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Effect of AM fungi on some biochemical parameters in *Capsicum annuum* L. (Chili)

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Abstract

One of the most effective biofertilizers amongst the biological fertilizers is Mycorrhiza. Majority of angiospermic plants show presence of mycorrhiza. Earlier Family Chenopodiaceae was considered to be one of the exceptions for mycorrhizal host plants, but now reports of mycorrhiza are available in some of the plants like spinach. This symbiotic association proved to be beneficial for both of the symbionts. The benefits of this symbiotic association can be easily checked in host plants which are often a higher plant like angiosperm. In present investigation Capsicum annuum L. plant commonly known as chili was screened and assessed for such benefits shared by mycorrhiza. In present paper, biochemical aspects were studied in controlled (non mycorrhizal) and treated (mycorrhizal) plants of chili. The estimation of alpha amino nitrogen, nitrate content, nitrate reductase activity from leaves and protein content of fruit was carried out in mycorrhizal chili plants and is compared with non mycorrhizal plants of chili. Results from above experiment showed that there is a significant increase in all the studied biochemical parameters in mycorrhizal chili plants than the non mycorrhizal chili plants. These results may contribute to the increment in overall yield of chili plants.

Keywords: Arbuscular mycorrhiza, *Capsicum annuum* L., alpha amino nitrogen, nitrate reductase, nitrate content, protein content.

Introduction

German botanist Frank coined the term mycorrhizae for the first time to designate the symbiotic relationship between the fungi and plant roots[1]. According to Harley and Smith mycorrhiza is an association between fungal hyphae and roots of higher plants concerned with absorption of mineral substances from the soil[2]. The term mycorrhiza (which literally means fungus-root) originates from two Greek words mycos meaning fungus and rhiza meaning root in its broadest sense is the non-pathogenic (or very feebly pathogenic) association of fungi and the roots of higher plants[3]. The root fungus association is symbiotic and the whole association is being considered as 'functionally distinct organ' involved in mineral uptake from the soil. This association is characterized by the movement of plant produced carbohydrates to the fungus and the fungus acquired nutrients and water to the plant. The benefit afforded by the mycorrhizal symbiosis can be derived by the autotrophs in terms of increased growth and yield and by the fungus in terms of flow of organic carbon compounds to the soil, called as soil nutrition[4]. According to Gerdeman mycorrhizal associations are so prevalent that the non-mycorrhizal plant is more of an exception than the rule[5].

Capsicum annuum L. is a perennial herbaceous plant in the family Solanaceae. Although the species name *annuum* means "annual" (from the Latin *annus* - year), the plant is not an annual. The numerous varieties that have been developed are categorized in five major groups: 1) Cerasiforme (cherry peppers); 2) Conoides (cone peppers); 3) Fasciculatum (red cone peppers); 4) Grossum (bell or sweet peppers); and 5) Longum (chili or cayenne peppers). Chilies are used fresh, cooked, or dried in an enormous variety of dishes characteristic of different regional cuisines. They are high in vitamins A and C. Some varieties have been developed to use as ornamentals, often for indoor pots; these often have small, brightly-coloured, persistent fruits. One of the major biochemical active compounds one can obtain from fruits of chili (*C. annuum* and other *Capsicum* species) is Capsaicin, is an intense skin and eye irritant, and is the ingredient used in pepper sprays sold for self-defense. However, it also has numerous medical uses, including topical pain relief for muscle soreness, shingles, skin irritations, and rheumatism, and as an anti-inflammatory. Recent medical research has also documented antimicrobial and antifungal activity of capsaicin obtained from several *Capsicum* species, and on-going studies are exploring its use in cancer treatment.

There are many references available on the mycorrhization in chili plant throwing a light of enhancement in yield of chili plant by use of mycorrhiza[6-9]. Kavitha *et al* studied the use of mycorrhiza in biocontrol of Damping-Off in Chili[10]. In present paper effect of mix culture of *Glomus* (*Glomus mosseae* and

Glomus fasciculatum) on *Capsicum annuum* L. plant is studied in terms of some of the biochemical parameters. They are Alpha amino nitrogen content of leaf, nitrate content of leaf, nitrate reductase activity in leaf, protein content of fruit. Estimation was done in mycorrhizal (controlled) plants of chili along with non mycorrhizal (controlled) plants of chili.

