Effect of dual inoculation of AM fungi and Pseudomonas with Phosphorus Fertilizer rates on growth performance, nutrient uptake and Yield of Soybean

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Abstract: A pot experiment was conducted to evaluate the effect of inoculation of two arbuscular mycorrhizal (AM) species viz *Glomus mosseae* and *Acaulospora laevis* alone and in combination (phosphate solubilizing bacteria *Pseudomonas fluorescens)* with different superphosphate levels were used on Soybean. Results indicate that AM fungi, *Pseudomonas* and phosphorus significantly affected all the measured traits. Among all the growth parameters, the following were the highest in *G. mosseae* + *A. laevis* + *P. fluorescens* combination at the low concentration (half the recommended superphosphate dose): Plant height(cm), fresh shoot weight(g), dry shoot weight (g), fresh root weight (g), dry root weight(g), root length (cm). The percentage mycorrhizal root colonization, AM spore number, shoot (%) and root (%) P content, acidic (IUg^{-1} FW) and alkaline phosphatase activity (IUg^{-1} FW), percent of oil and protein content were also found highest in combination *G. mosseae* + *A. laevis* + *P. fluorescens* at low concentration superphosphate (half recommended superphosphate dose) as compared with non-mycorrhizal plants. Under the conditions of superphosphate oversupply decrease in plant growth.
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Introduction:

Soybean also called "edible grain legumes" can be divided into two types: oilseeds and pulses. Together, Soybean oil and protein content account for about 60 per cent of dry Soybeans by weight (protein at 40% and oil at 20%). Soybean (*Glycine max* L.) is the world's important food legume of great nutritional value. It is the second only to groundnut in terms of oil content (20%) among food legumes (Bekere and Hailemariam, 2012). So to enhance the production of oil seed in India, researchers have to use various essential nutrients and biofertilizers. Among the essential nutrients, Nitrogen (N) and Phosphorus (P) are the primary nutrients in the soil which play crucial role in improving plant growth (Mohamed *et al*., 2011). Phosphorus is another most growth limiting nutrient for plant growth (Ezawa, 2002). Phosphorus is called "Key to life" because it is directly involved in most living process. Most minerals nutrients in soil solution are present in milimolar amount, only P is only present in micromolar or less (Goldstein, 1994). This is because in the soil, the mobility of this element is slow and cannot respond to its rapid uptake by plants and this causes the creation and development of P depleted zones near the contact area of roots and soil in rhizosphere. Instead of heavy fertilizer, the plants needs an assisting system which could extend beyond the depletion zones and help to absorb the P from a wider area by developing an extended network around root system (Salehrastin, 1999). Biological fertilizers like phosphate solubilizing microorganism (PSM) and plant growth promoting rhizobacteria (PGPR) are considered among the most important plant helper microorganism to supply nutrient at a favourable level and these fertilizers are absorbed on the basis of selection of beneficial soil microorganisms which has the highest efficiency to enhance plant growth by providing nutrients in a readily absorbable form.). Phosphate solubilizing Arbuscular mycorrhizal (AM) fungi and *Pseudomonas fluorescens* are known as effective organisms in this process (Reyes *et al*., 1999). Research activities aimed at achieving better use efficiency of fertilizers, including the use of AM fungi + PGPR as supplements to fertilizers have steadily increased in the last two decades (Adesemoye *et al*., 2009). Additive effects between AM fungi and plant growth promoting bacteria with reduction in fertilizer input level were observed by different workers (Gamalero *et al*., 2004; Meghvansi and Mahna, 2009; Soleimanzadeh, 2012; Patra *et al*., 2013; Valadabadi *et al*., 2013). The improved plant growth is generally attributed to the enhanced absorption of immobile nutrients especially P (Shibata and Yano, 2003; Pasqualini *et al.,* 2007) through extensive and highly branched extra radical hyphae. AM fungi are also known to enhance absorption of other nutrients such as N, Zn, Cu etc. (Liu *et al.,* 2000; Zhu *et al.,* 2001). Enhanced mineral nutrition helps in increased chlorophyll content thus helping in higher photosynthetic rate (Feng *et al.,* 2002). Despite the

substantial amount of total phosphorus in tropical soils, phosphorus deficiency is one of the most important fertility problems in tropical agriculture (Nyemba, 1986; Mengel and Kirkiby, 1987; Mamo *et al*., 2002). Keeping in view the above information, present study was undertaken to investigate first the efficacy of two AM fungi i.e. *Glomus mosseae* and *Acaulospora laevis* alone and in combination with PGPR i.e. *Pseudomonas fluorescens* and different rates of super-phosphate fertilizer for enhancing the growth and yield of Soybean important oil yielding crop under pot condition.

Materials And Methods:

Collection of soil sample

For isolation of dominant AM fungi, composite soil sample from rhizospheric soil of Soybean was collected. It was done by digging out a small amount of soil close to the plant roots up to the depth of 15 to 30 cm and kept in sterilized polythene bags at 10 °C for further processing.

