Agriways 3(1): 14-18 (2015)

ISSN: 2321-8614 (Print)
ISSN:2454-2318 (Online)

EFFECT OF CO-INOCULATION OF *B. JAPONICUM*, PSB AND AM FUNGI ON MICROBIAL BIOMASS CARBON, NUTRIENT UPTAKE AND YIELD OF SOYBEAN (*Glycine max* L. merril)

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ABSTRACT

A field experiment was conducted during Kharif 2004 *to study the effect of co-inoculation of B. japonicum, PSB and AM fungi microbial biomass carbon, nutrient uptake and yield of soybean (Glycine max l. merril). var. Pk – 416 on Tarai Mollisols. Sixteen treatment consisting inoculation of B. japonicum, PSB (Pseudomonas striata) and AM fungus (Glomus lamellosum) either alone or in combinations with 40 and 60 kg P_2O_5 per hectare were included in the study. Maximum N uptake (66.06 kg/ha) was shoot with obtained with AMF inoculation + 40 kg P_2O_5 per hectare, which was significantly higher than B. japonicum inoculation alone. The highest N content of 1.73 per cent in shoot was recorded by co-inoculation of AMF + PSB with 40 kg P_2O_5 per hectare and lowest with uninoculated control treatment (1.2%). The maximum P content (0.46%) in shoot was recorded with the treatment of B. japonicum + PSB + 40 kg P_2O_5 per hectare which was significantly higher than microbial inoculation alone or in combination. Microbial biomass carbon was significantly increased with AM fungi inoculation. P did not show any significant effect on microbial biomass carbon. The highest grain yield of 2296 kg per hectare was recorded with B. japonicum inoculation + 60 kg P_2O_5 per hectare which was 9.4 and 5.1 per cent more than B. japonicum inoculation alone and 60 kg P_2O_5 per hectare. Either alone or inoculation in combinations with 40 and 60 kg P_2O_5 per hectare did not show any significant effect on straw and test weight of grain.*

***Keywords*:** *B. japonicum*, PSB, AM fungi, Microbial biomass carbon, Soybean, Yield, NPK

Soybean requires high amount of nutrients due to its high yield potential. A good crop producing 6720 kg ha^{-1} biomass removes about 514 kg nitrogen, 480 kg phosphorus and 485 kg potash ha^{-1} (Nelson, 1989). In case of nitrogen, full nitrogen requirement is not met by symbiosis however; soybean plant has ability of fixing about 240-250 kg ha^{-1} (Chandel *et al.*, 1989) through symbiosis which gets reduced at seed development stage when requirement of nitrogen is maximum. Soybean utilizes atmospheric nitrogen by its symbiotic relationship with *Bradyrhizobium japonicum* to meet a major part of its nitrogen requirement under normal conditions. Soybean harvest about 200 to 250 kg nitrogen per hectare from atmosphere, enriching the soil, thus soybean is also economically profitable as compared to cereals and other oil seed crops. Phosphate solubilization by microorganisms is an important process in natural ecosystems, especially in agricultural land. Some types of microbes such as bacteria, fungi and actinomycetes were reported active in the conversion of insoluble phosphate into soluble phosphates. Some researchers reported that bacteria were more active than other types of microbes in the conversion of P (Sadia *et al*., 2002; Thakuria *et al*., 2004). Bacteria of the genus Bacillus and Pseudomonas can mobilize P of the form is not available through the mechanism of solubilization and increase the availability of P for plants (Richardson, 2001). Vesisular arbuscular micorrhiza (VAM) has an important role in helping to increase the uptake of P by plants due to the increase in absorption capacity through the external hyphae of VAM which is infect plant roots (Mosse, 1981). In P deficient soil inoculation of VAM on soybean plants, with or without P fertilizer was able to increase grain yields by 20% -50%, whereas the application of P fertilizer alone did not increase grain yield (Jalaluddin, 2005). *Rhizobium* inoculation on soybean plants increase mycorrhiza fungal colonization in the root zone (Xie *et al*., 1995). VAM inoculation on peanut enhancing nodule weight and plant N levels (Devi and Reddy, 2002). This suggests a synergistic relationship between mycorrhizal and *Rhizobium*. *Rhizobium* requires phosphorus for its growth and survival in soil, rhizospheric colonization and energy transformation during nitrogen fixation in root nodules. Therefore the study was undertaken to evaluate the influence of *B. japoniucm*, PSB (*Pseudomonas striata*) and AM fungus (*Glomus lamellosum*) on soybean with applied phosphorus.

