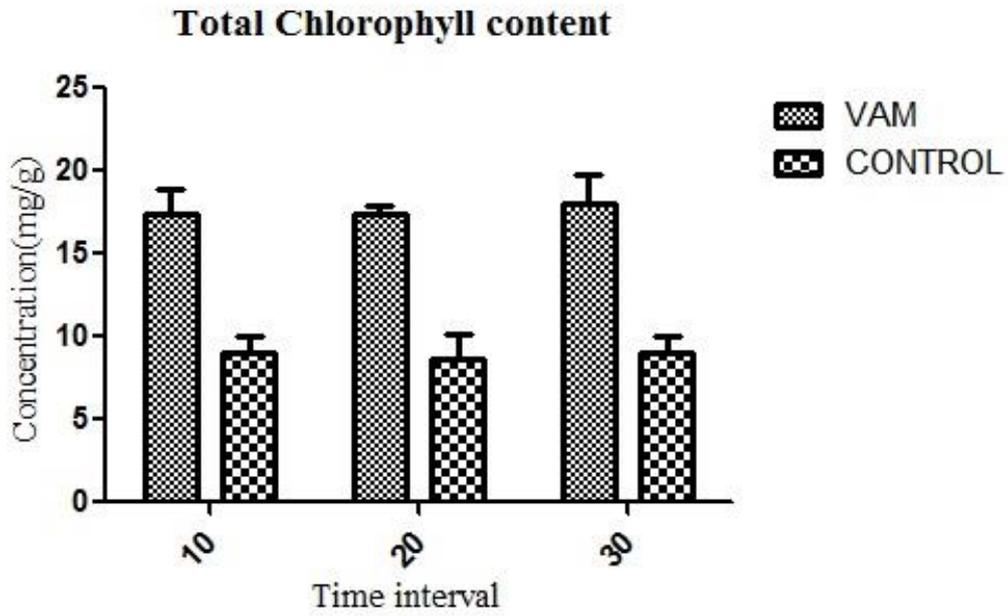


Fig. IX. Interaction of mycorrhizal fungus on the total chlorophyll content at varying time intervals.

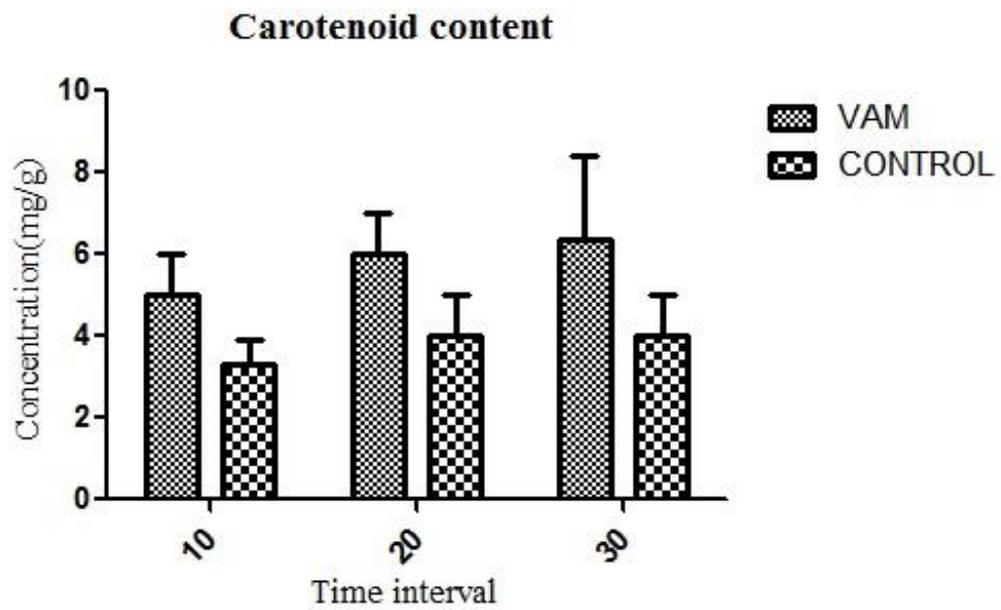


Fig. X. Interaction of mycorrhizal fungus on the carotenoid content at varying time intervals.