Experimental site: Experiments were designed out under poly house of Botany Department, Kurukshetra University, Kurukshetra, Haryana, India during 2011-2013. The poly house was maintained controlled temperature (20º±5ºC) and humidity (50–70%), with cool white fluorescent lamps (8000 lux) under a 16–h photoperiod during the experiments.

Isolation of dominant AM spores from soil samples

Isolation of dominant AM spores i.e., *G. mosseae* and *A. laevis* were done by using Wet Sieving and Decanting Technique of Gerdemann and Nicolson (1963). In this technique, 50 g of soil were soaked in 500 ml water for 24 h. The supernatant was then passed through a gradient of sieves with pore size ranging from 150 µm to 45 µm arranged one above the other in an ascending order. Each sieve was then washed in water and filtered through Whatmann No. 1 filter paper. This filter paper was then observed under stereobinocular microscope for the presence of various kinds of spores and mounted on polyvinyl lactic acid (PVLA) for further studies.

Mass culture of *Pseudomonas fluorescens*:

Pseudomonas fluorescens (MTCC N° B103) was procured from IMTECH (Institute of Microbial

Technology, Chandigarh, India) and multiplied in nutrient broth medium (1.25 g peptone, 0.75 g beef extract, 1.25 g NaCl, 250 mL distilled water) for 24 h for suitable bacteria growth.

Experimental design

The recommended dose of phosphate fertilizer for Soybean is 80 kg ha^{-1} (Anand, S. 2008; Huda, K. 2008). The experiment was conducted in a 3×6 factorial design employing three levels of single super-phosphate [half the recommended (F1), recommended (F2) and double the recommended (F3)] and six levels of different bioinoculants for super-phosphate. The following combination were taken control, *G. mosseae*, *A. laevis*, *G. mosseae* + *P. fluorescens*, *A. laevis* + *P. fluorescens* and *G. mosseae* + *A. laevis* + *P. fluorescens*.

Pot experiment set-up under polyhouse: The top soil (0–30 cm) from experimental site was sieved through 2 mm sieve, mixed with sand: soil (1:3) and autoclaved at 121ºC at 15 psi for 2 h for two consecutive days. Three different doses super-phosphate fertilizers were used. For Soybean (super-phosphate: 0.075 g/pot, F2–0.150 g/pot and F3–0.300 g/pot). Granules of super-phosphate fertilizer were taken, ground and dissolved in sterilized distilled water. Then, the solution was applied in all the pots (including control) soil. For AM fungi, 200 g of soil containing approximately 950-1050 spores and colonized root fragments of Maize plants with an infection level of around 90-95% were used as inoculum. While for *P. fluorescens* inoculum, 10 ml nutrient broth containing 1.8×10^{6} cfu g^{-1} was added as per the treatment allocation. The inoculum was applied as a broth in the soil in the pot and then mixed well. For bacterium inoculation, healthy, sterilized seeds of Soybean were suspended in 20-40 ml thick suspension (10^{9}cells/ml) of *B. japonicum* for inoculation. The seeds were then air dried for 30 minutes in sterile Petri plates. The seeds were transferred above the soil in earthen pot. In control pots, autoclaved soil (without any microbial inoculants) was used. The seed of Soybean crops (Soybean: SL525) were procured from Oil Seed Section, Department of Botany, Chaudhary Charan Singh Haryana Agricultural University, Hisar, Haryana-125004, India and sown in pots. The plants were irrigated regularly. Hoagland's nutrient solution without P (100 ml/pot) was applied every 15th day after transplantation.

Analysis of growth parameters:

Quantification of AM spores: It was done by Adholeya and Gaur 'Grid Line Intersect Method' (1994). Spores were counted under stereo binocular microscope by using a counter.

Identification of AM fungi: For identification of AM spores, the keys of Walker (1983), Scheneck and Perez (1990), Morton and Benny (1990), Mukerji (1996) were followed.

Growth parameters: After 120 days roots were uprooted, washed, blotted dry for determination of plant height, root and shoot fresh biomasses, fresh root weight and mycorrhizal root colonization and then oven dried for root dry weight and P content estimation.

Isolation and quantification of AM spores: AM spores were isolated by wet sieving and decanting technique (Gerdemann and Nicolson, 1963) and quantified by grid line intersect method (Adholeya and Gaur, 1994).

Percent of root colonization: For assessment of root colonization, rapid clearing and staining method of Philips and Hayman (1970) was followed and the percent infection was calculated by the following equation (Giovannetti and Mosse, 1980):

$$\text{Root Colonization (\%)} = \frac{\text{No. of root segments colonized}}{\text{Total no. of root segments observed}} \times 100$$

Leaf area: Leaf area (cm^2) was assessed by using leaf area meter (Systronics 211, Ahmedabad, India).

Estimation of total chlorophyll: The chlorophyll content was estimated by using Arnon's method5 by using 80% acetone as solvent. Total chlorophyll (total chl), chlorophyll a (chl a) and chlorophyll b (chl b) was calculated by the standard formula.