MATERIALS AND METHODS

A field experiment was conducted at the Crop Research Centre of Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, during *Kharif* 2004 to evaluate the effect of PSB and AM inoculation on soybean *Bradyrhizobium* symbiosis on silty clay loam soil having organic carbon 0.75 per cent, available nitrogen (N) 295.62 kg per hectare, available phosphorus (P) 19.71 kg per hectare, available potash (K) 140.12 kg per hetare and pH 7.2. Treatments consisted of inoculation of *B. japoniuc*, PSB (*Pseudomonas striata*) and AM fungus (*Glomus lamellosum*) either alone or in combinations with 40 and 60 kg P_2 O_5 per hectare applied as basal through SSP and uninoculated control treatment with three replicaions. The experiment was laid out in randomized block design (RBD) in plots of 3 m × 4 m size. Soybean (*var*. PK-416) seed was treated with 20 g inoculants of *B. japoniucm*, PSB (*Pseudomonas striata*) and AM fungus (*Glomus lamellosum*) per kg see at the time of sowing. The microbial biomass C was estimated by Vance *et al.* (1987) method. Pour plate serial dilution method was used for estimating the population of total bacteria, fungi and actinomycetes in soil (Subba and Rao, 1986). Nitrogen and phosphorus content in plant was determined by modified Kjedhal (Jackson, 1967) and vanadomolybdo phosphoric yellow colour method in nitric acid system (Jackson, 1973) after grinding the sample to 40 mesh After threshing and proper cleaning, the grain yield of individual plot was recorded with single pan balance and expressed as kg per hectare after conversion.

RESULTS AND DISCUSSION

Nitrogen and phosphorus content

The application of phosphorus increased plant nitrogen content because phosphorus in required for nodular tissue formation and as a source of ATP for activation of nitrogenase enzyme. Singh *et al*. (2001) reported similar results in soybean and found that nitrogen content was significantly more with the application of 60 kg P_2O_5 per hectare. The highest nitrogen content of 1.73 per cent (Table 1) was recorded by co-inoculation of PSB and AMF along with application of 40 kg P_2O_5 per hectare and lowest in uninoculated control (1.2%), which may be due to more availability of energy for N_2 fixation. Inoculation of AMF and PSB significantly increased phosphorus content in plant over uninoculated control treatment probably due to phosphorus solubilizing action of PSB and phosphorus mobilizing effect of AM fungi which increased available phosphorus to the plant roots. Application of *Rhizobium*, PSB and VAM has resulted in improved nodulation in plant roots and resulted in supplying higher amount of nitrogen for growth and yield attributes which inturnhelped to realize higher growth parameter and dry matter of soybean. Similar observations were earlier reported by Dubey (1995) and Thenua *et al*. (2010). In addition to this, combination of *Rhizobium*, PSB and VAM has resulted in cumulative effects such as supply of N and P to the crop along with production of growth promoting substances like auxin, gibberlins and cytokinins. These results are in agreement with the findings of Singh (2005), Tomar (2011) Nagaraju and Mohankumar (2010). Sreenivasa *et al*. (1995) also found significant increase in phosphorus content of plant with dual inoculation of *B. japonicum* + *Glomus fasciculatum.* Inoculation of *B. japonicum* and AM fungi recorded significant increases of 64.4 and 43.9 per cent in total nitrogen uptake, respectively, over uninoculated control and *B. japonicum* alone inoculation. It seems that the combined effect of both organisms on the availability of nitrogen to plant through more supply of phosphorus by AM fungi needed for more fixation of nitrogen by *B. japonicum.* Similarly, Singh and Singh (1993) reported that maximum uptake of N was obtained by the combined inoculation of *B. japonicum* + *Aspargillus awamori* in soybean. The highest uptake of P 10.51 kg per hectare (Table 1) was recorded by AM fungi inoculation with 40 kg P_2O_5 per hectare which was similar to that obtained by combined inoculation of *B. japonica* and PSB with 40 kg P_2O_5 per hectare, possibly because of increased amount of available phosphorus by fertilizer, PSB and AM funi to the plant and N supply by *B. japonicum.* Similar results were reported by Bardet *et al*. (1986) who found that super phosphate fertilization along with *B. japonicum* inoculation significantly increased P uptake by soybean plant. Suryantini (2014) also showed in his experiment results a synergistic effect of Rhizobium, PSB and VAM in increasing growth, root nodules dry weight, N and P uptake, grain yield and yield components of soybean. Inoculation of *B. japonicum* alone resulted numerical increase of 27 per cent in microbial biomass carbon over uninoculated control treatment by AM fungi alone inoculation

