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SYNERGISTIC EFFECT OF BIO-INOCULANTS ON GROWTH OF *GUIZOTIA ABYSSINICA (L.F) CASS VAR*, RCR-18, A MINOR OIL YIELDING PLANT

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Keywords:

Arbuscular mycorrhizal (AM) fungi, *Scutellospora nigra*, Mycorrhiza Helper Bacteria (MHB), *Azotobacter chroococcum, Aspergillus luchensis, Guizotia abyssinica (L.f) Cass*, Biovolume index

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ABSTRACT

Pot Experiments were undertaken on *Guizotia abyssinica (L.f) Cass var*, RCR-18 to examine the combined effect of bioinoculants i.e. AM fungus *Scutellospora nigra*, *Aspergillus luchensis* and Mycorrhiza helper bacteria (MHB), *Bacillus coagulans* and *Azotobacter chroococcum*. The results revealed that plant height, root length, biomass yield, number of seeds, per cent root colonization, spore number and N, P uptake in shoots and root. Significantly increased plant growth, biomass production, was recorded after 90 days in *Guizotia abyssinica (L.f) Cass*, which received *Scutellospora nigra* combined inoculations with *Bacillus coagulans* and *Azotobacter chroococcum*, *Aspergillus luchensis* over the control or uninoculated plants. The results revealed that AM fungus dual and triple inoculation of *S. nigra* with microbial inoculants like MHB (*B.coagulans*), biocontrol agent (*A.luchensis)* and N fixer (*A. chroococcum*) enhanced, the growth and nutrition. When the plant inoculated with all the bioinoculants viz;*Scutellospora nigra* + *Bacillus coagulans* + *A. chroococcum* + *A.luchensis* gave the best growth and nutrition of *Guizotia abyssinica (L.f) Cass var*, RCR-18 maximum enhancement in growth, nutrition, biovolume index and quality index occurred .Therefore, this work suggests that application of microbial consortia MHB and mycorrhiza is positive role of inoculation more suitable to get yield in *Guizotia abyssinica (L.f) Cass var*, RCR-18.

INTRODUCTION

The importance of Arbuscular mycorrhizal (AM) fungi a unique group of soil fungi forming symbiotic association with higher plants, facilitate uptake of diffusion limited plant nutrients such as P, Z, and Cu etc., (Tinker, 1984;Bagyaraj and Reddy, 2005). Interest in these associations is mainly because of the manifold benefits conferred on the host by the fungus. Further these fungi show a preferential colonization to the hosts, and thereby, the extent to which, a host benefited depends on the fungal species involved in the symbiosis (Abott and Robson, 1982,; Lakshman ,2009)Mycorrhizal fungi interact with a wide range of other soil organisms in the root or in the rhizosphere of the soil. Some form a symbiotic, association and in turn modify the host of physiology (Fitter and Garbaye, 1994). Interaction between AM fungi, fixing bacteria and Mycorrhiza helper bacteria (MHB) has been proved to form a consortium benefitting growth of a few plant species (Jayanthi Srinath *et al,* 2003). N2-fixing and P-solubilizing bacteria may be important for plant nutrition by increasing N and P uptake by the plants, and playing a significant role as plant growth promoting rhizobacteria (PGPR) in the biofertilization of crops (Zaidi .,2006) Therefore ,microbial inoculants can help maintain good soil health and fertility that contribute to a greater extent to a sustainable yield and quality of products . The synergistic effects of PGPR and mycorrhizal fungi have gained more importance in the last two decades for its beneficial effects to many crops, in terms of biocontrol efficiency, improvement of nutrient absorption, and phytoremediation. Among co-inoculation plant growth promoting rhizobacteria and Arbuscular mycorrhizal fungi combination is considered to be more beneficial for plant growth, and nutrient acquisition (Barea *et al.,* 2002), Information related to the complicated mechanism of interaction between PGPR and AMF in the rhizosphere is scarce , one of such mechanisms evolved by Carpenter *et al*. (1995). These fungi show a preferential colonization to the hosts, and thereby, the extent to which a host is benefited depends on the fungal species involved in the symbiosis (Smith and Read, 1997).For these reasons, the interactions between AMF and PGPR might be useful for the development of re-vegetation in soils having water and nutrients limitations (Marulanda *et al.,* 2009). The role of AM fungi and PGPR's in improving crop plants growth is well documented (Bhowmik and Singh, 2004). The beneficial effect of these AMF and PGPR under drought conditions in sterile soil has recently been reported (Marulanda-Aguirre *et al.*, 2008)

