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COMPARATIVE STUDY OF VESICULAR ARBUSCULAR MYCORRHIZA (VAM) AND BRASSINOSTEROID (BRs) ACTIVITY ON DIFFERENT METABOLITES IN *LUFFA CYLINDRICA* (L.) ROEM.

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ABSTRACT

Mycorrhizal associations especially VAM in plants have shown to increase plant growth, improve tolerance to drought and provide protection against root pathogens. Similarly Brassinosteriods (BRs) elicit diverse physiological responses and ameliorate various biotic and abiotic stresses. It is the known fact that VAM and BRs treated plants boosts not only the growing ability of plants but also perform an integral role in establishing their metabolism. This research focused on how the plants efficiency be affected by using VAM and BRs applications and it's further aim is to explore and elaborate their role by undertaking physiological and morphological aspects in this research. Both BRs and VAM are highly recommended for boosting plant growth and metabolism but the combined application of both these treatments showed extra ordinary boosting effects towards plant growth and metabolism during this research.

KEYWORDS: Vesicular arbuscular mycorrhiza, *Luffa cylindrica* (L.) Roem., Brassinosteroid activity.

INTRODUCTION

The VAM fungi are the best known for their ability to improve plant growth in low phosphate soils by exploiting large areas of soil and actively transporting the phosphate back to the plants. VAM increases the efficiency of plants in phosphate utilization (Gray and Gerdemann, 1969; Mosse *et al*., 1973). Other benefits to the plants due to VAM colonization include increased absorption of nitrogen, potassium, magnesium, copper, zinc, boron, sulphur, molybdenum and other elements that are made available to the plant (Rai *et al*., 2013). VAM also helps in retaining moisture around the root zone of plants.

VAM form a beneficial interaction with a wide range of plants, including horticultural and forage field crops. The fungi promote efficient nutrient absorption and increase plant growth and yield. These interactions with the soil play an important role in controlling soil fertility, soil erosion and plant water stress. VAM also act as facilitating partner to the root hairs in the process of nutrient translocation for the soil. Mali *et al*. (2009) reported that VAM enter root cortical parenchyma to xylem elements for facilitation of nutrient translocation from the soil and in to plants. Hence provide conditioning to soil and imparts greater drought tolerance in plants.

VAM fungus provides the alternative survival strategies to host plant for their growth under drought conditions or in mineral lacking soils (Auge *et al*., 2001). The importance of VAM fungi to sustainable agriculture and the ecosystem has led to its commercial development (Dar and Reshi, 2017). However, for the past thirty years, progress on VAM cultivation has not been very successful, probably because the research is difficult and very time consuming. This Fertilizer is suitable for all kinds of crops.

Brassinosteriods (BRs) are required for normal plant growth, reproduction and metabolism. Brassinosteriods and Reactive oxygen species (ROS) act as vital secondary messengers for the induction and regulation of antioxidant systems in plants under stress (Mazzora *et al*., 2002). BRs treatment shows positive approach towards agriculture as it performs certain functions which have beneficiary effects like it act as protective agent against different diseases on the other hand it stimulates certain macromolecules which regulate the plant growth and metabolism under different environmental conditions such as salinity, temperature and water stress.

BRs have been reported to protect plant against toxic effects of pesticides (Krishna 2003). BRs prove to be beneficial in nutrient deficiency (Khripach *et al*., 2000; Anuradha and Rao, 2002). BRs generally play a multitude of protective and stimulatory roles in improving plant quality and quantity (Khripach *et al*., 2000).

This study is designed to investigate the most promising and cheapest biocontrol and biofertililizer to enhance the growth of *Luffa cylindrica* (family Cucurbitaceae). This species is reported to associate with VAM species such as *Glomus leptotichum*, *G.claroideum* and *Acaulospora laevis* with around 2125 VAM spore/100g soil under field conditions and root colonization of up to 16.57% (Rauf *et al.*, 2016). The present research is focused on the efficiency of VAM and BRs applications for physiological and morphological improvement in Luffa plants.

MATERIALS AND METHODS

Viable and healthy seeds of *Luffa cylindrica* plant were selected for the study. The seeds were surface sterilized with mercuric chloride. Six months old and fresh seeds of *Luffa cylindrica* seeds with or without Topsin-M dressing were sown in earthen pots containing 5 kg autoclaved sandy clay loam soil. Five seeds were sown in each of the three replicate pots. The pots were watered regularly to maintain the moisture regime. VAM spores were obtained from the collected soil samples by wet sieving and decanting method of Gerdemann and Nicolson (1963) modified by Vilarino and Arines (1990). The extracted VAM spores were identified after reference to Schenck and Perez (1990). The constituents of VAM species were *Glomus fasiculatum, Glomus callosum* and *Acaulospora rugosa.*. We have used prepared solution of Brassinosteroid which is 100% pure so we have prepared 50% from that solution.