Stomatal conductance estimation: The stomatal conductance of all experimental plants was measured by using Porometer (AP4- Delta T devices, Cambridge, UK) after 120 days of inoculation in morning and evening.

Phosphorus estimation: The phosphorus content of roots and shoots of all experimentally plants was estimated by 'Phospho-vanadomolybdate yellow colour method' (Jackson, 1973) after 120 days.

Phosphatase estimation: Phosphatase activity was assayed by using p-nitrophenyl phosphate (PNPP) as substrate which is hydrolyzed by the enzyme to pnitrophenol. For this ice cold sodium acetate buffer (0.05M with pH 4.8) for acid phosphatase and sodium carbonate-bicarbonate buffer (0.05M with pH 10) for alkaline phosphatase activity was used and was measured in terms of IU/g FW.

Protein estimation: Protein was estimated by the method of Bradford12 using coomassive brilliant blue G-250 dye.

Oil extraction: Oil was extracted by petroleum ether of boiling range between 40-600C using the Soxhlet's procedure4. Five replicates of each treatment were taken.

Statistical analysis: All results were analyzed using analysis of variance (ANOVA), followed by post hoc test through computer software SPSS 11.5 version. Means were ranked at P d"0.005 level of significance using Duncan's Multiple Range Test for comparison.

Results:

The present research was conducted in polyhouse conditions in order to evaluate the effectiveness of mycorrhizal and/ or bacterial amendments. All inoculants showed marked improvement in plant growth and P acquisition. In case of soybean, inoculation with AM fungi significantly increased plant growth compared to uninoculated plants at all levels of P.

Plant growth parameters

While studying influence of different levels of super-phosphate on growth improvement (Table- 1.1) of Soybean, it was observed that inoculation with AM fungi significantly increased the plant height compared to uninoculated plants at all levels of P fertilizers. The growth behavior of a crop is measured in terms of plant height. The comparison of plant height of inoculated and un-inoculated seed depicts that plant height of inoculated seed was significantly more than that of un-inoculated seed. Highest value of plant height was recorded in mix inoculation of *G. mosseae* and *A. laevis* along with *P. fluorescens* (150±1.58) at half of the recommended dose of super-phosphate. On the other hand dual inoculation of *G. mosseae* + *P. fluorescens* synergistically increased root length (47.26±3.98), shoot biomass (fresh-30.35±3.16, dry-3.52±0.04) at half of recommended dose and while, root biomass (fresh-4.98±0.02, dry-1.10±0.01) were foun

d highest in mix consortium.

AM spore number and root colonization

The data generated by this study shows that root colonization as well as AM spore number was maximum at low level of super-phosphate in plants treated with mix consortium of all the bioinoculants. High rate of fertilizers application adversely affects the survival of AM fungi. After 120 days, *G. mosseae* alone produced maximum AM spore number (98.46±3.89) and root colonization (97.6±5.39) followed by mix consortium *G. mosseae* + *A. laevis* + *P. fluorescens* (AM spore-98.00±3.16, Root colonization-95.98±3.92) at half of the recommended dose of superphosphate. It can be concluded that the high P application (double recommended dose) effectively suppressed the percentage of root colonization and AM spore number in soybean plant. (Table- 1.1).

Leaf area, stomatal conductance and leaf chlorophyll content

Chlorophyll content was found to be increased in all treated plants than control. Application of half of recommended dose of superphosphate fertilizer with AM fungi and *P. fluorescens* inoculation markedly improved the chlorophyll content in soybean (Table-1.2). Recommended dose of fertilizers (P) increases leaf area, high value of chlorophyll and stomatal conductance and further increase did showed inhibitory/decline effect. At recommended dose of superphosphate the mix consortium of *G. mosseae* + *A. laevis* + *P. fluorescens* resulted in maximum leaf area (32.16±2.55); chlorophyll content (chla-0.827±0.004, chlb- 0.051±0.003, and total chl-0.878±0.007) and stomatal conductance morning (lower-357.27±4.23, upper-29.77±3.06,) and evening (lower-103.28±2.75, upper-21.32±1.48,) after 120days of inoculation respectively. Second best results were obtained at half the recommended dose. Photosynthetic parameters showed increasing trend with increasing fertilizers from medium to low and afterwards it decline at high dose.

Plant nutrient uptake

It is clear that inoculation of soil with AM fungi, *P. fluorescens* and different levels of superphosphate markedly improved P content in Soybean in comparison to control (Table 1.3). Higher P content was found in all inoculated plants in both shoot and root as compared to uninoculated plants at all levels of super-phosphate. The utmost accretion in P content was observed at half of the recommended dose of super-phosphate in plants treated with *G. mosseae* + *P. fluorescens* (shoot-0.26±0.043, root-0.290±0.003).