It has been observed that *Guizotia abyssinica (L.f.) Cass* is effective in the treatment of various disease and disorders. Moreover, it possesses some potent phytochemical which will

be useful as anti-microbial, anti-fungal and anti-inflammatory activities. The seed oil it is eaten by the tribal people as vegetable oil. The species is under cultivation but fast disappearing and vulnerable in Madhya Pradesh and require urgent ex-situ conservation before it become extinct. Therefore, it requires detailed documentation and standardization for the formulation of valuable drugs of therapeutic importance. (Dwivedi**,** 2014)The oil content of *Niger* is reported to be in the range of 30-50% of the seed weight and the oil has four major fatty acids which include two main unsaturated fatty acids viz., linoleic acid (18:2) and oleic acid (18:1); and two major saturated fatty acids viz., palmitic acid (16:0) and stearic acid (18:0) (Geleta *et al,* 2011). In India, it provides only about 3% of the edible oil requirement of the country and addition to its use as an edible crop; its oil is used for industrial and pharmaceutical purposes also (Ramadan., 2003).

Knowledge regarding AMF and PGPR interactions and with the host plants is essential for sustainable agriculture. This make farming relied on biological processes and resources, rather than the use of chemicals for maintaining soil fertility and plant growth (Artursson *et al.,* 2006).However, the information available on the use of these beneficial microorganisms in oil yielding plants is meager. Hence the present investigation was aimed to study the effect of AM fungus *Scutellospora nigra*, and PGPR's, *Bacillus coagulans*, *Azotobacter chroococcum* and *T. harzianum,* singly and in combination on the growth, biomass, nutrient uptake of *Guizotia abyssinica (L.f) Cass* raised under green house condition.

MATERIAL AND METHODS

This study was carried out under green house conditions. The seedlings of *Guizotia abyssinica (L.f) Cass var,* RCR-18 were raised for 90 days using the sterilized garden soil and pure sand in (1:1) ratio and the earthen pots measured 25X 30 cm (BxL) .The potting mix used for filling earthen pots was a mixture of unsterilized sand: soil: FYM @ 1:0.25 by volume. AM fungi, *Scutellospora nigra* used in the study was maintained in the polyhouse Department of Botany, Karnatak University with using sand-soil (1:1 v/v) as the substrate and *Rhodes* grass as the host. The AM fungal inoculums was added to the planting hole at the rate of 125-135 infective propagules per pot, based on most probable number estimation (Porter, 1979) *Bacillus coagulans*, which is not only a PGPR but also mycorrhizal helper bacterium (MHB), was grown in Nutrient broth, *Aspergillus luchensis* in Potato dextrose broth and *Azotobacter chroococcum* in Bijernick medium containing 800 ml medium. After 3 days growth of *B. coagulans,* 5 days growth of *A.luchensis* and 7 days growth of *A.*

chroococcum, the cultures were used for inoculation along with *S .nigra* of transplanting and the plants were maintained in a glass house for 180 days.

Plant growth parameters viz., plant height and stem girth were recorded on 30, 60, 90 days after transplanting (DAT). However, only observations recorded on day 90 are presented in this paper. Plants were harvested 90DAT. Shoot and root biomass was determined after drying the plant samples to a constant weight at 70°C in a hot air oven. The phosphorus content of the plant was determined by employing vanado/molybdate phosphoric acid yellow colour method (Jackson, 1973). The nitrogen content was estimated following Microkjeldahl method (Jackson, 1973). Soil Samples (50 g) were collected from each earthen pot and subjected to wet sieving and decanting as outline by (Gerdemann and Nicolson 1963) to estimate the population of AM Fungal spores. Fine terminal feeder roots were stained using 0.05% tryptophan blue as described by (Philips and Haymann,1970) and the per cent root colonization was estimated by adopting the gridline intersect method (Giovannetti and Mosesse, 1980). Quality Index and Biovolume Index of the seedlings were calculated using formulae suggested by Hatchell, (1985).