Materials and methods

Eighteen cm diameter, bottom holed plastic pots twelve in number were used for the experiment. Out of twelve pots, six were maintained as control and six were used for the treatment with mycorrhiza culture. From nursery suppliers garden soil was obtained in bulk. Sand was collected from Girgaon Chowpatty sea shore and thoroughly washed with plenty of tap water to remove soluble salts. Mixture of soil and sand (volume / volume) in 3: 1 proportion was made in trays. As mycorrhizae are aerobic microorganisms, aeration in pot is essential and that is why sand was added to support aeration.

The above mixture was sterilized at 121^0C for 1 hour in autoclave at 15 lbs pressure to kill microorganisms and insect present if any. This sterilized mixture was allowed to cool down to normal room temperature and was used as a growth medium for pot experiment.

Initially $3/4^{th}$ capacity of each pot was filled up with sterilized soil mixture. 10 g of AM inoculation was added to six pots as experimental or treated pots. The inoculum was distributed evenly in the pot and was covered with a layer of 4 cm of sterilized soil mixture. Fifteen water soaked seeds of *Capsicum annuum* L. (Chili) were sown in each pot, both control and treated and covered with a layer of soil. The pots were watered with watering can having small pores to avoid the disturbance of the soil surface. After intervals of 15 days after sowing (15 DAS), 30 DAS, 45 DAS and 60 DAS, chili plants were carefully uprooted and all necessary precautions were taken unless and until plant leaves were processed for making aliquots for estimation of the biochemical parameters. Various standard biochemical assays were followed as mentioned. An alpha Amino Nitrogen content of leaf was estimated in the fresh leaf materials of mycorrhizal (experimental) and non mycorrhizal (controlled) plants by the method of Moore and Stein[11]. The method of Johnson and Ulrich was used to determine nitrate content of fresh leaves from mycorrhizal and non mycorrhizal leaves of *Capsicum annuum* L. plant[12]. The *in vivo* assay of nitrate reductase [NR] activity in the leaves of the mycorrhizal and non mycorrhizal plants was carried out according to the method of Kleeper *et al*[13]. Soluble protein contents in the fruit material were analysed by the method of Lowry *et al*[14]. Unpaired t-test is carried out to check the effect of mycorrhizal inoculation of *Capsicum annuum* L. plants. All the statistical analysis are carried out with the help of R i386 3.3.3. Ink.

Results and discussion

Alpha amino nitrogen (mg / g of leaf material) of *Capsicum annuum* L. leaf: Table-1 reflects the alpha amino nitrogen of *Capsicum annuum* L. leaf in experimental and control (mycorrhizal and non – mycorrhizal respectively) plants. It was observed that experimental plants were showing more alpha amino nitrogen (mg / g of leaf material) than that of control plants.

Table-1: Alpha amino nitrogen of *Capsicum annuum* L leaf in experimental and control (mycorrhizal and non – mycorrhizal respectively) plants.

Alpha amino nitrogen (mg / g of leaf material)	15 DAS	30 DAS	45 DAS	60 DAS
Control	0.062	0.097	0.140	0.156
Experimental	0.121	0.181	0.250	0.300
Calculated t	-92.655	-1.0023	-112.68	-58.871
Level of significance (p)	0.05	0.05	0.05	0.05

