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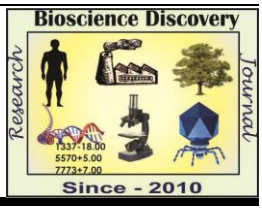

Positive effect of Am fungi and PGPR on growth Biomass yield of (sunflower) *Helianthus annus* LSH_1 var.

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Abstract

Microbial inoculants on plant growth and biomass improvement have been well documented in recent days on majority of crop and trees. In the present work the positive effect of AM Fungi such as; Rhizophagus fasciculatus, *Glomus macrocarpum*, Glomus bayagyarajii and Sclerocystis dussii with combinatiohn of PGPR *Bacillus polymyxa* have studied on *Helianthus annus L.* (sunflower) LSH_1.Among the AM fungi Solerocystis dussii combined with *Bacillus polymyxa* had significancantly influenced on shoot length, root length, stemwidth, dry weights of shoots and roots, per cent root colonization, spore number, Chlorophyll content on leaves, number of seeds per plant, lipid content in seeds and proline accumulation in then roots compared non-inoculated (Control) plants.The second most effective AM Fungus is *Rhizophagus fasciculatus* with *B. Polymyxa*. The third most effective influenced AMF is *Glomus macrocarpus* with *B. Polymyxa* and the least influenmced AMF is *Glomus bagyarajii* with *B. Polymyxa*. However, there was favourable increase in all growth parameters over the non-inoculated (Control) plants. Therefore, it can concluded that there is synergistic interaction between two microbial inocualation lead to positively improved on Heliantus annus L.

INTRODUCTION

Modern agriculture which is characterized by intensive cultivation methods, is totally dependent on regular input of numerous types of inorganic fertilizers. The long term effects of such massive fertilization on the environment are now a matter of serious concern. Current trends in agriculture are focused on the reduction of the use of pesticides and inorganic fertilizers, forcing the search for alternative ways to improve a more sustainable agriculture. Therefore, there is need of development of eco-friendly technologies to escape from the adverse effects caused by the synthetic or chemical fertilizers used in the modern agricultural practices. One possible approach is to explore soil microbial diversity for beneficial microbes having combination of plant growth promoting activities and well adapted to particular soil environment.These microorganisms play an important role in effecting the availability of soil phosphorous to plant roots, and increasing phosphate (P) mobilizatuion in soil. The ability of soil microorganisms to convert insoluble forms of phosphorus to a soluble form is an important trait in plant growth promoting bacterial (PGPB) for increasing plant yields (Lakshman, 2010; Hosmani *et al.*,2011; Meng *et al.*, 2015).

Today to overcome the problems of modern agricultural practices, soil microbiologists are searching for the microorganisms associated with root zone. The root zone represented with highest microbial activity is referred as Rhizosphere.

Among the Rhizosphere microbial population, rhizobacteria and beneficial AM Fungi were found to be more important for the sustainable agricultural practices. (Johnson *et al.,* 2004; Smith and Read, 2007 Smith and Liu *et al.,* 2012a). As, these group of microbes are actively involved in the plant growth promotion activities which can improve agricultural developments. Thus these micro-organisms appear as a research target with regard to sustainability purposes (Johanson *et al.,* 2004).

Mycorrhizal plants, can take up more phosphorous than non-mycorhizzal plants, mainly from the same soluble pool. Inoculation with phosphate-solubilizing bacteria (PSB) may help to solubilize native soil P as well as P from rock phosphates. The soluble P released by the activity of phosphate solubilizing microbes (PSM) is actively taken up by mycorrhizal roots (Barea *et al.,* 1983, Che *et al.,* 2006). Plant-associated bacteria that are able to utilize/solubilize the phosphorus from the soil to make it available for the plant are refereed as Phosphate solubilizing bacteria (PSB). These are also called as rhizobacteria that stimulate plant growth are usually referred to as plant growth promoting rhizzobacteria PGPR.(Glick, 1995). Phosphorus solubilizing bacteria play role in P nutrition by enhancing its availability to plants released from inorganic and organic soil P pools by solubilization and mineralization.