RESULTS AND DISCUSSION

In general, mycorrhizal inoculation resulted in a significant increase in plant height, stem girth, plant phosphorus and plant nitrogen content of *Guizotia abyssinica (L.f) Cass var,* RCR-18 inoculation with *Scutellospora nigra* along significantly increased plant height compared to uninoculated control. Plant height was further increased significantly when co-inoculated with *Scutellospora nigra + Bacillus coagulans + A. chroococcum* differing significantly from all other treatments except *Scutellospora nigra + A.luchensis + A. chroococcum* and *Scutellospora nigra +B.coagulans + A. Chroococcum* (Table-1). Similar, observations have been made earlier in cowpea (Caroline and Bagyaraj, 1995). There was significant increase in stem grith plants inoculated with *Scutellospora nigra + Bacillus coagulans + A. chroococcum + A.luchensis* when compared, to all other treatments except *Scutellospora nigra + Bacillus coagulans + A. chroococcum*. The treatments *Scutellospora nigra* alone, *A.luchensis* and *Scutellospora nigra +A.chroocaccum* were on par with uninoculated plants .Inoculation of AM fungi with PGPRs enhancing stem girth has been observed by earlier workers (Jayanthi Srinath *et al*., 2003; Earanna and Bagyaraj, 2004).The shoot, root and total plant (shoot+ root) biomass varied, significantly with the different treatments, of the single inoculation treatments, treatment with *A.luchensis* and *A. chrooccum* significantly enhanced plant biomass compared to uninoculated plants. Compared to single

inoculation with *Scutellospora nigra* dual inoculation *Scutellospora nigra* + *Bacillus coagulans* significantly enhanced the plant biomass; addition *A.luchensis* to it further enchanced plant biomass significantly (Figure 1-2) . Plants responding better to microbial consortia rather than inoculation with one or two organisms have been reported earlier (Sumana and Bagyaraj, 1998; Lakshman, 2012). Those similarly inoculated with *G. aggregatum* +*B. coagulans* + *T. harzianum* showed maximum shoot and root dry weight. This may be due to synergistic interaction of the AM fungi and PGPR's in the rhizosphere of the plants (Lakshmipathy et al., 2002; Muthuraju et al., 2002; Sivakumar et al., 2002).

P uptake was significantly more in all the inoculated plants compared to uninoculated plants (Table -2). The plants inoculated with *S. nigra* + *B. coagulans* + *A.luchensis*.+ *A. chroococcum* showed highest P content and different significantly when compared to other treatments. Enhanced P uptake by mycorrhizal plant is well documented. Various mechanisms have been suggested for increased P uptake by mycorrhizal plant viz., a) the external hyphae of AM exploring greater volume of soil for phosphorus away from the root. b) Small radical hyphae related to roots enabling them to exploit smaller spores and adding surface area to the absorption system and c) effective phosphorus acquisition by external hyphae by production of extracellular acid phosphatases which catalyze the release of phosphorus from organic complexes in the soil (Marschner and Dell., 1994). MHB enhancing the P uptake by mycorrhizal fungi has been reported earlier (Syvertsen and Graham, 1999). Several species of *Bacillus* spp, are known to solublize forms of P and convert them to available from thus enabling better P uptake by AM fungi (Srihari and Sreenivasa, 1992; Singh and Kapoor, 1999). The phosphorus, potassium, zinc, copper, manganese and iron content were maximum in the plants treated with *G. aggregatum* +*B. coagulans*+*T. harzianum* probably due to the enhanced mycorrhizal colonization resulting in efficient uptake (Lakshmipathy *et al.,* 2002; Selvaraj *et al.,* 2008).It was noticed that plants inoculated with *S. nigra* + *B. coagulans* + *A.luchensis*.+ *A. chroococcum* had the higher N content which differed significantly highest content was observed(Figure -2)in plants treated with *S. nigra* + *A.luchensis*.+ *A. chroococcum* followed by those inoculated with *S. nigra* + + *B. coagulans* + *A.luchensis* both differing significantly. The uninoculated plants recorded the lowest N content, which differed significantly from other treatments. A similar result was reported by Barea *et al.,* (1983) in corn and rye grass and Azcon and Barea (1975) in tomato. As phosphorus essential for the process of N fixation (Barea *et. al,* 1992), inoculation of AM fungus and the 3 PGP must have helped the plants with N nutrition. Plants inoculated with *S.*