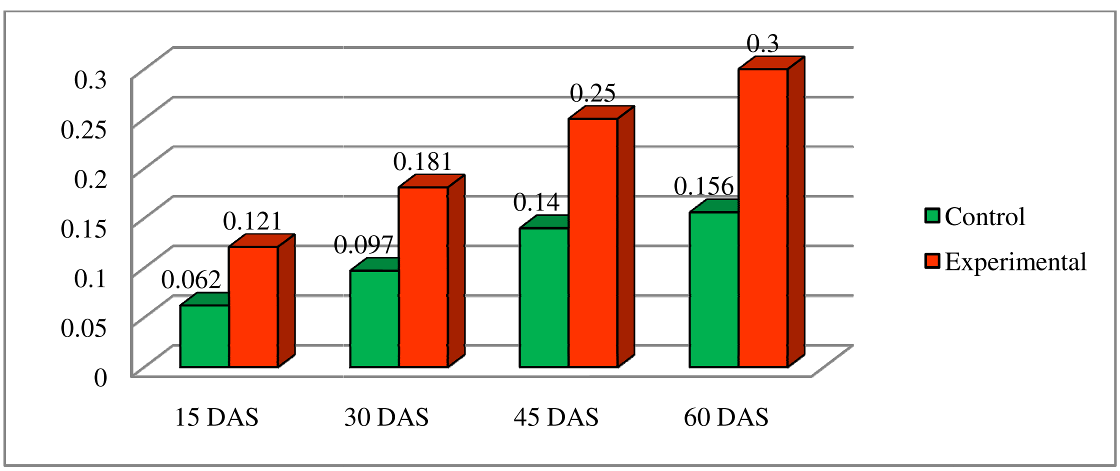

Figure-1: Alpha amino nitrogen of *Capsicum annuum* L leaf in experimental and control (mycorrhizal and non – mycorrhizal respectively) plants.

Nitrate content (mg / g of leaf material) of *Capsicum annuum* L. leaf: Table-2 throw a light on trend of nitrate content in leaves of *Capsicum annuum* L. experimental and control sets. It is in higher range in experimental set than of control set.

Table-2: Nitrate content of *Capsicum annuum* L leaf in experimental and control (mycorrhizal and non – mycorrhizal respectively) plants.

Nitrate content (mg / g of leaf material)	15 DAS	30 DAS	45 DAS	60 DAS
Control	0.276	0.285	0.377	0.412
Experimental	0.412	0.531	0.629	0.795
Calculated t	-87.788	-389.91	-229.86	-250.51
Level of significance (p)	0.05	0.05	0.05	0.05

Nitrate reductase activity (mg / g of leaf material / hour) of *Capsicum annuum* L. leaf: Table-3 shows that nitrate reductase activity in experimental plants is more than that of observed in controlled plants.

Table-3: Nitrate reductase of *Capsicum annuum* L leaf in experimental and control (mycorrhizal and non – mycorrhizal respectively) plants.

Nitrate reductase activity (mg / g of leaf material / hour)	15 DAS	30 DAS	45 DAS	60 DAS
Control	2.587	2.663	2.701	2.708
Experimental	3.892	3.997	4.081	4.092
Calculated t	-1201.7	-1089.7	-593.69	-502.25
Level of significance (p)	0.05	0.05	0.05	0.05