Sunflower (*Helianthus annuus* L) LSH_1 Var. is an important crop plant in the world. It is easily cultivated and is grown mainly under rainfed conditions on a wide range of soils. It is used for animal feed and is the second most important crop producing edible oil after soybean. Recently, sunflower has also been cultivated to produce biodiesel to some extent (Sokorich, 1992). Sunflower seeds improve digestion, brain power and functioning of cardiovascular system..Sunflower oil prevents heart disease and it is a great skin moisture retainer.The seeds have high oil content and have been one of the primary source used to acquire polysaturated oil. Sunflower oil is used in industry for making paints and cosmetics.The roasted seeds make a coffee type drink. The dried stems have also been used for fuel.The Chinese have used the fiber from stems for fabrics and paper.

Hence, in view of the importance of sunflower the present study was undertaken to investigate the effect of phosphate solubilizing bacteria and arbuscular mycorrizal fungi on the growth and biochemical contents of *Helianthus amnus* L., under greenhouse conditions.

MATERIALS AND METHODS

Experiments were conducted under green house conditions of P.G. Department of Botany, Karnatak University, Dharwad – 580003, India,

Healthy, seeds were Procured from University of Agricultural Sciences (UAS), Dharwad – 580005, Karnataka. These seeds were washed under running tap water and the surface sterilization was done by treatment of seeds with mild solution of Tween 20 (detergent). Seeds were germinated in vitro condition with petri dishes containing moist filter papers and the whole set up was kept in dark condition for 24 hrs. this was to check the germinating percentage of seeds. The soil based inoculums containing chlamydospores and colonized roots, rhizosphere soil of *sorghum* i.e., host plant used for mass multiplication of all AM fungal species having mycelia was served as AM fungal inoculums. AM Fungus *Rhizophagus fasciculats,Glomus macrocarpum*, *Glomus bagayarajii and Selerocystis dussii* were was mass multiplicated on *Sorghum vulgare* and *Selerocystis dussii* as a potential host. 10g mixed inoculants (5g rhizospare of *Sorghum vulgare* contain spores approximately115-201 spores/1g soil with hyphae, other 5g was highly colonized chopped root bits Inoculams was placed 4cm below the surface of each experimental pot except (control pots). Gum Arabic plus Jaggery mixed semisolid culture was pasted on each Sunflower seed peat based carrier material was mixed with *B. Polymyxa*. For isolation of phosphate solubilizing bacteria 10 g rhizosphere soil was suspended in 100 ml of distilled water. An aliquot (100 ml) from decimal dilutions was inoculated on Pikcovskaya's medium (PH 7.2) (Pikcovskaya, 1948), incubated at 30^0C Colonies (cfu) of PSB were counted after 24 hours.The solubility of phosphate was observed as a zone of clearance with a diameter that was measured in millimeters and observations were noted in triplicates. Single colonies appearing on Pikcovskay's agar plates were transferred in liquid nutrient broth and on agar slants and maintained at 4^0C for further study. Colony and cell morphological futures of selected bacterial isolates were observed by culturing on nutrient agar medium in Petri plates for 48 hours. Biochemical tests such as Indole acetic acid production test, Citrate utilization test, Methyl red test, Voges Proskauer test, Oxidation fermentation test,

Catalase test. Starch hydrololysis test, Nitrate reduction test, Casine production test and Gas production from glucose were performed to identify the bacterial isolates as suggested by Lakshman *et al.,* (2006).In all there were 21 treatments and each treatment was maintained in triplicates of random block design (RBD). The pots were treated with 10 ml of Hoagland nutrient. (without phosphorous) once in fifteen days. To maintain moisture, the pots were watered early in the morning every alternate day. The shoot length, root length, dry weight of the shoot and root was recorded; number of Seeds, percent root colomizatic spore number, chlorophyll content in leaves and lipid content in seeds and the number were recorded 45 and 90 days respectively.

Earthern pots measuring about 30 x 25 cm diameter containing 12 kg growth media (sand: soil: FYM=1:2:1 ratio v/v) were used for all the the experimented except root control ones. The surface sterilized seeds (4 seeds per pot) were sowed in the pots containing growth media. After 15 days of seed germination four different AM Fungal inoculums along with *Bacillus Polymyxa* were inoculated and the following treatments were maintained under green house conditions.