ISSN: 2321-8614 (Print)
ISSN:2454-2318 (Online)

significantly increased microbial biomass carbon over uninoculated control treatment, *B. japonicum*, PSB alone inoculation and dual inoculation of AM fungi + *B. japonicum*. Dual inoculation of *B. japonicum* + AM fungi showed numerical increase (20.1%) in microbial biomass carbon over uninoculated control treatment. However, phosphorus application @ 60 kg per hectare recorded numerical increase of 55.3 per cent in microbial biomass carbon over 40 P_2O_5 kg per hectare. The increase in microbial biomass carbon due to microbial inoculation showed the increase in microbial cells in soil over the uninoculated control treatment. AM fungi inoculation recorded significant increase in microbial biomass carbon over *B. japonicum* and PSB alone inoculation treatments due to development of more fungal mycelium in soil. Application phosphorus with microbial inoculants recorded significant increase in microbial biomass carbon over microbial inoculation without applied phosphorus showed the effect of applied P due to more cellular components were synthesized. Moharram *et al.* (1999) conducted a green house pot experiment and observe that soybean cultivar clark inoculated with *B. japonicum* + compost increase biomass carbon.

Yield and yield attributes

An increase of 14.9 per cent in the seed yield of soybean (Table2) over uninoculated control was obtained with inoculation of *B. japonicum*. It might be due to *rhizobium* is a symbiotic N fixer in pulses and oilseed crops and is known to produce growth promoting substances like IAA, gibberellins and cytokinins etc., which help in enhancing the plant growth and yield. Many research studies have showed that dual or triple microbial inoculation is highly beneficial to crop growth in obtaining higher crop yield (Iraj *et al*. 2009). Praharaj and Dhingra (2001) also reported that *B. japonicum* inoculation significantly enhanced the seed yield of soybean by 10.40 per cent over uninoculated control. However, when *B. japonicum* was inoculated with AM fungi or PSB yield increased significantly showing the combined effect of nitrogen and phosphorus availability to the plants due to combined action of these organisms. These findings are in corroboration with the result reported by Suryantini (2014) who observed that the highest increased in grain yield (102%) over control (without biofertilizer) was obtained from the combination of the three biofertilizers (Bradyrhizobium+ PSB + VAM). The highest grain yield of 2296 kg per hectare was recorded with *B. japonicum* inoculation + 60 kg P_2O_5 per hectare which was 9.4 and 5.1 per cent more than *B. japonicum* inoculation alone and 60 kg P_2O_5 per hectare, respectively, because of more availability of phosphorus supplied through chemical fertilizer along with N fixed by *B. japonicum*. Mausumi *et al.* (1997) also reported that *Rhizobium a*nd phosphorus interaction significantly increased grain yield of soybean.

The applied treatments did not show significant effect on the straw yield over uninoculated control treatment. However, highest straw yield of 2498 kg per hectare was recorded with combined inoculation of AM + PSB with 40 kg P_2O_5 per hectare. All the treatment showed numerical increase in straw yield over the uninoculated control treatment. Alone inoculation of *B. japonicum*, AM fungi and PSB resulted in 15.2, 12.2 and 26.0 per cent numerically higher straw yield over uninoculated control treatment. Similar results were recorded by Singh and Singh (1993) who found that application of rock phosphate, *Rhizobium*, VAM and PSM increase in straw yield of soybean.

