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Original Research Article

Composite Rhizobium Inoculation on Soybean and Its Residual Effect on Chickpea

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Composite *Rhizobium*, Soybean, Chickpea, *B. japonicum*, *R. leguminosarum*

A B S T R A C T

Application of composite *Rhizobium* culture (*B. japonicum* + *R. leguminosarum*) to soybean exhibited either similar effect to that of alone application of *B japonicum* or slight synergistic effect in terms of nodulation to soybean i.e.2.00, 16.83 and 21.67 nodules/plant at 30, 45 and 60 DAS respectively compared to 1.33, 14.00 and 19.17 nodules/plant at 30, 45 and 60 DAS respectively in alone application of *B. japonicum*. Nodule dry weight and plant dry weight at 30, 45 and 60 DAS and nitrogen uptake by the plant was also more in inoculation of composite culture compared to alone application of *B. japonicum.* In chick pea, fresh inoculation of *R. leguminosarum* increased the nodulation i.e. 1.00, 6.17 and 9.17 nodules/plant at 30, 45, and 60 DAS respectively, compared to 0.67, 2.83 and 6.00 nodules/plant at 30, 45 and 60 DAS respectively in the residual composite culture of *B. japonicum* and *R. leguminosorum* which was applied to soybean during kharif. Residual composite culture yielded less nodules dry weight and plant dry weight at 30, 45 and 60 DAS as well as nitrogen uptake by the plant compared to fresh inoculation of *R. leguminosorum*.

Introduction

In modern days, for highest crop production within available area without environment and soil hazard, it is necessary to have alternate source of expensive chemical fertilizers which have low cost along with renewable source of input. One of the potent tools which can effectively be used for increasing crop production under tropical condition is the use of biofertilizer Most of legume crops are naturally gifted with nitrogen fixing bacteria with the “Nodule” in which the atmospheric nitrogen is fixed and supplied partly to intact plant there by increasing grain yield and diffused through roots on the surrounding soil. Soybean and chickpea are the legumes that can make use of nitrogen fixing bacteria called *Rhizobium*.

Generally soybean is followed by chickpea in Vidarbha region. *Bradyrhizobium japonicum* and *Rhizobium leguminosarum* are host specific to soybean and chickpea crop respectively.

The farmer has to apply *Bradyrhizobium japonicum* and *Rhizobium leguminosarum* to soybean and chickpea during respective crop season. *Bradyrhizobium japonicum* of soybean cannot be used for chickpea and vice versa due to host specificity of *Rhizobium*. Whether one species of *Rhizobium* when applied along with another species is having any adverse or synergistic effect on the applied host or any advantage to the next crop as residual effect is not known. If composite culture of *Rhizobium* containing species for both the crop is applied to kharif crop shows any additional effect on rabi crop the cost and method of application can be made easy and user friendly. In this context the present study was conducted.

Materials and Methods

Five days old broth culture of *Bradyrhizobium japonicum* and *Rhizobium leguminosarum* were used for preparation for preparation of composite culture so as to adjust moisture level of final product to 50% water holding capacity of the carrier. Individual culture was made by mixing respective broth in carrier in 1:2 proportion. The composite culture was made by mixing equal proportion of broth culture (1:1) and then mixing it in carrier in 1:2 proportion.

Initial *Rhizobial* population at the time of mixing was adjusted to 10^9 cell/g in both the cultures. The *Rhizobial* population after curing in each carrier was quantified by following usual dilution plate method using YEMA medium. The carrier based composite culture as well as host specific culture was stored at room temperature (28 ± 2^0C). The treatments were applied to soybean seeds of var. JS-335 during kharif and grown in pots (containing sterilized soil) in triplicate. After harvest of the soybean in the same pots chickpea seeds of var. JAKI 9218 was sown by applying treatments as per details while residual effect of composite culture was observed without any treatment to chickpea seeds. Treatments were given as

Crop: Soybean (during kharif)

T_1 - Control

T_2 - *B. japonicum* @ 25 g/kg

T_3 - *B. japonicum* + *R leguminosarum* (Composite culture @ 25 g/kg)