1. Control.
2. Inoculation of *Bacillus polymyxa*.
3. *Rhizophagus fasciculutus* with Bacillus Polymyxa.
4. Inoculation of *Glomus macrocarpum* with *Bacillus polymyxa*.
5. Inoculation of *Glomus bagyaranjii* wuth *Bacillus polymyxa.*
6. Inoculation of *Sclerocystis dussii* with *Bacillus polymyxa*.

All the experimental plants were harvested to analyze the effect of different AM Fungal inoculums on growth. First harvest was done at 45 days after sowing and second harvest was done after 90 days of sowing. The harvested plants were subjected for analysis of growth parameter such as shoot length, root length, number of leaves, and fresh weight of both root and shoot. Dry weight of root and shoot was determined after drying at 70^{o}C for 48 hours under hot air oven. All the growth parameters were measured in triplicate. AM fungal spores were recovered from the rhizosphere from soil of *Helianthus annuus* I., inoculated with different AM fungi, by adapting wet sieving and decanting method described by Gerdemann and Nicoloson (1963). Mycorrhizal spore number per 50g of rhizospore soil was estimated by using the procedure described by Gour and Adholeya (1994) were recorded for all AM fungal inoculated and non inoculated *Helianthus annuus. Helionthus annuus* L,fine roots were collected and maintained in lactoglycerol solution. Roots were then cleared in 10 % KOH and stained with 0.05% try phan blue in lacto phenol to reveal AM fungal structure. Stained roots were cut in to lenth fragments and macerated on slides according to (Philips and Hayman, 1970)..

$$\text{PMC (\%)} = \frac{\text{Number of root bits colonized}}{\text{Total Numbr of root bits examined.}} \times 100$$

RESULTS AND DISCUSSION

Helianthus annuus L., (LSH_1Var) had significant positive response in each AM fungi and *Basillus polymyxa* inoculation. Experimental results showed increased growth of plants inoculated with AM fungi and *Bacillus polymyxa* compared to non inoculated ones. But the rate of extent of increased growth was varied with each AM fungi inoculation. *H. annuss L.*, showed maximuim growth with inoculation of AM fungus *Selerocysis dussii* and *Bacillus polymyxa* compared to other treatments. (Table 1-2).It was observed that the four bioinoculants inoculated plant shows significantly increased plant height, root length, weight over the non inoculated plants. Most efficient strains in the order of *Selerocystis dussii* and *Bacillus polymyxa, Rhizophagus faciculatus* of *polymyxa* showed the third most effective strain *Glomus macrocarpua* with *B. Polymyxa* for the plant-growth. *Bacillus Polymyxa* individually (alone) showed moderately influenced favourable results compared to *Glomus* and *Glomus bagyarajii* with *Bacillus polymyxa*. But, it was significantly higher than the non mycorrhizal plants. Experimental results also revealed that almost 60% increase in plant growth was observed at 90days when compared at 45 days. (Figs. 1- 2).

H. annus L., plants showed that significantly increased shoot and root fresh weight as well as dry weight of both AM fungi with *Bacillus polymyxa* inoculated plants showed increased biomass production over control plants. The rate of extent of increase in biomass was varied with each AM fungi and *Bacillus polymyxa*.

The inoculation of AM fungus *Solerocysis dussii* with *Bacillus polymyxa* considered to be the efficient strain for *H. annus* L. compared to other five inoculunts. The second best influenced bioinoculants were AM fungus, *Rhizophagus fasciculatus* with *Bacillus polymyxa.*