e) Root phosphatase activity

With reference to phosphatase (acidic and alkaline) activity, acid phosphatase was found to be more active than alkaline phosphatase. All the plants inoculated with bioinoculants harboured higher enzyme activity than non-inoculated plants. However, higher activity was recorded in mix consortium of *G. mosseae*, *A. laevis* and *P. fluorescens* at all levels of super-phosphate with maximal at low concentration followed by medium and high concentrations Root phosphatase activity increased with increase in the application of super-phosphate fertilizers from Medium to low and after that it decreases in high. Maximum phosphatase activity was recorded in *G. mosseae* + *A. laevis* + *P. fluorescens* (acidic-1.489±0.007, alkaline-0.327±0.003) at recommended dose of super-phosphate.

f) Yield Parameters

Persual of the data shows that the protein content (26.79±1.71) was highest in the plants inoculated with both AM fungi and *P. fluorescens* at half of recommended dose. While the highest value of oil content (43.00±0.70) were obtained with *G. mosseae* + *A. laevis* + *P. fluorescens* at half recommended dose of superphosphate after 120 day of inoculation.

Table-1.1: Efficacy of AM fungi, *Pseudomonas fluorescens* and super-phosphate on mycorrhization and growth parameters of Soybean after 120 days of inoculation

Super phosphate Concentration (g/pot)	Parameters→ Treatments ↓	Plant height (Cm)	Shoot biomass (g)		Root biomass (g)		Root length (Cm)	AM root colonization (%)	AM Spore number/10 g soil
			Fresh	Dry	Fresh	Dry			
F1 Half Recommended (0.075 g/pot)	Control	85±3.16^{fg}	12.47±3.55^{gh}	1.08±0.04^{g}	1.40±0.04^{f}	0.30±0.05^{f}	24.30±3.19^{gh}	0^{h}	0^{h}
	G. *mosseae*	128±3.16^{cd}	24.25±3.74^{bcd}	2.48±0.05^{c}	3.03±0.01^{cd}	0.97±0.03^{abc}	36.26±4.04^{cde}	97.6±5.39^{a}	98.46±3.89^{a}
	A. *laevis*	120±6.08^{de}	20.89±3.14^{cdef}	1.99±0.04^{d}	2.90±0.05^{de}	0.89±0.05^{cd}	34.38±2.89^{cdef}	76.64±3.02^{fg}	91.12±3.16^{cdefg}
	G+Pf	140±1.58^{b}	30.35±3.16^{a}	3.52±0.04^{a}	3.56±0.03^{bc}	1.05±0.03^{ab}	47.26±3.98^{a}	90.50±3.93^{cde}	93.37±2.60^{bcde}
	A+Pf	135.±4.52^{abc}	25.55±3.83^{abc}	2.56±0.02^{bc}	3.48±0.03^{bcd}	0.99±0.04^{b}	37.26±4.33^{cd}	87.32±3.14^{cde}	94.03±2.94^{abcd}
	G+A+Pf	150±1.58^{a}	26.96±4.74^{ab}	2.66±0.04^{b}	4.98±0.02^{a}	1.10±0.01^{a}	45.26±3.15^{a}	95.98±3.92^{ab}	98.00±3.16^{a}b
F2 Recommended (0.150g /pot)	Control	82 ±4.43^{g}	11.03±3.16^{gh}	1.02±0.05^{g}	1.25±0.04^{g}	0.22±0.03^{g}	20.46±3.67^{h}	0^{h}	0^{h}
	G. *mosseae*	120±2.23^{de}	20.12±3.68^{def}	1.79±0.04^{de}	2.90±0.03^{de}	0.90±0.05^{bc}	34.16±4.05^{cdef}	94.36±3.10a^{bc}	95.25±3.02^{abcd}
	A. *laevis*	110±7.90^{ef}	18.88±6.02^{def}	1.65±0.07^{f}	2.78±0.03^{ef}	0.81±0.05^{de}	31.38±3.90^{efg}	71.40±3.07^{fg}	88.45±5.85^{efg}
	G+Pf	137±1.58^{b}c	20.50±2.28c^{def}	1.89±0.03^{de}	3.50±0.04^{bc}	0.99±0.05^{b}	39.24±1.61^{bc}	85.64±3.28^{de}	93.18±2.26^{bcdef}
	A+Pf	130±3.80^{cde}	20.03±2.22^{def}	1.80±0.04^{de}	3.35±0.06^{cd}	0.93±0.02^{bc}	35.12±3.83^{cdef}	82.40±4.06^{def}	90.38±2.79^{defg}
	G+A+Pf	141±3.16^{b}	23.79±4.16^{bcde}	2.42±0.04^{c}	4.81±0.04^{ab}	1.03±0.04^{ab}	43.26±4.70^{ab}	95.38±2.35^{ab}	95.50±2.29^{abc}
F3 Double Recommended (0.300g/pot)	Control	75±3.16^{h}	10.28±4.06^{h}	1.00±0.06^{g}	1.00±0.66^{h}	0.19±0.03^{h}	18.20±3.21^{i}	0^{h}	0^{h}
	G. *mosseae*	115±5.09^{ef}	15.82±1.50^{efg}	1.75±0.04^{ef}	2.82±0.04^{def}	0.80±0.03^{de}	30.26±4.69^{fg}	80.46±5.49^{efg}	87.26±3.82^{fg}
	A. *laevis*	104±5.56^{f}	15.46±2.05^{fg}	1.56±0.03^{f}	2.73±0.01^{ef}	0.73±0.04^{e}	28.08±3.46^{gh}	69.3±5.12^{g}	83.41±3.53^{g}
	G+Pf	130±1.58^{bcd}	18.04±2.53^{ef}	1.82±0.04^{de}	3..00±0.05^{cde}	0.92±0.05^{bc}	33.20±5.42^{defg}	92.34±4.51^{bcd}	92.20±2.20^{cdef}
	A+Pf	125±1.58^{cd}	16.32±4.37^{efg}	1.75±0.03^{ef}	2.87±0.06^{def}	0.84±0.03^{de}	32.32±3.53^{defg}	83.16±4.11^{def}	89.22±4.13^{efg}
	G+A+Pf	137±2.54^{ab}	18.68±1.30^{def}	2.34±0.03^{cd}	4.72±0.02^{b}	0.98±0.04^{abc}	36.28±4.64^{cde}	93.36±2.30^{abc}	94.72±3.20^{abcd}
Annova (F)		78.339	11.743	948.464	3434.719	0.775	19.918	282.543	152.782
LSD (P≤0.05)		13.6788	9.1862	0.1168	0.1064	5.2572	9.804	9.6682	8.4454
F values	Fertilizer (f)	144.836	684.395	886.158	684.395	1.023	106.136	99.982	17.56
	Parameter (p)	233.101	7867.342	3046.791	7867.342	0.247	101.807	6835	1048.053
	f x p	3.513	51.222	116.779	51.222	0.992	0.548	1.320	0.585