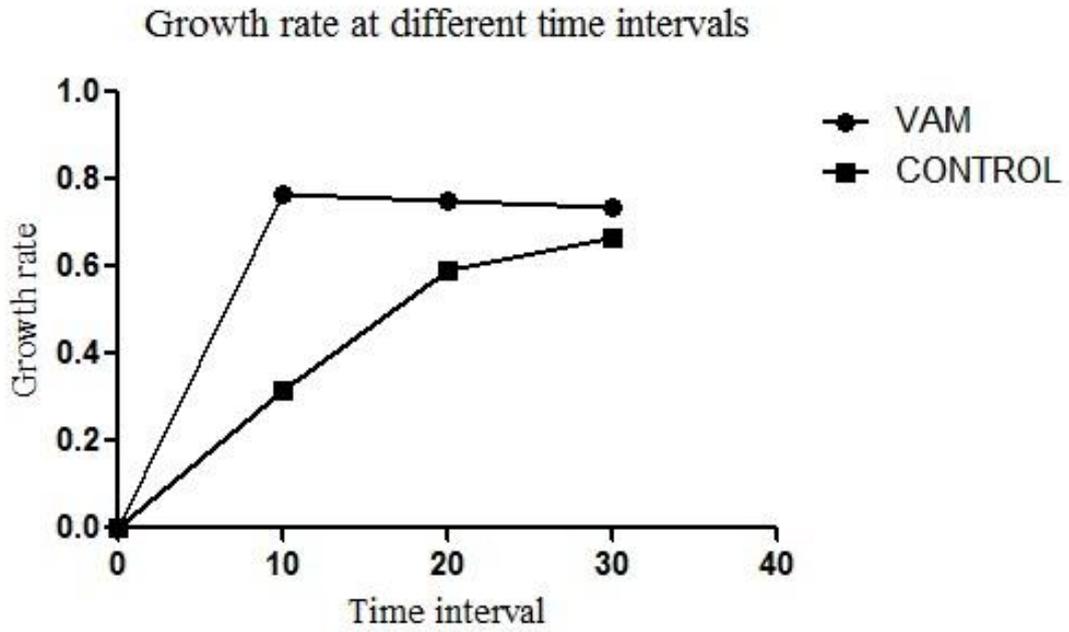


Fig. XI. Interaction of mycorrhizal fungus on the growth rate at varying time intervals.

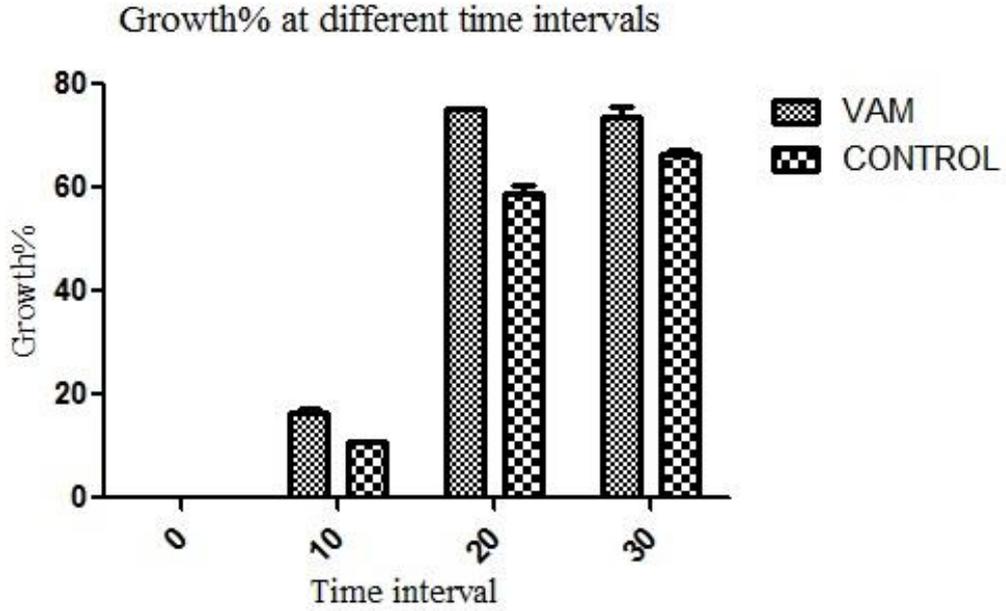


Fig. XII. Interaction of mycorrhizal fungus on the growth percentage content at varying time intervals.

## 4. RESULT

### The content of root mycorrhizal colonization

Root colonization in inoculated plants with *Glomus fasciculatum* was determined. This indicates that *Glomus fasciculatum* can significantly colonized in root. Fig I shows the penetration of fungi into root cells.

### Number of roots and leaves

The results of this study showed that number of root and leaves was found to be high in number at treatment with VAM than the control. Number of root and leaves were increased was meaningful at  $p \leq 0.0001$  and have significant difference was seen between time intervals (10- 30day) of analysis. In mycorrhiza fungus treatment there was an increase in number of root and leaves growth, was found to high as  $5.6 \pm 0.3$  and  $8.10 \pm 0.3$  at 30<sup>th</sup> day of analysis respectively (Fig. II and III).