Inoculation of *Bacillus polymyxa* influenced increased in fresh weight as well as dry weight over the two in oculants. The least values for plant fresh weight as well dry weight was recorded wkith *Glomus bagyarjii* with *B. Polymyxa* increased weight in fresh as well as dry weight was observed in other four bioinoculants. However, it was was significantly higher than that of non mycorrhizal plants. (Table – 1, Figs 1-2). Results also indicated that increased number of AM fungal spores in the rhizosphere of *Helianthus annuus* L., in all the inoculated with four different AM fungi over the control plants, but it was varied with each fungal species. More number of AM spores were recorded in *H. onnuus L*., with the inoculation of AM Fungus *Selerocys dusii* and least in *Glomus bagyarajii.* It was observed that there was an on correlation between percentage of AM fungal colonization and spore density in the rhizospher of *Helianthus annuus* L.,with dual inoculation of *selerosystis dussii* and *Bacillus Polymyxa,* Similarly, Selerosystis dussii and *B. Polymyxa* inoculated plants showed maximum chlorophyll and lipid content over the non inoculated plants. And it, was determined that the second highest lipid and chlorophyll content was seen in those plants inoculated with *Rhizophagus fasciculatus* and *Bacillus polymyxa*. The third inoculation effect was with the inoculation of *Glomus macrocarpum* and B. Polymyxa, whereas, *Glomus Bagyarjii* with *Bacillus polymyxa* showed least content of chlorophyll and lipid content but greater than that of the non inoculated plants. (Tables -1-2, Figs. 1 – 4).

Experimental results revealed that the sunflower inoculated with AM fungus and *Bacillus polymyxa* have showed synergy. The increased growth parameters were encountered with each treatment. The similar results were reported by many earlier researchers, that synergistic microbial interaction between AMF and phosphate solu-bilizing bacteria (PSB) in improving P supply to plant has been reported (Barea *et al.,* 1975; Doss dayal, 2008; Madagaonkar and Lakshman,2013). The increased plant growth was recordedon sunflower inoculated with AM fungus and *Bacillus polymyxa*. This indicated that microorganisms act synergistically perform better function when, inoculated simultaneously (Muthukumar *et al.*, 2001, Lakshman, and Romana, 2014' Jyothi and Lakshman, 2017). There is addition to enhancing nutrient absorption capacity of their plant host by the soil beneficial microbes. The hyphae of AM fungi provide an area for the interaction of plants with other soil microorganisms that have an effect on root development and performance (Lakshman *et al.,* 2006; Ramakrishnaiah and Vijaya, 2013). This interaction can be positive, neutral, or negative (Smith and Read, 1997).

Phosphate solubilizing bacteria (PSB) have great prospects to improve plant growth under given conditions mainlyas in P deficient soils when, used in combination with AM fungi (Gryndler, 2000; Andress *et al.,* 2015). They are known to mobilize phosphate ions from sparingly soluble organic and inorganic P sources. However, the released P does not reach the root surface as a result of inadequate diffusion. It was proposed that AM fungi could improve the uptake of the solubilised P; Hence, this combined interaction should improve P nutrition and supply to plants. The interactive effects of AM fungi and PSB on plant use of soil P in the form of either endogenous or added rock P was studied using a soil microcosm system integrated with ^{32}P isotopic dilution. (Johnnson *et al.*, 2004; Che *et al.*, 2006).. The microbial inoculantions to the sunflower plants have shown much significant results with dual inoculation over the single inoculation of *Bacillus polymyxa* alone. Effects of these interactions may be exploited for the benefit of sustainable agriculture. A number of studies on the interaction of AM fungus with wide variety of soil microorganisms (Barea and Olveres 1983; Linderman, 1988; Swetha and Lakshman, 2008; Jalaluddin, 2011; Herter *et al.,* 2011; Martin, 2018) that exist under various agroclimatic conditions. The experimental results confirmed that, the dual inoculation of AM fungus and PSB yielded positive results confirmed that, the dual inoculation of AM fungus and PSB yielded positive results over the remaining four inoculation treatments. Since, both are beneficial microorganism, their synergistic or additive effect could be more beneficial for increasing growth and yield (Vaddar, 2009); (Baset and Shamsuddin, 2010; Cardoso *et al.,* 2011; Hosmani *et al.,* 2013). Since the soil contains extremely rich pool of microbial entities with highly diversified and complex relationships, this characteristic of soil may sometimes contribute difficulty to reproduce

Table 1: Showing the effect of different AM fungi on growth parameters of *Helianthus annuus* L., inoculated with four different AM fungi with *Bacillus polymyxa* at 45 and 90 Days.