†G: *Glomus mosseae*, A: *Acaulospora laevis*, Pf: *Pseudomonas fluorescens*, F: Super phosphate fertilization, AM: Arbuscular mycorrhiza

‡Each value is a mean of five replicates, ±: standard deviation, values in columns followed by the same alphabet is not significantly different, $P \leq 0.05$, least significant difference test

Table- 1.2: Efficacy of AM fungi, *Pseudomonas fluorescens* and super-phosphate on chlorophyll and stomatal conductance of Soybean after 120 days of inoculation

Super phosphate Concentration (g/pot)	Parameters→ Treatments ↓	Leaf area (sq.cm.)	Chlorophyll content (mg g^{-1} Fresh weight)			Stomatal conductance ($mmol^{-2}$ s^{-2})			
						Morning		Evening	
			Chlorophyll a	Chlorophyll b	Total Chlorophyll	Lower	Upper	Lower	Upper
F1 Half Recommended (0.075 g/pot)	Control	23.38±3.65^{f}	0.420±0.005^{h}	0.026±0.003^{h}	0.446±0.007^{gh}	95.46±6.87^{h}	11.27±3.11^{hi}	27.22±2.90^{hi}	07.70±1.44^{g}
	G. *mosseae*	27.22±3.17^{c}	0.617±0.003^{ef}	0.042±0.004^{de}	0.658±0.007^{ef}	210.00±5.24^{ef}	23.49±4.79^{ef}	85.70±3.05^{d}	20.16±4.08^{bc}
	A. *laevis*	25.18±1.56^{e}	0.557±0.004^{fg}	0.039±0.003^{ef}	0.596±0.009^{f}	184.04±2.58^{fg}	22.76±2.58^{efg}	68.32±4.04^{gh}	17.32±2.56^{de}
	G+Pf	30.28±4.67^{b}	0.795±0.003^{b}	0.045±0.004^{bcd}	0.838±0.005^{b}	270.44±5.24^{cd}	25.58±3.99^{cd}	79.74±2.81^{def}	19.42±4.05^{bcd}
	A+Pf	29.17±2.54^{bc}	0.643±0.002^{d}	0.046±0.004^{bcd}	0.686±0.005^{d}	236.18±2.14^{def}	24.36±4.12^{de}	75.16±4.08^{ef}	18.34±2.51^{cd}
	G+A+Pf	32.16±2.55^{a}	0.820±0.004^{a}	0.043±0.003^{cde}	0.866±0.008^{ab}	350.21±6.07^{ab}	27.35±3.72^{bc}	99.26±2.46^{b}	23.20±3.39^{a}
F2 Recommended (0.150g /pot)	Control	20.06±2.15^{g}	0.427±0.005^{h}	0.029±0.003^{gh}	0.456±0.007^{gh}	100.37±4.13^{g}	17.18±4.73^{h}	30.40±3.37^{h}	09.20±2.59^{a}
	G. *mosseae*	25.31±1.54^{e}	0.627±0.004^{e}	0.046±0.003^{bcd}	0.673±0.007^{de}	276.37±4.41^{c}	25.31±3.39^{cd}	92.50±4.83^{bc}	22.34±3.08^{ab}
	A. *laevis*	21.78±2.14^{fg}	0.527±0.003^{g}	0.043±0.003^{cde}	0.576±0.016^{f}	189.45±3.61^{fg}	24.55±5.07^{cde}	73.40±1.53^{ef}	19.44±3.06^{bcd}
	G+Pf	27.26±3.11^{c}	0.797±0.003^{b}	0.049±0.001^{ab}	0.845±0.004^{b}	276.28±4.53^{c}	27.59±4.13^{bc}	83.40±3.25^{de}	21.48±4.42^{abc}
	A+Pf	26.26±2.19^{d}	0.647±0.006^{d}	0.048±0.002^{abc}	0.695±0.008^{d}	243.39±3.86^{d}	26.56±4.04^{bcd}	81.34±3.90^{de}	20.24±1.56^{bc}
	G+A+Pf	30.20±2.29^{b}	0.827±0.004^{a}	0.051±0.003^{a}	0.878±0.007^{a}	357.27±4.23^{a}	29.77±3.06^{a}	103.28±2.75^{a}	21.32±1.48^{abc}
F3 Double Recommended (0.300g/pot)	Control	19.53±1.91^{h}	0.410±0.003^{i}	0.017±0.003^{i}	0.427±0.006^{h}	88.28±3.18^{i}	10.27±4.17^{i}	22.54±3.63^{i}	06.48±1.95^{h}
	G. *mosseae*	20.30±4.72^{g}	0.590±0.005^{f}	0.032±0.004^{fgh}	0.622±0.009^{def}	197.56±2.90^{efg}	20.17±3.62^{gh}	80.34±4.70^{de}	18.46±4.68^{cd}
	A. *laevis*	19.744±2.53^{gh}	0.445±0.003^{h}	0.030±0.004^{gh}	0.475±0.007^{g}	176.12±5.81^{fg}	18.15±4.04^{h}	65.20±3.67^{gh}	15.30±4.47^{ef}
	G+Pf	25.20±2.98^{e}	0.780±0.005^{c}	0.034±0.003^{fg}	0.814±0.008^{c}	240.36±3.98^{de}	22.14±2.19^{fg}	72.28±2.22^{efg}	17.16±3.48^{de}
	A+Pf	23.27±3.07^{f}	0.630±0.002^{e}	0.033±0.003^{gh}	0.662±0.005^{de}	230.43±5.27^{def}	21.08±3.08^{gh}	71.36±3.86^{fg}	16.36±3.13^{def}
	G+A+Pf	26.10±4.31^{d}	0.790±0.004^{bc}	0.035±0.003^{fg}	0.824±0.010^{c}	299.88±2.43^{b}	23.22±2.80^{ef}	93.44±2.99^{bc}	20.12±4.11^{bc}
Annova (F)		1519.392	5787.416	34.326	2109.815	2337.269	68.086	1233.663	125.145
LSD (P≤0.05)		0.5604	0.0108	0.0088	0.0188	9.0706	3.2212	3.268	2.1404
F values	Fertilizer (f)	7050.510	995.954	154.730	518.345	666.522	157.578	490.805	78.274
	Parameter (p)	2215.224	46140.373	202.058	20193.795	8214.801	172.634	8644.675	289.351
	f x p	52.015	196.904	0.823	104.750	106.408	0.952	37.096	5.260

†G: *Glomus mosseae*, A: *Acaulospora laevis*, Pf: *Pseudomonas fluorescens*, F: Super phosphate fertilization, AM: Arbuscular mycorrhiza
‡Each value is a mean of five replicates, ±: standard deviation, values in columns followed by the same alphabet is not significantly different, $P \leq 0.05$, least significant difference test

Table-1.3: Efficacy of AM fungi, *Pseudomonas fluorescens* and super-phosphate on Phosphorus content, phosphates activity and yield of Soybean after 120 days of inoculation