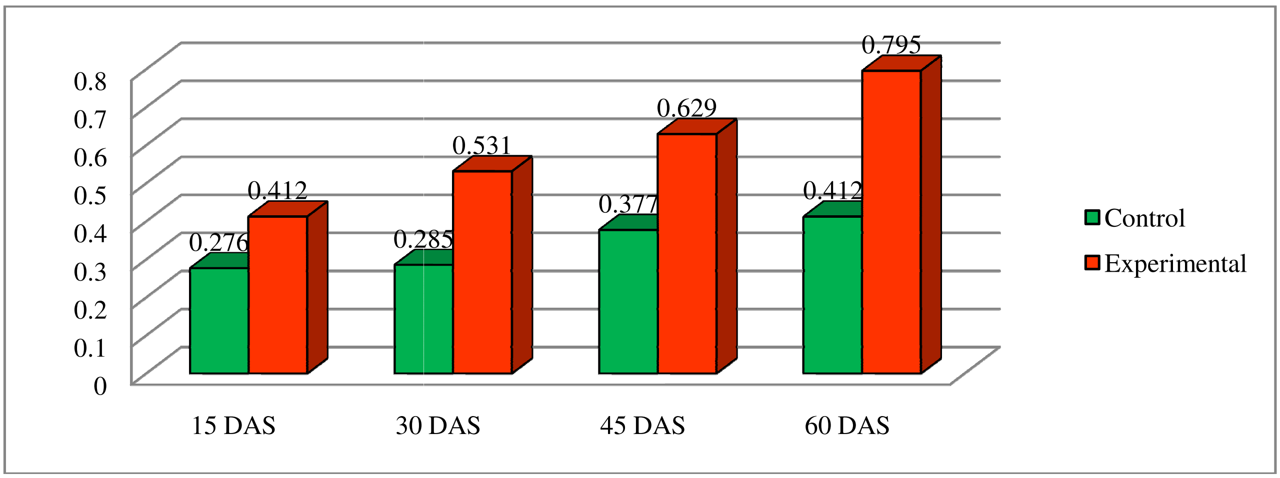

Figure-2: Nitrate content of *Capsicum annuum* L leaf in experimental and control (mycorrhizal and non – mycorrhizal respectively) plants.

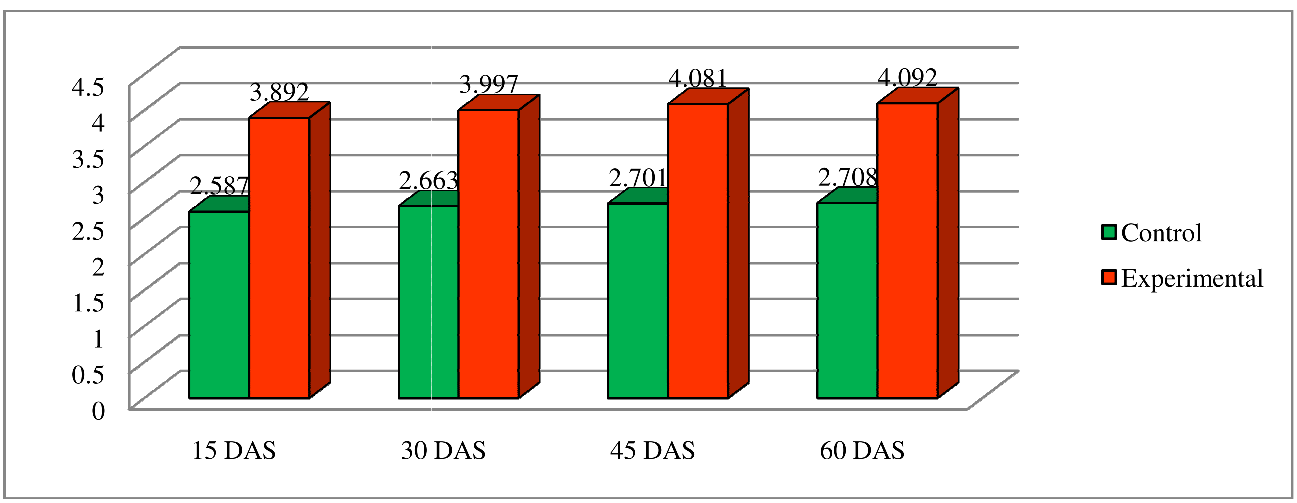

Figure-3: Nitrate reductase of *Capsicum annuum* L leaf in experimental and control (mycorrhizal and non – mycorrhizal respectively) plants.

Protein content (mg / g) of *Capsicum annuum* L. fruit: Table-4 indicate higher amount of protein (mg / g) in experimental (mycorrhizal plant) fruit with the comparison to control (non – mycorrhizal plant) fruit.

Table-4: Protein content of *Capsicum annuum* L fruit in experimental and control (mycorrhizal and non – mycorrhizal respectively) plants.

Fruit	Protein content (mg / g of fruit)
Control	1.628
Experimental	2.590
Calculated t	-84.115
Level of significance (p)	0.05

Discussion: It is seen that in the above experiments, enhancement in all the four biochemical parameters studied in mycorrhizal (experimental) plants of *Capsicum annuum* L. in comparison with the non mycorrhizal (controlled) plants of *Capsicum annuum* L. plants. Similar results have been reported by various researchers in various other plants

From Table-1 it can be understood that for throughout the study period, alpha amino nitrogen in mycorrhizal plants is significantly high that of non mycorrhizal plants. In grafting experiment of Rangpur lime, Barman *et al* reported the significant increase in nitrogen and protein content of mycorrhizal plants than that of non mycorrhizal one[15].