nigra + B. coagulans + A.luchensis + A. Chroococcum were found to have maximum root colonization differing significantly from all other treatments. Spore numbers in the root zone soil followed a similar trend and the lowest was found in uninoculated plants. *B. coagulans* is known to secrete to enzymes which help in the easy penetration of the intercellular spaces by the AM fungal hyphae by dilating the host cell wall in the cortex region. Perhaps this is the reason for higher percent colonization, in the *S. nigra + B. coagulans* treatment compared to *S. nigra* alone treatment. In the present study, there was positive correlation between mycorrhizal root colonization and plant growth. This finding supports the observations made by earlier workers in other plants (Raj *et al.,* 1981, Sreenivasa *et al.,* 1993). Biovolume index and quality and quality index was observed to be highest in *S. nigra* among single inoculation followed by plants inoculated with *A. chroococcum* alone, *B.coagulans* alone and *A.luchensis* alone (Table-2) .Treatment with dual and triple inoculation further increased the index and maximum biovolume and quality index values were observed (Figure 3-4) in plants treated with *S. nigra + B. coagulans + A.luchensis + A. Chroococcum*. Maximum plant biomass was obtained in the treatment *Scutellospora nigra + Bacillus coagulans + A. chroococcum* which were significantly different from all other treatments. Improved response in plant growth with AM and PGPR's obtained in the present investigation supports Earanna *et al.* (2002) for periwinkle Selvaraj *et al.,* (2008) for *Begonia melabonica* and of Sivakumar *et al*. (2002) for *Pelargonium graveloleus* inoculated with *Glomus spp.* and some PGPR's Single inoculation with *G. aggregatum* or dual inoculation with *G. aggregatum + B. coagulans* also significantly enhanced the total dry weight, biomass of *S. viarum* plants. Inoculation with mycorrhizal fungus improving the quality and biovolume indices of plants, which is further enhanced when co-inoculated with PGPRs, has been observed earlier in other plants (Rajan *et al*., 2000; Sumana and Bagyaraj, 2002). Overall the response of *Guizotia abyssinica (L.f) Cass var,* RCR-18 with the different microbial inoculants was found to be good, further application of this consortium for other oil yielding plants could be a significant research.

CONCLUSION

The present investigation clearly brings out that inoculation of the potting mixture with *S. nigra* improves P and N nutrition of *Guizotia abyssinica (L.f) Cass var,* RCR-18 raised in the nursery, Dual and triple inoculation of *S. nigra* with microbial inoculants like MHB (*B.coagulans*), biocontrol agent (*A.luchensis)* and N fixer (*A. chroococcum*) further enhanced, the growth and nutrition. When inoculated all together gave the best growth and nutrition of *Guizotia abyssinica (L.f) Cass var,* RCR-18 maximum enhancement in growth,

nutrition, biovolume index and quality index occurred when all the four microorganisms were added together suggested that it is the best 'microbial consortia' for inoculating *Guizotia abyssinica (L.f) Cass var,* RCR-18 in the nursery.

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Fig-1 Effect of AM fungus and PGPR on plant root length, biomass yield stem diameter and seed number of *Guizotia abyssinica (L.f) Cass* at 90 days.