Super phosphate concentration (g/pot)	Parameters→ Treatments ↓	Phosphatase (IUg^{-1}Fresh weight)		Phosphorus content(%)		Yield	
		Acidic	Alkaline	Shoot	Root	Protein content (%)	Oil content (%)
F1 Half Recommended (0.075 g/pot)	Control	1.105±0.003^{h}	0.184±0.003^{f}	0.13±0.029^{fgh}	0.128±0.003^{h}	19.07±0.74^{ef}	36.07±1.57^{ef}
	G. *mosseae*	1.305±0.003^{e}	0.228±0.004^{cd}	0.21±0.038^{bcd}	0.259±0.002^{def}	24.14±2.62^{cd}	41.03±1.94^{bc}
	A. *laevis*	1.251±0.001^{f}	0.200±0.003^{ef}	0.19±0.042^{bcde}	0.194±0.003^{g}	23.33±1.72^{cde}	40.57±1.47^{cd}
	G+Pf	1.450±0.003^{bc}	0.295±0.003^{c}	0.26±0.043^{a}	0.290±0.003^{a}	25.03±1.57^{bc}	42.17±1.61^{ab}
	A+Pf	1.423±0.003^{cd}	0.252±0.004^{d}	0.23±0.040^{ab}	0.270±0.001^{b}c	25.03±1.58^{bc}	42.04±1.58^{ab}
	G+A+Pf	1.480±0.003^{ab}	0.318±0.005^{ab}	0.24±0.022^{ab}	0.271±0.003^{bc}	26.79±1.71^{a}	43.00±0.70^{a}
F2 Recommended (0.150g /pot)	Control	1.114±0.003^{g}	0.132±0.003^{g}	0.11±0.044^{gh}	0.121±0.004^{h}	18.35±2.47^{f}	34.12±2.23^{efg}
	G. *mosseae*	1.317±0.004^{e}	0.235±0.003^{de}	0.19±0.015^{bcde}	0.253±0.003^{ef}	23.12±1.57^{cde}	40.19±1.61^{cd}
	A. *laevis*	1.261±0.002^{f}	0.221±0.003^{def}	0.16±0.022^{def}	0.191±0.002^{g}	22.17±1.56^{def}	38.17±1.56^{de}
	G+Pf	1.465±0.002^{bc}	0.301±0.003^{b}	0.21±0.031^{bcd}	0.269±0.003^{bcd}	25.08±1.53^{bc}	41.32±1.39^{b}
	A+Pf	1.437±0.004^{cd}	0.257±0.038^{d}	0.20±0.031^{bcd}	0.265±0.004^{cd}	24.44±1.48^{cd}	41.04±1.54^{bc}
	G+A+Pf	1.489±0.007^{a}	0.327±0.003^{a}	0.23±0.035^{ab}	0.286±0.003^{ab}	26.22±1.83^{ab}	42.45±1.61^{ab}
F3 Double Recommended (0.300g/pot)	Control	1.199±0.004^{g}	0.110±0..003^{g}	0.09±0.047^{h}	0.120±0.002^{h}	16.42±1.60^{g}	33.08±1.51^{h}
	G. *mosseae*	1.300±0.004^{e}	0.221±0.003^{def}	0.15±0.043^{efg}	0.250±0.004^{ef}	22.81±1.55^{de}	39.29±1.54^{de}
	A. *laevis*	1.245±0.004^{fg}	0.196±0.002^{ef}	0.14±0.015^{fg}	0.190±0.004^{g}	22.11±1.56^{def}	37.79±1.57^{def}
	G+Pf	1.431±0.001^{cde}	0.288±0.003^{bc}	0.17±0.020^{cdef}	0.266±0.001^{cd}	24.13±1.57^{cd}	40.87±1.60^{bcd}
	A+Pf	1.418±0.004^{de}	0.248±0.003^{d}	0.16±0.033^{def}	0.263±0.002^{cde}	22.83±1.54^{de}	40.04±1.56^{cde}
	G+A+Pf	1.478±0.004^{ab}	0.300±0.004^{b}	0.22±0.036^{abc}	0.280±0.004^{ab}	24.81±1.57^{bcd}	41.84±1.58^{b}
Annova (F)		4962.377	1393.509	9.333	1.001	4378.133	3697.026
LSD (P≤0.05)		0.0102	0.0092	0.087	68.7722	0.2474	0.2472
F values	Fertilizer (f)	491.205	6054.565	43.537	1.001	5067.050	3034.262
	Parameter (p)	15156.369	35245.146	119.223	1.003	7513.384	9680.831
	f x p	193.980	2385.293	1.488	1.000	152.358	148.470