T_4 - *B. Japonicum* @ 25 g/kg + Molybdenum @ 4 g/kg

T_5 - *B. japonicum* + *R leguminosarum* (Composite culture @ 25 g/kg) + Molybdenum @ 4 g/kg

T_6 - RDF (25: 50: 0 NPK)

T_7 - 75% RDF

T_8 - 50% RDF

T_9 - 25% RDF

T_{10} - Molybdenum @ 4 g/kg of seed

Crop: Chickpea (during rabi)

T_1 - Control

T_2 - *R. leguminosarum* @ 25 g/kg

T_3 - *B. japonicum* + *R leguminosarum* (Residual composite culture)

T_4 - *R leguminosarum* @ 25 g/kg + Molybdenum @ 4 g/kg

T_5 - *B. japonicum* + *R leguminosarum* (Residual composite culture) + Molybdenum @ 4 g/kg

T_6 - RDF (25: 50: 0 NPK)

T_7 - 75% RDF

T_8 - 50% RDF

T_9 - 25% RDF

T_{10} - Molybdenum @ 4 g/kg of seed

Results and Discussion

Effect of composite *Rhizobium* inoculation on soybean

In soybean highest germination percentage (81.67), shoot length (8.38cm), root length (7.35cm) and seedling vigour index (1279.37) was recorded in treatment T_6 i.e.100% RDF (Table 1). The seedling vigour index (1107.07) recorded in treatment T_3 i.e. application of *B. japonicum* + *R. leguminosarum* was at par with application of *B. japonicum* alone (T_2) i.e.1078.25. The application of molybdenum in both the treatments yielded increased seedling vigour index. However molybdenum alone in treatment (T_{10}) was not encouraging and was at par with control (T_1). The increase in germination percentage, shoot length, root length and seedling vigour index in *Rhizobium* applied treatment over control (T_1) and alone application of molybdenum (T_{10})was might be due to various plant growth promoting substances secreted by *Rhizobium* which get more activated due to molybdenum. Many workers reported increase in germination percentage, SVI and growth promoting activity of *Rhizobium* including Krishna *et al.,* (2008), Alahadadi *et al.,* (2009), Poonam Gautam and Pant (2002) and Nezarat and Gholami (2009).

In this experiment as soil was sterilized only inoculated treatments showed nodulation. At 30 DAS the treatment T_5 i.e. *B. japonicum* + *R. leguminosarum* + molybdenum produced at par number of nodules (3.17) with treatment T_4 i.e. *B. japonicum* molybdenum (2.50) and this was followed by treatment T_3 (*B. japonicum* + *R. leguminosarum*) i.e. 2.00 nodule par plant (Table 2) Further enhancement in number of nodules was noticed at 45 and 60 DAS. Treatment T_5 produced significantly higher root nodules per plant i.e.19.17 nodule/plant at 45 DAS and 25.83 nodule/plant at 60 DAS which was at par with treatment T_4 (17.83 nodule/plant at 45 DAS and 24.33 nodule/plant at 60 DAS).The increase in nodulation due to application of *B. japonicum* to soyabean have also been recorded by Sharma and Namdev(1999), Pramila Rani and Kondandaramaiah (1997), Bhuiyan *et al.*,(1997) and Chendrayan *et al.*, (2003). The result showed that the application of *R. legunimosarum* with B *japonicum* to soybean either having similar effect to that of alone application of *B. japonicum* or slight synergistic effect in term of nodulation. Although Islam (1980) observed more nodulation due single inoculation of *Rhizobium* than combination of strains, they found more or less same number of nodule per plant due to single or multiple strains inoculation in chickpea.

Treatment T_5 i.e. *B. japonicum* + *R. legunimosarum* +molybdenum yielded at par nodule dry weight (4.17 mg/plant) with B. *japonicum* + molybdenum (3.78 mg/pant) at 30 DAS exhibiting neither synergistic nor antagonistic effect of *R. leguminosoram* on soybean. However at 45 and 60 DAS application of *B. japonicum* with *R. legunimosarum* and molybdenum resulted significantly superior nodule dry weight (18.67 and 22.33 mg/plant respectively) as compared to application of B. *japonicum* + molybdenum (16.83 and 20.33 mg/plant). This showed that *R. leguminosaram* boosted the performance of *B. japonicum* in soybean which may be due to plant growth promoting activity of *Rhizobium* irrespective of host specificity for nodulation. Mahamoud *et al.,* (1994) were also of the same opinion. Increase in nodule dry weight due to *Rhizobium* was also recorded by Chore and Shastri (1991), Dubey (1997), Bhuiyan *et al.,* (1997), Thakre *et al.,* (1996), and Chandrayan *et al.,* (2003). The present study showed that addition of molybdenum enhanced the positive effect of treatments. The significant effect of molybdenum on yield contributing factors of soybean was also opined by Heidarzade *et al.,* (2016).