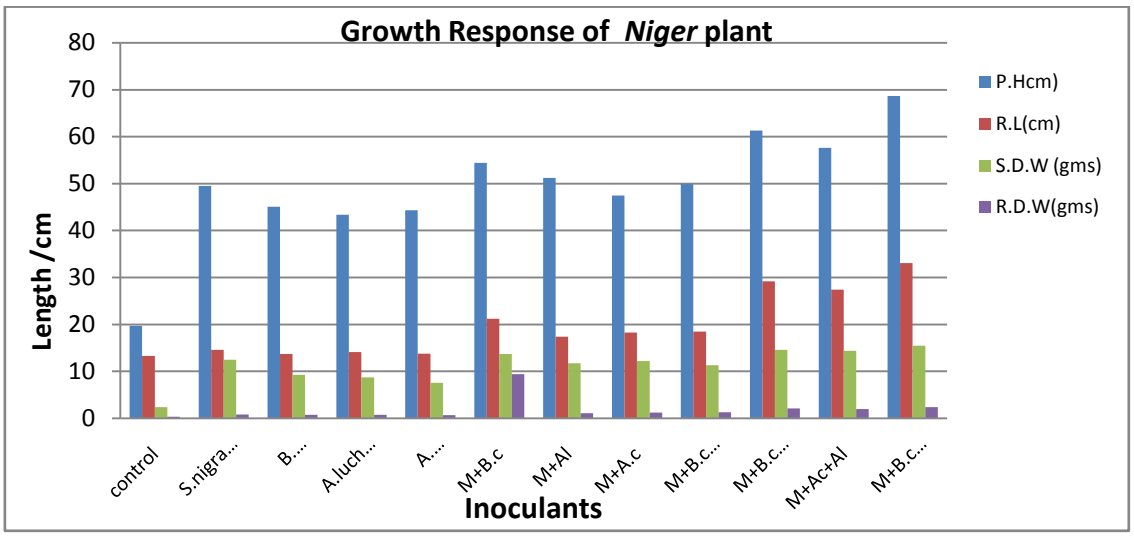


Fig-2 Effect of AM fungus and PGPR on on P and N content in Shoot(s) and Root(R) of *Guizotia abyssinica (L.f) Cass* at 90 days

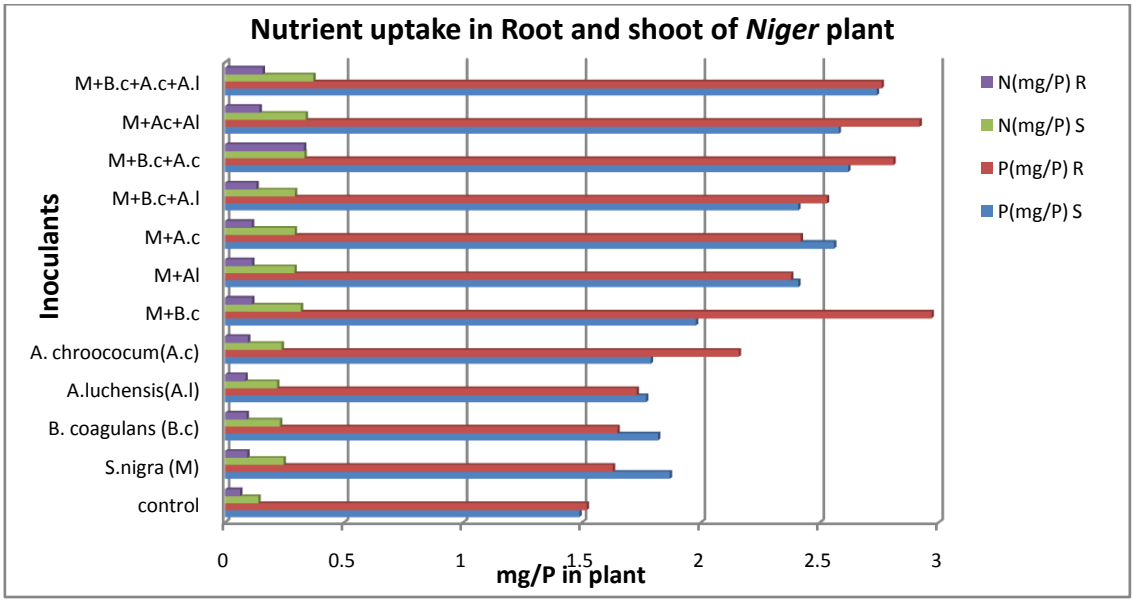


Table-1 Effect of AM fungus and PGPR on root length, biomass yield stem diameter and seed number of *Guizotia abyssinica (L.f) Cass* at 90 days