Four sets of treatments were maintained in pots, 1st set of pots maintained as a control, 2nd as VAM (164ml in each pot as 1mL contains 10 VAM spores so 164 mL contains 1,640 VAM spores per plant), 3rd as BRs (Twenty five mL of this BRs solution was provided in each pot) and 4th set of pots was treated with combined application of VAM + BRs. After germination, the treatments of VAM, BRs and combined application of both treatments were given to *Luffa* plants once only. All treatments were replicated three times. The research was conducted to study morphological and biochemical aspects of *Luffa cylindrica* plant on weekly basis up to 4 weeks from November 20 – December 20, 2013. In morphological attributes overall fresh and dry weight of plant was determined. Biochemical analysis was performed by determined some of the biochemical aspects of plants such as chlorophyll and carotenoids were determined by the method of Lichtenthaler (1987), Carbohydrates by Yemm and Willis (1956), reducing sugar (Miller, 1959) and protein was determined by the method of Bradford (1976).

RESULTS AND DISCUSSION

The data presented are the means and standard errors (S.E.) of three independent replicates. To ensure that the assumptions for statistical analysis were fulfilled, the equality of variances and the normality of the data were tested.

Effect on fresh and dry weight of plants: The data for fresh and dry weight of *Luffa cylindrica* plant showed significant effect at ($p<0.05$) during the course of experiment. Significant increase was observed in fresh and dry weight of plants treated with BRs alone and combined VAM+BRs treated plants as compared to all other treatments as shown in Tables 1 and 2. The lowest fresh and dry weights were observed in control. Mycorrhizal inoculation is known to significantly increase leaf area, shoot, root, fresh and dry weight of Basil (*Ocimum basilicum*), a member of the Lamiaceae family (Sharifi *et al*., 2011).

The VAM fungi have the capability to enhance growth and overall biomass of the plants (Jinying *et al*., 2003). Plant showed normal function for growth and development in the presence of BRs. (Haubrick and Assmann, 2006). Plants showed better performance against environmental stresses like temperature, salinity and drought in the prence of BRs (Bajguz and Hayat 2009).

Effect on protein content: The data for protein content of *Luffa cylindrica* plant showed significant effect at ($p<0.05$) during the course of experiment. An enhanced increase was observed in VAM + BRs treated plants as compared to control and significant increase as compared with other treatments as shown in (Table 3). The application of BRs has been found to enhance protein content in normal plants as well as those subjected to different kinds of stresses (Bajguz, 2000). Vardhini and Rao (1998) reported that BRs substantially increase the growth of the plant which was associated with enhanced levels of DNA, RNA, soluble proteins and carbohydrate. Protein role in Mycorrhizal plants reflects the growth and development under dual symbiosis and showed influenced of fungi in mature plants (Singh 2012; Sgherri *et al*., 1998). The results of (Martins *et al*., 1997) in chest nut plants and (Mathur and Vagas, 1995) in *P. cineria* also showed that protein contents were higher in mycorrhiza-associated plants.

Table 1. Fresh weight (g) of *Luffa cylindrica* plants with different treatments.

Weeks	Control	VAM	BRs	VAM+BRs
1^{st}	1.94 ± 0.01	2.31 ± 0.01	2.97 ± 0.01	3.24 ± 0.01
2^{nd}	2.3 ± 0.01	3.28 ± 0.01	4.28 ± 0.01	4.86 ± 0.01
3^{rd}	2.42 ± 0.02	3.32 ± 0.01	4.66 ± 0.01	4.92 ± 0.01
4^{th}	2.61 ± 0.01	3.66 ± 0.02	4.89 ± 0.01	4.96 ± 0.02

Table 2. Dry weight (g) of *Luffa cylindrica* plants with different treatments.

Weeks	Control	VAM	BRs	VAM+BRs
1^{st}	1.35 ± 0.01	1.53 ± 0.02	2.34 ± 0.01	2.65 ± 0.01
2^{nd}	1.86 ± 0.02	2.56 ± 0.01	2.76 ± 0.01	2.81 ± 0.02
3^{rd}	1.91 ± 0.02	2.62 ± 0.01	2.98 ± 0	2.94 ± 0.01
4^{th}	1.98 ± 0	2.77 ± 0.01	2.91 ± 0.02	2.98 ± 0

Table 3. Protein content (µg/mL) of *Luffa cylindrica* plants with different treatments.

Weeks	Control	VAM	BRs	VAM+BRs
1st	115 ± 0.70	188.33 ± 0.81	191 ± 0.70	204 ± 0.70
2nd	131.66 ± 0.81	281± 0.70	296 ± 0.70	316 ± 0.70
3rd	152 ± 0.70	311 ± 0.70	321 ± 0.70	361 ± 0.70
4th	187 ± 0.70	343.66 ± 0.81	361 ± 0.70	391 ± 0.70

Table 4. Carbohydrate content (µg/mL) of *Luffa cylindrica* plants with different treatments.