†G: *Glomus mosseae*, A: *Acaulospora laevis*, Pf: *Pseudomonas fluorescens*, F: Super phosphate fertilization, AM: Arbuscular mycorrhiza
‡Each value is a mean of five replicates, ±: standard deviation, values in columns followed by the same alphabet is not significantly different, $P \leq 0.05$, least significant difference test

Discussion:

Analysis of the present experimental results shows that inoculation of Soybean plants with AM fungi along with microbial inoculants resulted in significant impact on biomass production by improving soil physical and biological properties, directly affecting root growth, production of phosphatase enzymes, enhanced mineral uptake and transfer of P to the plant. Inoculation of AM fungi along with PGPR exhibited significant increase in growth, physiological as well as yield parameter.

Present results corroborate well with the finding of Babaei *et al*. (2012) who reported the mix consortium of AM fungi along with *Pseudomonas fluorescens* under low phosphorus level to be superior in influencing the growth and oil content of Sunflower. Sabannavar and Lakshman (2009) studied the interactive potential benefits of inoculation with AM fungi (*Glomus fasciculatum* and *Acaulospora laevis*) and phosphate solubilizing bacteria

(*Pseudomonas striata*) in the presence of different doses of rock phosphate and found significant improvement in shoot and root growth, biomass, colonization rate and shoot P content. Likewise, a significant increase in shoot and root biomass in *L. usitatissimum* was found by Cavagnaro *et al*. (2005) and Neetu *et al*. (2012) when inoculated with AM fungi and with lower doses of P fertilizer. Similar was the results made by Ardakani and Mafakheri (2011) and found that application of 90kgP/ha without AM inoculation gave significantly the same wheat grain yield as 30kgP/ha with AM inoculation. This finding proves the ability of AM fungi to compensate for lower P application rates. The results were also supported by Ibiremo *et al*. (2012) who found enhanced nutrient uptake and dry matter of Cashew with dual inoculation of AM fungi and PGPR with single super- phosphate. The results are in accordance with the study of Prasad and Bilgrami (2002), who observed that inoculation of *Saccharum officinarum* with *Glomus fasciculatum* and lower dose phosphate exhibited triggering effect on chlorophyll content in comparison to control. AM fungi inoculation also show effective results in chlorophyll content increment. Increase in chlorophyll content in mycorrhizal treated plant indicates the increase in rate of photosynthesis which can be due to more absorption of nutrients.