Maximum plant dry weight (0.84, 2.10 and 4.42 g/plant at 30, 45 and 60 DAS) was recorded in treatment T_6 i.e. 100% RDF which was at par with treatment T_5 i.e. *B.japonicum* + *R.leguminosrum* +molybdenum and also with T_7 i.e. 75% RDF where plant dry weight was recorded as 0.81, 2.06 and 4.23 g / plant at 30, 45 and 60 DAS respectively in T_5 and 0.81, 2.03 and 4.17 g /plant at 30, 45 and 60 DAS respectively in T_7. More plant dry weight due to application of *Rhizobium* and Mo have also been noticed by Bhuiyan *et al.,* (1997). The increase in plant dry weight in RDF was might be due to more amount of nutrient supplied through chemical fertilizer at sowing which provided more nutrient as compared to other treatments. Rapid availability of nutrient through fertilizer might resulted more uptake of nutrient there by increase in plant dry weight over other treatments.

The significant differences in N uptake by plant over control were observed. Maximum N uptake was noticed in treatment T_6 i.e. RDF (20.73 kg/ha). The treatment T_7 i.e.75% RDF recorded 18.60 kg/ha N uptake by plant followed by T_8 (17.38 kg/ha) which was at par with treatment T_5 i.e. *B. japonicum* + *R. leguminosarum*+ molybdenum (16.07 kg/ha). Addition of *R. leguminosarum* with B *japonicum* (T_3) showed 16.43 kg/ha N uptake which was more than the alone application of *B. japonicum* (14.79 kh/ha). Significant variation in respect of nitrogen uptake by the plant due to various treatments over uninoculated control was shown by Dudeja (1981) and Jain and Rewari (1973).Khan *et al.,* (1997) recorded increase in N content and uptake by shoot in *Rhizobium* inoculated plant as compared to control.

Residual effect of composite *Rhizobium* inoculation on chickpea

Table 3 showed that treatments vary significantly over control in terms of seed germination and seedling vigour index. Although highest germination percent, shoot length, root length and seedling vigour index was recorded in RDF i.e. 84.33%., 8.58 cm,7.15 cm and 1326.56 respectively, the application of *R.leguminosarum* with molybdenum yielded at par SVI (1218.18) with 50% RDF which may be due to PGPA of rhizobia. Khan *et al.,* (1997) also recorded increase in shoot and root length due to *Rhizobium* in Chickpea. However the residual *Rhizobium* treatment (T_3) was not encouraging and yielded at par SVI (783.47) with control (765.60). The increase in SVI due to application of *Rhizobium* is well documented, but the poor performance of residual *Rhizobium* was might be due to decrease in plant growth promoting substances secreated by *Rhizobium* with time span which already benefitted the kharif crop as PGPR.

Table 4 revealed more nodulation due to fresh application of *R. leguminosarum* with molybdenum compared to residual *R.leguminosarum*. However residual *Rhizobium* inoculation significantly increased number of nodule over control at 30, 45 and 60 DAS. In present study addition of molybdenum to *Rhizobium* inoculation increase the nodulation which confirms the finding of Rao *et al.,* (1978) in soybean.