### Shoot length

The results of this study showed that length of shoot was found to be high at treatment with VAM than the control. Length of shoot was increased was meaningful at  $p \leq 0.0001$  and have significant difference was seen between time intervals (10-30 day) of analysis. In mycorrhiza fungus treatment there was an increase in length of shoot growth, was found to high as  $8.34 \pm 0.2$  and for control was about  $5.54 \pm 0.2$  at 30<sup>th</sup> day of analysis respectively (Fig. IV).

### Root dry and fresh weights

The results of this study showed that root dry and fresh weights were found to be high at treatment with VAM than the control. Root dry and fresh weights were increased was meaningful at  $p \leq 0.0001$  and have significant difference was seen between time intervals (10 -30day) of analysis. In mycorrhiza fungus treatment there was an increase in Root dry and fresh weights were found to high as  $1.98 \pm 0.1$ gms,  $1.95 \pm 0.1$ gms and for control was about  $1.15 \pm 0.1$  gms,  $1.01 \pm 0.04$  gms at 30<sup>th</sup> day of analysis respectively (Fig. V and VI). The amount of this parameter was meaningfully increased in treating plants with *Glomus fasciculatum* increase in roots dry and fresh weights were observed relative to control plant at 30<sup>th</sup> day of analysis.

### Shoot dry and fresh weights

The results of this study showed that root dry and fresh weights were found to be high at treatment with VAM than the control. Root dry and fresh weights were increased was meaningful at  $p \leq 0.0001$  and have significant difference was seen between time intervals (10 -30day) of analysis. In mycorrhiza fungus treatment there was an increase in Root dry and fresh weights were found to high as  $2.21 \pm 0.3$ gms,  $2.17 \pm 0.3$ gms and for control was about  $1.34 \pm 0.3$  gms,  $1.01 \pm 0.04$  gms at 30<sup>th</sup> day of analysis respectively (Fig. VII and VIII). The amount of this parameter was meaningfully increased in treating plants with *Glomus fasciculatum* increase in roots dry and fresh weights were observed relative to control plant at 30<sup>th</sup> day of analysis.

### **Chlorophyll A**

Chlorophyll A content in VAM treatments meaningfully increased at  $p \leq 0.001$ . However, treating plants with mycorrhiza fungi, meaningfully increased chlorophyll A content than the control (Fig. IX). Chlorophyll A content significantly varies with the time intervals between the VAM and control. Chlorophyll A content was found to be high at VAM treatment as  $15.6 \pm 0.5$  mg/g and for control as  $8.3 \pm 0.5$  mg/g.

### **Chlorophyll B**

Chlorophyll B content in VAM treatments meaningfully increased at  $p \leq 0.001$ . However, treating plants with mycorrhiza fungi, meaningfully increased chlorophyll B content than the control (Fig. X). Chlorophyll B content significantly varies with the time intervals between the VAM and control. Chlorophyll B content was found to be high at VAM treatment as  $7 \pm 0.5$  mg/g and for control as  $3.6 \pm 0.5$  mg/g.

### **Total chlorophyll**

Increasing in total chlorophyll content, which is meaningful at  $p \leq 0.05$  in VAM treatment. In inoculated plants with mycorrhiza fungi, chlorophyll content had meaningfully increased at  $p \leq 0.05$  relative to control plant at various time intervals (Fig. XI). In this experiment, increasing effects of *Glomus fasciculatum* were more prominent on total chlorophyll content than the control. Total chlorophyll content was found to be high at VAM treatment as  $18 \pm 0.7$  mg/g and for control as  $9 \pm 0.5$  mg/g.

### **Carotenoids**

The results of this study showed that increase in carotenoids content in VAM treatment. Furthermore, the increase in carotenoids content was meaningful at all time intervals to treat with *Glomus fasciculatum* than the control plants (Fig. XII). Carotenoids content was found to be high at VAM treatment as  $6.3 \pm 0.2$  mg/g and for control as  $4 \pm 0.3$  mg/g.

### **Growth rate and growth percentage**

Growth rate and growth percentage were showed to be high at VAM treatment at all time interval analysis. Growth rate was meaningfully increased in treatment than the control as  $0.72 \pm 0.3$ ,  $0.67 \pm 0.2$  respectively at 30<sup>th</sup> day of analysis. At all time analysis of analysis growth percentage significantly increases in treatment than the control as 72 % and 67 % respectively.