Table-2 depicts the significant increase in nitrate content in *Capsicum annuum* L. plant throughout the investigation period in mycorrhizal (experimental) plants than that of non mycorrhizal (controlled) plants. Table-3 shows significant increase in nitrate reductase in mycorrhizal (experimental) plants with respect to non mycorrhizal (controlled) plants of *Capsicum annuum* L. Mycorrhizal *Ziziphus mauritiana* showed increase activities of Glutamine synthetase and nitrate reductase enzyme than that of non mycorrhizal one. According to Singh *et al* plant roots take up nitrogen from the soil both in the form of nitrate nitrogen or ammonium nitrogen[16]. In nitrate assimilation, nitrate is first reduced to nitrite by the cytoplasmic enzyme nitrate reductase. Nitrite is then reduced to ammonia by nitrite reductase activity. An amino group from ammonia is added to amino acid glutamate to give the amide glutamine. This reaction is catalysed by the enzyme glutamine synthetase. AM fungi are known to produce both nitrate reductase enzyme and ammonium assimilating enzymes. Manjunath *et al* reported increment in nitrogen content of shoot and root in *Leucaena* inoculated with *Glomus fasciculatum*[17]. Increased nitrate content in mycorrhizal plants than non mycorrhizal plants of papaya has been reported[18]. Anil Kumar and Muraleedhara reported in *Vigna sinesis* L. grown in saline soil with mycorrhizal inoculation showed significant increase in nitrogen content as compared to plants having no mycorrhiza[19]. It was observed that percentage of mycorrhization and nitrogen uptake was directly proportional. Similar trend in mycorrhizal plants as compared to non mycorrhizal plants for nitrogen content has been reported[20]. Bucking and Kafle in their review paper suggest the similar increase trend of nitrogen uptake by mycorrhizal plants in comparison with non mycorrhizal plants[21]. Mathan and Sevanan Rajesh Kumar reported increase nitrate reductase activity in *Wedallia chinensis* because of mycorrhizal fungi. They worked on 7 different mycorrhizal fungi[22].

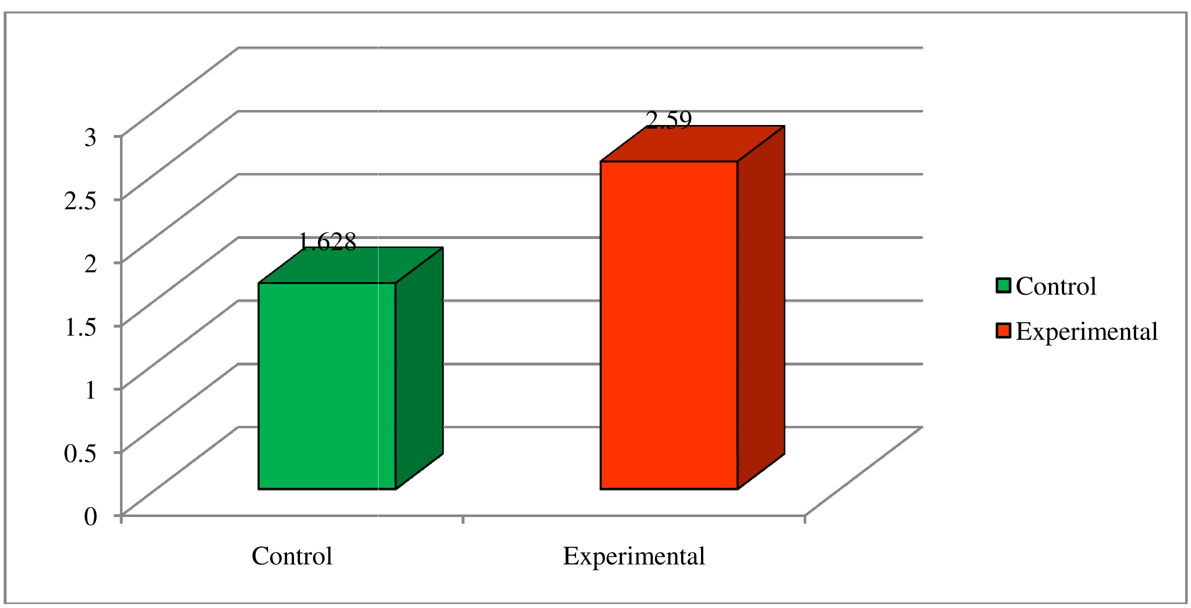

Figure-4: Protein content of *Capsicum annuum* L fruit in experimental and control (mycorrhizal and non – mycorrhizal respectively) plants.