The applied treatments did not show significant effect on test grain weight over uninoculated control treatment. The test grain weight ranged from 86.01 to 95.45 gm. The highest test grain weight (95.45 gm) was recorded with AM fungi inoculation followed by *B. japonicum* + PSB inoculation with application of 40 kg P_2O_5 per hectare (94.39 gm).

Table-1: Effect of *B. japonicum*, PSB and AM fungi inoculation with applied phosphorus doses on microbial biomass carbon, microbial population, N, P, content and uptake at harvest and yield of soybean

Treatment	Nutrient content in shoot (%)		Nutrient uptake at harvest (kg / ha)		Microbial biomass carbon (µ g / gm soil)
	N	P	N	P	
Uninoculated control	1.20	0.29	36.35	5.61	252.05
B. japoniucm alone	1.61	0.30	41.55	8.25	321.08
AMF alone	1.21	0.37	51.21	6.51	409.30
PSB alone	1.42	0.35	49.85	9.58	265.33
B. japoniucm + AMF	1.66	0.34	59.83	7.31	302.81
B. japoniucm + PSB	1.64	0.38	57.48	7.28	274.78
AMF + PSB	1.63	0.31	56.21	6.29	251.72
B. japoniucm + SSP @ 60 kgP_2O_5 ha^{-1}	1.55	0.34	62.03	7.51	248.27
B. japoniucm + SSP @ 40 kgP_2O_5 ha^{-1}	1.55	0.45	62.03	7.51	308.29
AMF + SSP @ 40 kgP_2O_5 ha^{-1}	1.68	0.38	66.01	10.51	250.79
PSB + SSP @ 40 kgP_2O_5 ha^{-1}	1.42	0.31	48.58	8.21	248.60
B. japoniucm + AMF + SSP @ 40 kgP_2O_5 ha^{-1}	1.57	0.46	60.36	7.48	386.67

ISSN: 2321-8614 (Print)
ISSN:2454-2318 (Online)

B. japoniucm + PSB + SSP @ 40 kgP_2O_5 ha^{-1}	1.59	0.31	60.69	10.51	349.36
AMF + PSB + SSP @ 40 kgP_2O_5 ha^{-1}	1.73	0.34	63.23	9.05	360.49
SSP @ 40 kgP_2O_5 ha^{-1}	1.56	0.37	58.42	7.77	237.87
SSP @ 60 kgP_2O_5 ha^{-1}	1.59	0.21	59.82	8.42	293.19
SEm (±)	0.14	0.21	6.01	1.23	2.15
CD (P = 0.05)	NS	0.06	17.38	NS	73.08
CV (%)	8.55	15.18	10.51	16.28	14.13

Table-2: Effect of co-inoculation of *Bradyrhizobium japonicum*, PSB, AM fungi with phosphorus on yield and yield attributes of soybean

Treatments	Grain yield (kg ha^{-1})	Straw yield (kg ha^{-1})	100 grain weight (gm)
Uninoculated control	1827	1905	86.01
B. japoniucm alone	2099	2196	89.07
AMF alone	1994	2138	95.45
PSB alone	1976	2401	90.69
B. japoniucm + AMF	2116	2148	90.52
B. japoniucm + PSB	2138	1998	86.58
AMF + PSB	2074	1971	93.01
B. japoniucm + SSP @ 60 kgP_2O_5 ha^{-1}	2296	2148	93.55
B. japoniucm + SSP @ 40 kgP_2O_5 ha^{-1}	2296	2240	93.41
AMF + SSP @ 40 kgP_2O_5 ha^{-1}	2044	2296	90.22
PSB + SSP @ 40 kgP_2O_5 ha^{-1}	2034	2148	93.87
B. japoniucm + AMF + SSP @ 40 kgP_2O_5 ha^{-1}	2155	2333	90.68
B. japoniucm + PSB + SSP @ 40 kgP_2O_5 ha^{-1}	2106	2277	94.39
AMF + PSB + SSP @ 40 kgP_2O_5 ha^{-1}	2261	2498	87.94
SSP @ 40 kgP_2O_5 ha^{-1}	2130	2231	90.66
SSP @ 60 kgP_2O_5 ha^{-1}	2184	2259	92.09
SEm (±)	97.09	312	3.13
CD (P = 0.05)	281.0	NS	NS
CV	8.09	-	5.95

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