Treatment .	SL (cm)	RL (cm)	SW (cm)	FWS (g)	DWS (g)	FWR (g)	DWR (g)	No of Seeds/Plant
				45 days				
Control	42.0	3.8	2.0	4.31	1.96	0.69	0.36	**NA**
B.p.	43.1	4.2	2.1	4.28	1.98	0.71	0.37	**NA**
R.f+B.p	105.0	7.2	2.3	11.30	4.132	0.90	0.51	**NA**
G.m+B.p	67.2	5.6	2.2	9.49	3.110	0.79	0.42	**NA**
G.b+B.p	61.5	5.2	2.0	7.91	3.148	0.64	0.39	**NA**
S.d+B.p.	109.2	8.2	2.5	13.48	15.121	1.21	0.97	**NA**
				90 days.				
Control	92.11	4.7	2.0	7.56	1.93	1.23	0.93	98
B.p.	93.10	7.5	2.1	8.32	2.241	1.26	0.94	105
R.f+B.p	107.5	8.3	2.6	16.16	4.27	1.78	1.22	151
G.m+B.p	82.50	7.4	2.4	14.5	3.84	1.56	1.10	131
G.b+B.p	78.50	6.0	2.1	13.90	3.12	1.43	1.11	117
S.d+B.p	119.5	9.1	3.2	19.40	5.16	1.84	1.34	197

Note: SL-Shoot length, RL-Root len gth, SW. Shoot width, F.W.R. – Freswh weight of root, WR-Dry weight of root, B.p. Bacillus polymyxa, R.f-Rhizophagus fasciculatus, G.m. = Glomus macrocarpum, G.b.-Glomus bagyarajii, S.d-Sclerocystis dussii.

Table 2: Showing the effectof AM fungi and PSB on percent colonization, Spore number, Cholorophyll content, Liopid content and proline content I roots of *Helianthus annuus* L., for 45 - 90 days.

Treatment	*Percent mycorrhizal colonization in roots.*		*Spores Number/50g soil.*		*Chlorophyll mg/g tissue*	*Lipid content mg/g seeds*	*Proline inroots u mole per g tissue*
	45 Days	**90 Days**	**45 Days**	**90 Days**	**45Days**	**90 Days**	
Control	9.21	18.3	0.00	0.00	1.173	0.332	0.072
B.p.	0.00	0.00	0.00	0.00	1.178	0.346	0.058
R.f+B.p	35.15	66.86	115	139	1.461	0.514	0.081
G.m+B.p	29.59	29.59	82.2	97	1.246	0.391	0.0141
G.b+B.p	22.32	51.19	73	78	0.161	0.176	0.051
S.d+B.p.	39.53	76.32	98	102	1.432	0.633	0.131
0.000.0124							

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Note: B.p. – Bacillus polymyxa, R.f-Rhizophagus fasciculatus, G.m-Glomus macrocarpum, G.b-Glomus bagyarajii.

S.d.-Sclerocystis dussii.

Fig.1. Showing the effect of different AM fungi with *Bacillus Polymyxa* on shoot length of H. annus L. at 45 days.