Table-4 indicates the results obtained for protein content in fruits of *Capsicum annuum* L. plant. It is very clear from the results that protein content of fruits in mycorrhizal (experimental) plants is significantly high than that of non mycorrhizal (controlled) plants.

Barman *et al* reported that Rangpur lime has the significant increase in nitrogen content and protein content in mycorrhizal plants with comparison to non mycorrhizal plants[15].

According to him such increased in protein content could be attributed to AM fungal association in terms of % root colonization and spore density. Azcon *et al* reported increased protein content in lettuce, *Lactuca sativa* plant inoculated with *Glomus fasciculatum* than the non mycorrhizal plant[23]. Fattah and Asrar reported increased protein content in leaves of mycorrhizal maize plant than the non mycorrhizal plants in saline conditions[24]. Shinde and Thakur carried out detail experiments on pea plants in water stress condition with mixture of AM fungi species of *Acaulospora denticulata, A. gerdemannii, Glomus macrocarpum, G. maculosum, G. fasciculatum and Scutellospora minuta*. It was observed that protein content of leaf decreases in water stress conditions in non mycorrhizal plants whereas no such decrease was observed by them in mycorrhizal plants of pea in water stress conditions. They concluded that such increased in protein content was because of mycorrhiza, as mycorrhiza is well known stress reducing symbiont[25]. Shinde and Khanna reported that proteins in mycorrhizal plants are significantly high than that of non mycorrhizal plants of potato[26].

Conclusion

So from the present research work, one can conclude that mycorrhizae are one of the best biofertilizers enhancing the biochemical parameters in chili. This enhancement must be contributing the overall increased growth of chili plant, ultimately resulting into enhancement in production of fruits of chili.

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References

1. Frank B. (1885). Uber die unf Wurzelsymbiose beruhende Ernahrung gewisser Baume, durch unterirdische, Pilze. *Ber. Duet. Bot. Ges*, 3, 128-145.
2. Harley J.L. and Smith S.E. (1983). Mycorrhizal Symbiosis. London: Academic Press, 53.
3. Verma N., Chaturvedi S. and Sharma A.K. (2008). Mass multiplication of AMF using soilless substrates. *Mycorrhiza News*, 20(1), 11-15.
4. Bethenfalvay G.J. (1992). Mycorrhiza and crop productivity. *mycorrhizae in sustainable Agriculture,* American Society of Agronomy special publication N. O. 54, Madison, Wisconsin, 1-27.
5. Gerdeman J.W. (1968). Vesicular-arbuscular mycorrhiza and plant growth. *Annual Review of Phytopathology,* 6, 397-418.
6. Bhuvaneswari G., Reetha S., Sivaranjani R. and Ramakrishnan K. (2014). Effect of AM fungi and Trichoderma species as stimulations of growth and morphological character of chili (*Capsicum annuum* .L). *International Journal of Current Microbiology and Applied Science*, 3(3), 447-455.
7. Selvakumar G. and Thamizhiniyan P. (2011).The Effect of the Arbuscular Mycorrhizal (AM) Fungus *Glomus intraradices* on the Growth and Yield of Chili (*Capsicum annuum L.*) Under Salinity Stress. *World Applied Sciences Journal,* 14(8), 1209-1214.
8. Gaur Atimanav, Adholeya Alok and Mukerji Krishna G. (1998). A comparison of AM fungi inoculants using *Capsicum* and *Polianthes* in marginal soil amended with organic matter. *Mycorrhiza*, 7(6), 307-312.
9. Vyas Mohnish and Vyas Anil (2014). Field Response of Capsicum Annuum Dually Inoculated with AM Fungi and PGPR in Western Rajasthan. *International Journal of Research Studies in Biosciences*, 2(3), 21-26.
10. Kavitha K., Meenakumari K.S. and Sivaprasad P. (2004). Arbuscular Mycorrhizal Fungi for Biocontrol of Damping-Off in Chili. *Journal of Mycology and Plant Pathology*, 34(2), 349-353.
11. Moore S. and Stein W.H. (1948). Methods in Enzymology. Eds. Colowick, S. P. and N. D. Kaplan Academic Press New York, 3:468
12. Johnson C.M. and Ulrich A. (1950). Determination of nitrate in plant material. *Analytical Chem,* 22(12), 1526-1529.
13. Kleeper L., Flesher D. and Hageman R.H. (1971). Generation of reduced nicotinamide adenine dinucleotide for nitrate reduction in green leaves. *Plant Physiol*, 48(5), 580-590.
14. Lowry O.H., Rosebrought S., Farr A.l. and Randall R.J. (1951). Protein measurement with Folin phenol reagent. *Journal of Biological Chemistry*, 193(1), 265-275.
15. Barman P., Swamy G.S.K., Patil P.B., Patil C.P. and Thammaiah N. (2008). Response of Rangpur lime to different mycorrhizal species for germination, growth parameters and graft success. *Mycorrhiza news,* 19(4), 13-16.