Sl No	Inoculants	Plant Height(cm)	Root Length(cm)	Shoot Dry weight (gms)	Root Dry weight (gms)	Stem Grith(mm)	Number of Seeds/Plant
1	control	19.7	13.3	2.4	0.33	1.2	0
2	*Scutellospora nigra (M)*	49.5	14.6	12.5	0.83	1.7	453
3	*Bacillus coagulans (B.c)*	45.1	13.7	9.3	0.76	1.5	445
4	*Aspergillus luchensis(A.l)*	43.4	14.1	8.7	0.74	1.4	441
5	*Azotobacter chroococum(A.c)*	44.3	13.8	7.6	0.68	1.4	446
6	M+B.c	54.4	21.2	13.7	9.4	2.7	519
7	M+Al	51.2	17.4	11.7	1.1	2.4	517
8	M+A.c	47.5	18.3	12.2	1.2	2.3	419
9	M+B.c+A.l	50.02	18.5	11.3	1.3	2.8	507
10	M+B.c+A.c	61.3	29.2	14.6	2.1	3.4	978
11	M+Ac+Al	57.6	27.4	14.4	2	3.1	943
12	M+B.c+A.c+A.l	68.7	33.1	15.5	2.4	4.2	1096

Table -2 Effects of AM Fungus and PGPR on P and N content in shoots and root of *Guizotia abyssinica (L.f) Cass* at 90 days

Sl No	Inoculants	P content(mg/plant)		N content(mg/plant)		Biovolume Index	Quality Index
		Shoot	Root	Shoot	Root		
1	control	1.49	1.52	0.14	0.063	602	0.67
2	*Scutellospora nigra (M)*	1.87	1.63	0.246	0.094	783	0.791
3	*Bacillus coagulans (B.c)*	1.82	1.65	0.231	0.091	912	0.813
4	*Aspergillus luchensis(A.l)*	1.77	1.73	0.219	0.086	837	0.818
5	*Azotobacter chroococum(A.c)*	1.79	2.16	0.238	0.097	944	0.813
6	M+B.c	1.98	2.97	0.319	0.114	1121	0.843
7	M+Al	2.41	2.38	0.292	0.114	1098	0.826
8	M+A.c	2.56	2.42	0.293	0.113	1019	0.841
9	M+B.c+A.l	2.41	2.53	0.294	0.132	1228	0.799
10	M+B.c+A.c	2.62	2.81	0.334	0.332	1247.2	0.864
11	M+Ac+Al	2.58	2.92	0.339	0.145	1449.5	0.872
12	M+B.c+A.c+A.l	2.74	2.76	0.371	0.158	1573.3	0.889

Fig-3 Effect of AM fungus and PGPR on Biovolume Index of *Guizotia abyssinica (L.f) Cass* after 90 days.