Weeks	Control	VAM	BRs	VAM+BRs
1st	172 ± 0.70	162 ± 0.70	182 ± 0.70	200 ± 0.70
2nd	241 ± 0.70	220.33 ± 0.40	264 ± 0.70	271 ± 0.70
3rd	260.33 ± 0.40	257 ± 0.70	283 ± 0.40	290.66 ± 0.80
4th	301 ± 0.70	291 ± 0.70	321 ± 0.70	341 ± 0.70

Effect on carbohydrate content: The data for protein content of *Luffa cylindrica* plant showed significant effect at ($p<0.05$) during the course of experiment. Control plants significantly showed higher carbohydrate content than VAM alone treated plants and lower than BRs alone and combined VAM+BRs treated plants as shown in (Table 4). Soluble sugars and soluble starch contents inhibited to some extent in VAM treated plants. According to (Allen, 1991) decrease in soluble sugars and soluble starch in the leaves may be due to the fact that VAM fungi utilize 10-20% of net photosynthesis in exchange for the transfer of nutrients to the host to lead a symbiotic life. The drain of Carbon compounds to the fungus stops the accumulation of Carbohydrate in the leaves which in turn enhances the Carbon assimilation through a higher rate of photosynthesis (Martins *et al.*, 1997) that fungi gives support to the plants by their association. Increased carbohydrate was observed under the influence of BRs in tomato pericarp by Vardhini and Rao (2002). Wu *et al.* (2008) also observed increased carbohydrate content due to BRs in rice.

Effect on reducing sugar: The data for protein content of *Luffa cylindrica* plant showed significant effect at ($p<0.05$) during the course of experiment. Results showed that VAM+BRs treated plants showed enhanced increase in reducing content of *Luffa cylindrica* plant as compared with control and VAM alone and an average increase as compared with BRs alone treated plants as shown in (Table 5). Influence of BR was found to be significant on the level of total soluble CHO and proteins in both the cultivars (Verma *et al.*, 2011). Zullo and Adam (2002) reported that BRs can also occur in conjugated forms especially with sugars or fatty acids. Filter (1991) reported that decrease in soluble sugars and soluble starch may be due to translocation of CHO produced by host to the fungal partner. Glucose as a likely substrate for VAM fungi in the symbiotic state, but VAM plants accumulated less glucose and fructose in leaves and roots than control plants (Gouk *et al.*, 1999).

Table 5. Reducing sugar content (μg/mL) of *Luffa cylindrica* plants with different treatments.

Weeks	Control	VAM	BRs	VAM+BRs
1st	32 ± 0.70	30.66 ± 0.81	41 ± 0.70	51 ± 0.70
2nd	60 ± 0.70	52.33 ± 0.40	79 ± 0.70	95 ± 0.70
3rd	82 ± 0.70	78.33 ± 0.81	97 ± 0.70	102 ± 0.70
4th	110 ± 0.70	91.33 ± 0.81	121 ± 0.70	123.66 ± 0.40

Table 6. Total photosynthetic pigment content (μg/mL) of *Luffa cylindrica* plants with different treatments.

Weeks	Control	VAM	BRs	VAM+BRs
1st	23.56 ± 0.02	25.60 ± 0.03	29.42 ± 0.03	32.24 ± 0.07
2nd	27.34 ± 0.03	28.67 ± 0.05	31.96 ± 0.05	36.62 ± 0.05
3rd	32.57 ± 0.07	34.17 ± 0.07	38.83 ± 0.08	42.26 ± 0.08
4th	38.85 ± 0.08	40.12 ± 0.08	43.12 ± 0.08	49.81 ± 0.07

Effect on total photosynthetic pigments: The data for protein content of *Luffa cylindrica* plant showed significant effect at ($p<0.05$) during the course of experiment. Control plants showed lower photosynthetic pigments over all other treatments. The highest photosynthetic pigments were observed in VAM+BRs treated plants as shown in (Table 6). The type of applied BR is main determinant in the manifestation of BRs effect on the content of photosynthetic pigments and plant spp., is probably the second deciding factor (Verma *et al*., 2012). In the literature application of BRs has been shown to increase total chlorophyll content and hence net photosynthetic rate in *Brassica juncea* (Alam *et al*., 2007) is also increased. These results are in agreement with previously reported work where mycorrhizal plant exhibit enhanced pigment contents (Manoharan *et al*., 2008). Gemma *et al*. (1997) reported that the chlorophyll content in VAM-associated plants is higher than un-inoculated plants.

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