16. Singh A., Singh R. and Adholeya A. (2007). Development of green cover at solid waste dumping site of a soda lime industry by mycorrhizal technology. *Mycorrhiza News*, 18(4), 25-30.

17. Manjunath A., Bagyaraj D.J. and Gopala Gowda H.S. (1984). Dual inoculation with VA mycorrhiza and *Rhizobium* is beneficial to Leucaena. *Plant and soil*, 78(3), 445-448.

18. Shivaputra S.S., Patil C.P., Swamy G.S.K. and Patil P.B. (2004).Effect of vesicular arbuscular mycorrhiza fungi and vermicompost on drought tolerance in papaya. *Mycorrhiza News*, 16(3), 12-13.

19. Anilkumar K.K. and Muraleedhara Kurup G. (2003). Effect of Vesicular Arbuscular Mycorrhizal Fungi Inoculation on Growth and Nutrient Uptake of Leguminous Plants Under Stress Conditions. *Journal of Mycology and Plant Pathology*, 33(1), 33-36.

20. Miransari Mohammad (2011). Arbuscular mycorrhizal fungi and nitrogen uptake. *Archives of microbiology*, 193(2), 77-81.

21. Bücking Heike and Kafle Arjun (2015). Role of Arbuscular Mycorrhizal Fungi in the Nitrogen Uptake of Plants: Current Knowledge and Research Gaps. *Agronomy*, 5(4), 587-612.

22. Mathan Nisha C. and Sevanan Rajesh Kumar (2010). Influence of Arbuscular Mycorrhizal Fungi on Biochemical Changes in Wedilla chinensis (Osbeck) Merril. *Ancient Science of Life*, 29(3), 26-29.

23. Azcon R., Gomez M. and Tobar R. (1996). Physiological and nutritional response of Lactuca sativa L. nitrogen sources and mycorrhizal fungi under drought conditions. *Biology and fertility of soils*, 22(1- 2), 156-161.

24. Fattah-Abdel G.M. and Asrar A.A. (2012). Arbuscular mycorrhizal fungal application to improve growth and tolerance of wheat (*Triticum aestivum* L.) plants grown in saline soil. *Acta Physiol Plant*, 34, 267-277.

25. Shinde B.P. and Thakur Jaya (2015). Influence of Arbuscular mycorrhizal fungi on chlorophyll, proteins, proline and total carbohydrates content of the pea plant under water stress condition. *International Journal of Current Microbiology and Applied Science*, 4(1), 809-821.

26. Shinde B.P. and Khanna Manjusha (2014). Impact of AM fungi on biochemical changes in potato plants. *International Journal of Current Microbiology and Applied Science,* 3(7), 1018-1027.