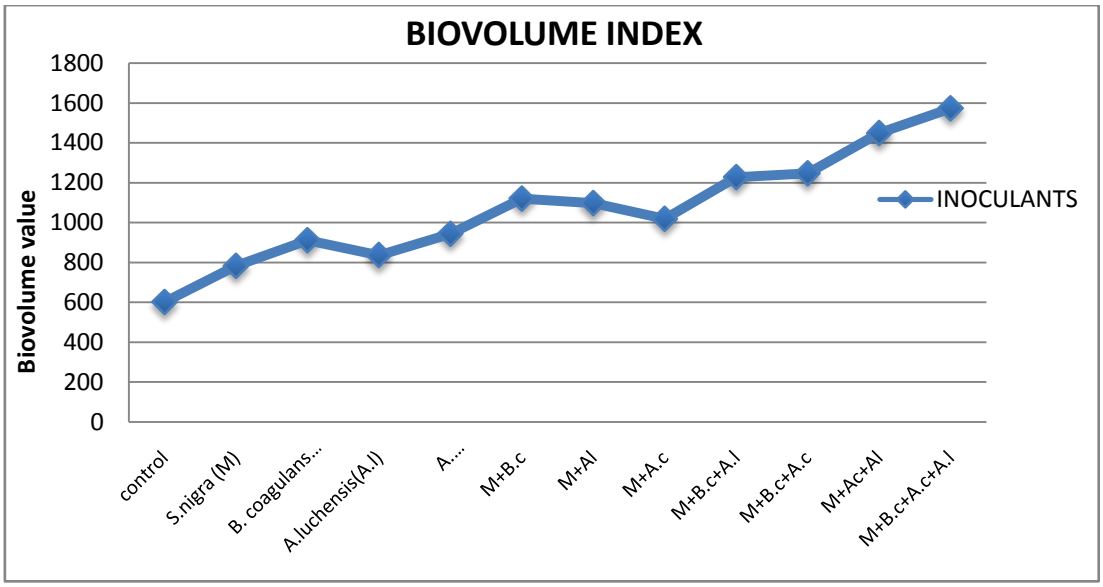
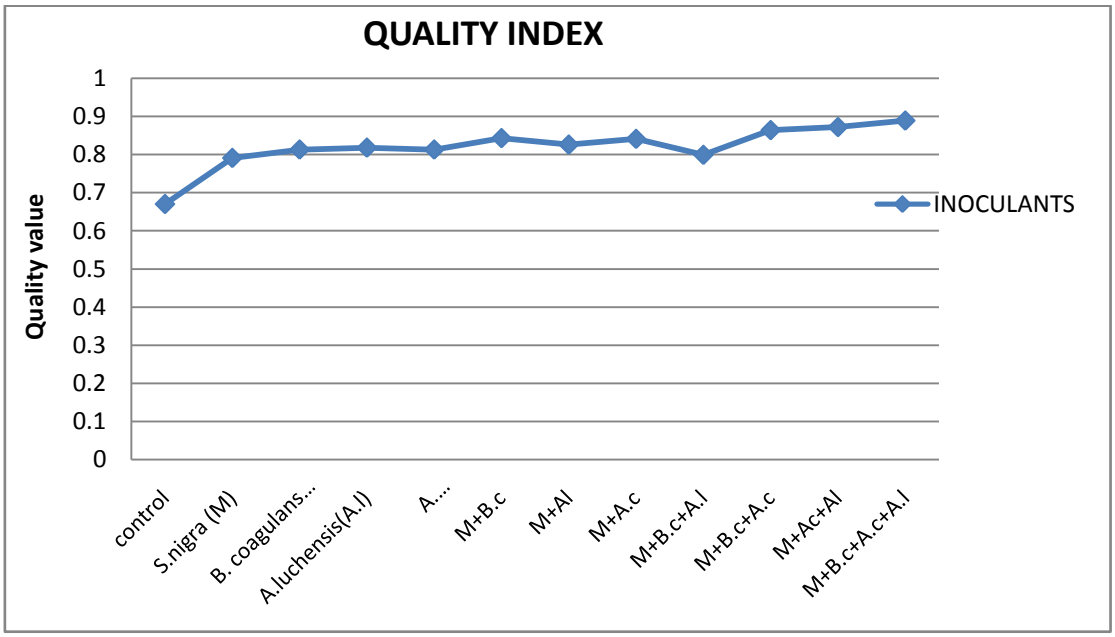


Fig-4 Effect of AM fungus and PGPR on Quality Index of *Guizotia abyssinica (L.f) Cass* after 90 days.



MHB- Mycorrhiza helper bacteria

PGPR-Plant Growth Promoting Rhizobacteria

AM- Arbuscular mycorrhizal

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