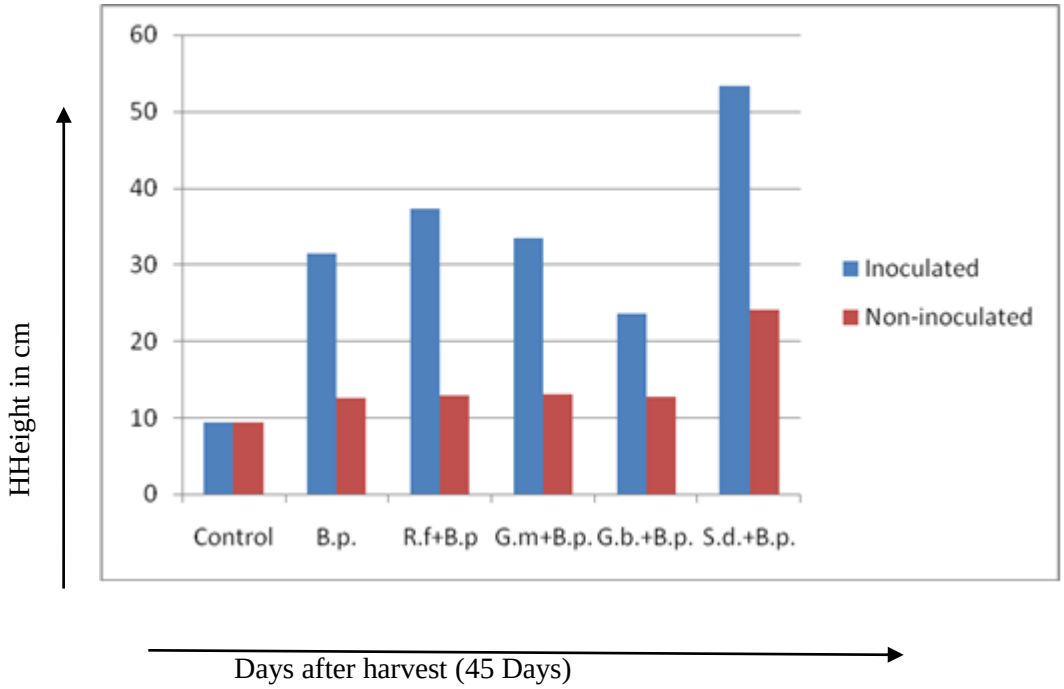


Fig 2. Showing the effect of different AM fungi with *Bacillus Polymyxa* on shoot length of H. annus L. at 90 days.

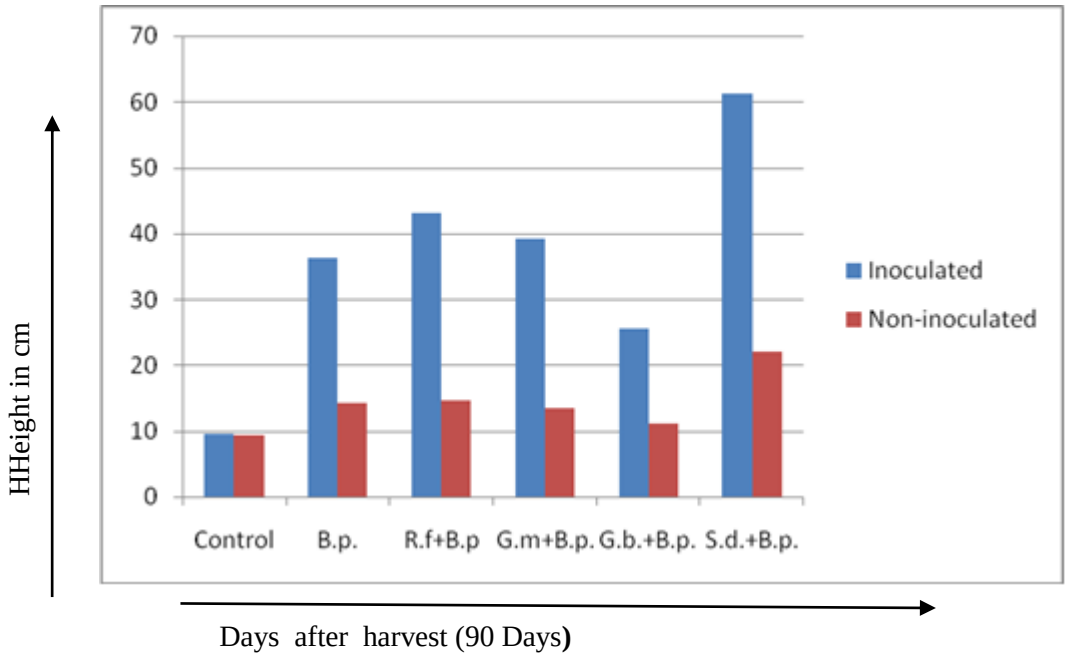


Fig.3. Showing the effect of different AM fungi and *Bacillus polymyxa* on chlorophyll content in the leaf at 90 days.