## **5. DISCUSSION AND CONCLUSIONS**

Inoculation of plants with arbuscular mycorrhizal [AM] fungi has the potential to increase or maintain yields and allow for reduced fertilizer and pesticide application (David et al., 2008). In these systems, the hosts studied are often the dominant species. Their endo mycorrhizal fungi produce a larger amount of extra radical mycelium that behaves as an ecosystem stabilizer, improving the nutrient flux among community components (Al-Agely and Reeves, 1995). The use of VAM fungi in forestry appears to be more important than in agriculture because in countries like India no large scale provisions exist to irrigate, fertilize and protect the plantation. The practical use of VAM fungi seems to be more appropriate as

they are effective in overcoming the stress conditions like draught, disease incidences and deficiency of nutrients (Peter and Rhodes, 1987). Although a few studies have been conducted on VAM interaction with the tropical trees (Marx et al., 1971), the results are encouraging as the growth of seedlings and productivity was found to be enhanced in VAM treated. The effect of growth parameters in VAM inoculated plants showed prompt response in terms of growth and flowering character when compared with Non-Mycorrhizal plants.

Low resistance to water movement through roots and conducting system to the shoots by an increase in vessels or vessel diameter also aids in maintaining water uptake under the stress (Hale and Oracett, 1987). The present investigation revealed that, the treatment VAM significantly increased the root length and shoots length at all time of investigation. In cycocel treated plants, there was increase in root length. Similar results were reported by Turner and Begg, (1978). In the present study VAM colonization was found in the root samples. The length and leaf surface area are considerably increased in the VAM infected plants. From the literature on the interactions between VAM- fungi and terrestrial plants, it is clear that VAM is mainly involved in facilitating nutrient uptake (Khan et al., 1975) although it has been shown that VAM is mainly involved in uptake of phosphorous, nitrogen and other nutrients and exchange for photosynthesis (Smith and Read, 2008). Microscopic pipelines hyphal structure of Mycorrhizal fungi that can transport carbon and minerals to and away from the plants (Barrow, 2004). The cultivators of Maize and *Sesemum* require more amount nutrients in the early stage of development of plant system completely based on the development and performance of roots (Cheung et al., 1987). In this study, VAM treated plants had root dry and fresh weights were increased due to osmotic potential of soil and disturbance in water absorption by plant. Existence of fungus hypha network increases nutrient and water absorption. Fibers of mycorrhizal fungus are divided into two groups; some of them enter the plant system and decrease density of abscisic acid and increase cytokinin content. This action increases water absorption and develops root system of the plant. Second group of fibers are out of root system and secrete organic acids solving phosphorus such as malic acid that increases phosphorus absorption by plant and its dry matter. Phosphorus as one of the elements required for plant increases dry matter because it has an important role in cellular division by regulating plant hormones. Moreover, it has an important role in producing photosynthetic matters and produces energy in plant and has an important role in cellular division by regulating plant hormones. Moreover, it has an important role in producing photosynthetic matters and produces energy in plant (Khalvati et al., 2005). The results of shoot dry and fresh weights were increased in this study under VAM treatment. In the plants inoculated with mycorrhiza fungus, increased fresh and dry weight of shoot was observed. This increase in weight can be resulted from the effects of mycorrhiza fungus on absorbing various nutriment such as nitrogen, calcium, potassium, copper, zinc and sulphur. Using mycorrhiza fungus increases plant growth and affects devoting and transferring nutriment between stem and root so that dry weight of shoot is increased by increasing absorption of nutriment and their transfer. Fresh weight of shoot was also increased in *Glomus mosseae* – treated plants in low salinity. Similar results were also obtained about mycorrhizal barley plant in salt stress conditions (Nourinia et al., 2007).

Chlorophyll is known to influence the photosynthetic rate and in turn influence growth and development of cotton. However, under VAM treated conditions there will be increase in pigment composition, which induce to increase chlorophyll content. Chlorophyll 'A', chlorophyll 'B', total chlorophyll and carotenoids content of leaves were increase in VAM treated conditions. Higher persistence of chlorophyll content under stress due to growth regulators and VAM may be attributed to decreased chlorophyll degradation and increased

chlorophyll synthesis. These results are in accordance with Jayakumar and Thangaraj (1998). VAM had significantly higher chlorophyll content at all time analysis of crop growth. The increase in total chlorophyll concentration of drought plants in response to mycorrhizal effects was positively correlated with respective levels of mycorrhizal infection in broad bean plants (Abdel et al., 2002). Such increases were related to the degree of mycorrhizal infection and Huixing (2005) also showed the effect of VAM on host plant in drought condition that enhanced resistance to drought stress by increase in chlorophyll content than non VAM plants. Thus, our results of enhanced chlorophyll content due to plant growth regulators application and VAM are in agreement with the above discussion.

Growth rate in VAM treatment were found to be high as VAM act as growth regulators and it influence even at stress conditions (Shekoofeh and Sepideh, 2011). The VAM fungal symbioses were proved to play a vital role in such stress conditions by supplying the nutrients to the host plant. The present study also established that the association of VAM fungi with Sesame as *Zea mays* enhanced the growth when compared with control.

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