In all the studies, mycorrhization status decreased with increased in concentration of P fertilizers. Soil with relatively high P content could have decreased mycorrhizal colonization levels and as consequence, the effects of AM fungi on the plants might have been less pronounced (Smith and Read, 2008; Zubek *et al*., 2012). However, in some cases medium recommended dose of superphosphate resulted in higher AM fungal sporulation and root colonization, which is favoured by Arpana and Bagyaraj (2007) that low levels of P favour the occurrence and distribution of AM fungi. Similar results reported by Kapoor *et al*. (2004) found that root colonization per cent in Fennel in inoculated treatments with two AM fungi (*Glomus fasiculatum* and *Glomus macrocarpum*) was substantially more than non-inoculated treatment at adequate level of P fertilizers. Researchers announced that inoculation of AM fungi with Mint root caused increase in per cent of colonization (Abdel-Fattah *et al*., 2002). Likewise, Linderman and Davis (2004) who found that in treatments with a high dose of fertilizers decreased AM spore number as well as root colonization was detected. Liu *et al*. (2000) reported that a high P level reduces both intra as well as extraradical AM development and thus inhibit AM colonization. *Pseudomonas* species are also effective root colonizers as they produce secondary metabolites that enhance AM fungi growth (O' Sullivan and O' Gara, 1992). It was found that plants with higher mycorrhizal root colonization had maximum phosphatase activity (alkaline and acidic). These enzymes help in mineralization of bound P into soluble form and make it available to the plants. This P is then absorbed by the plants through the AM colonized roots and thus absorbs maximum phosphorus from the soil.

In current investigation, AM fungi treated plants were found significantly higher P content in shoot as well as root as compared to uninoculated plants. Mycorrhizal association is known to increase the availability of diffusion limited nutrients, Phosphorus being the important of these nutrients (Sreeramulu and Bagyaraj, 1999). These results are in close conformity with Patil *et al.* (2013) to evaluate the effect of AM fungi with low dose of super-phosphate showed that an increase in all growth parameters of Maize and there was also increase in macro and micro nutrients. Microorganisms plays a significant role in P acquisition includes AM fungi and PGPR (Fankem *et al*., 2006). *G. mosseae* inoculated plants and treated with different levels of superphosphate grown in green house condition showed increase nutrient in shoot, when compared to uninoculated plants (Kerur and Lakshman, 2004). This is clear from our results where AM fungi + PGPR in all the plants appeared to be more effective at proper dose of fertilizers. In addition, a lot of studies have demonstrated that biofertilizers with proper doses of mineral fertilizers enhanced the growth and yield component (Son *et al*., 2007).

Plants inoculated with AM fungi and treated with different levels of superphosphate showed enhanced nutrient in shoot, oil content and root yield, when compared to uninoculated plants (Sani *et al*., 2010). This is clear from the present results that there was significant increase in growth and yield in all AM fungi + PGPR inoculated seeds. The present results here support the hypothesis that AM fungi along with PGPR can enhance growth and improve nutrient uptake of Soybean at proper dose of P fertilizers (Fattah, 2013). These results are in close conformity of those obtained in the current study and furthermore show consistency in performance of the tested microorganisms.

Similar trends has been reported by Chandrashekara *et al*. (1995) who observed that total biomass, oil content and P-uptake of Sunflower mycorrhizal plants at 38 kg P2O5 ha^{-1} more than non-mycorrhizal plants at 75 kg P2O5 ha^{-1}, the results indicated that AM fungi inoculation helps in saving 25 and 50 per cent of recommended dose of P fertilizers.

Conclusion:

The present study was conducted in order to assess the efficiency of AM fungi along with a

bioinoculants on the growth and P nutrition of Soybean plants at different levels of superphosphate with the possibility of reducing the use of Phosphate fertilizer. Result has shown that microbial inoculants have the capacity to sustain good healthy soil and fertility which add in large extent to yield and quality of products. An interaction of PGPR-plant-AMF is highly promising considering the capacity of both PGPR and AM fungi to help plants in uptake of nutrients, especially with the recommendation that AM fungi may work as a carrier to spread PGPR throughout the rhizosphere (Morrisey *et al*., 2004). Also, certain co-operative microbial activities can be used as low-input biotechnology that increases the stability and productivity of both agricultural systems and natural ecosystems and form a basis for a strategy to help sustainable, environmental-friendly practices.

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