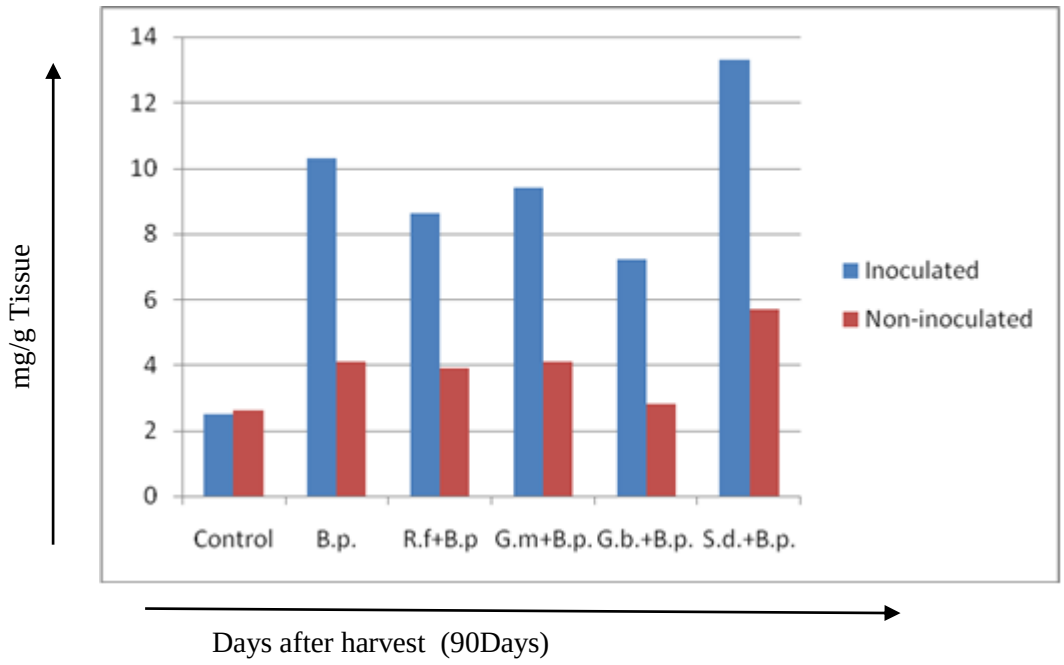
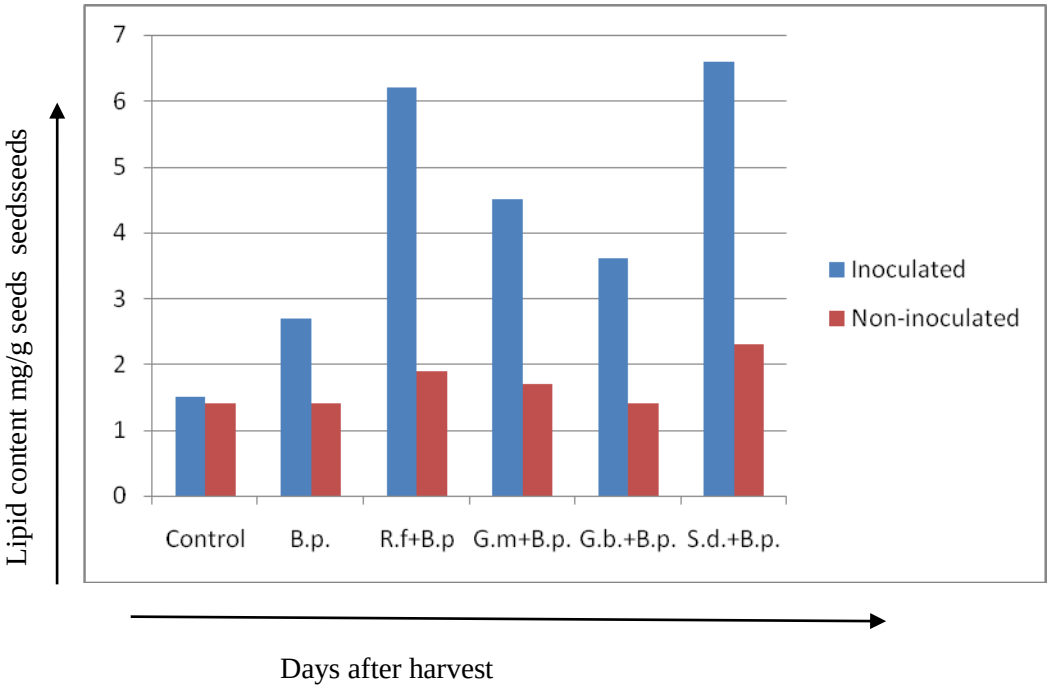


Fig.4. Lipid content showing the effect of different AM fungis and *Bacillus polymyxa* on lipid content in seeds of H. Annus L. at 90 days.



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