The treatment *R. leguminosarum* + molybdenum (T_4) recorded significantly higher nodule dry weight (1.83mg/plant) at

30 DAS followed by treatment of *R leguminosarum* i.e. T_2 (1.67mg/plant). The treatment *B. japonicum* + *R. leguminosarum* + molybdenum (T_5) and *B. japonicum* + *R. leguminosarum* (Residual) (T_3), recorded 1.00 mg/plant nodule dry weight and 0.89 mg/plant nodule dry weight respectively. The nodule dry weight increased up to 14.67 mg/plant at 60 DAS in treatment T_4 i.e. fresh application of *R. leguminosarum* + molybdenum. The application of fresh *Rhizobium* recorded more nodule dry weight over control. Vaisha and Dude (1991) also reported significant enhancement in nodule dry weight/plant with *Rhizobium* inoculation in chickpea. They further noticed that nodule dry weight per plant increased when *Rhizobium* +molybdenum (@4g/kg seed) treatment was combined which was superior over all other treatments. The results obtained in present study are in agreement of finding confirmed by Chandra (1995).

The performance of residual *Rhizobium* treatment was poor as compared to fresh inoculation and different level of RDF in respect of plant dry weight during observations at 30, 45 and 60 DAS. It was observed that fresh inoculation of *R. leguminosarum* increased the plant dry weight which further gets increased by addition of molybdenum as compared to residual treatment. However RDF recorded maximum plant dry weight at each observation. Bhuiyan *et al.,* (1997) also reported that plants along with chemical fertilizer produced significantly more shoot dry weigh over inoculated plant. In present study the increase in plant dry weight over control was recorded in fresh as well as residual application of Rhizobium. Chandra (1995) and Namdeo *et al.,* (1989) reported that dry matter weight/plant was significantly increased by Rhizobium inoculation. In regards to fresh application of *Rhizobium*, results are in accordance with the finding of Bhattacharya *et al.,* (1997) and Wankhede *et al.,* (1991). But the increase in plant dry weight due to residual *Rhizobium* could not be correlated with earlier finding due to lack of literature.

Table.1 Effect of composite *Rhizobium* inoculation on seedling vigour index in soybean

Treat No.	Treatment details	Germination (%)	Shoot Length	Root Length	Seedling vigour index
T_1	Control	72.00 (8.49)	6.56	5.66	879.84
T_2	*B. japonicum*	75.67 (8.70)	7.54	6.71	1078.25
T_3	*B. japonicum + R leguminosarum*	77.00 (8.76)	7.82	6.62	1107.07
T_4	*B. japonicum + Mo*	76.67 (8.77)	8.14	6.95	1161.93
T_5	*B. japonicum + R leguminosarum* + Mo	77.67 (8.81)	8.25	7.12	1193.74
T_6	RDF	81.67 (9.04)	8.38	7.35	1279.37
T_7	75% RDF	81.33 (9.01)	8.11	7.06	1238.88
T_8	50% RDF	80.33 (8.86)	8.05	6.85	1196.97
T_9	25% RDF	77.33 (8.79)	7.87	6.79	1133.71
T_{10}	Molybdenum	74.33 (8.62)	7.11	5.96	971.54
	F Test	Sig			Sig
	SE (m)±	0.06			17.92
	CD P=0.01	0.19			52.85

Table.2 Effect of composite *Rhizobium* inoculation on nodulation, nodule and plant dry weight and N uptake in soybean

Treat no	Treatment details	Number of nodule			Nodule dry weight (mg/plant)			Plant dry weight (g/plant)			N uptake by plant (kg/ha)
		30 DAS	45 DAS	60 DAS	30 DAS	45 DAS	60 DAS	30 DAS	45 DAS	60 DAS	
T_1	Control	0.00	0.00	0.00	0.00	0.00	0.00	0.64	1.21	2.50	8.61
T_2	*B.japonicum*	1.33	14.00	19.17	1.67	12.67	17.67	0.78	1.75	3.08	14.79
T_3	*B.japonicum + R.leguminosarum*	2.00	16.83	21.67	2.67	15.67	19.67	0.79	1.86	3.58	16.43
T_4	*B.japonicum + Mo*	2.50	17.83	24.33	3.78	16.83	20.33	0.80	1.93	3.75	15.74
T_5	*B.japonicum + R.leguminosarum* + Mo	3.17	19.17	25.83	4.17	18.67	22.33	0.81	2.06	4.23	16.07
T_6	RDF	0.00	0.00	0.00	0.00	0.00	0.00	0.84	2.10	4.42	20.73
T_7	75% RDF	0.00	0.00	0.00	0.00	0.00	0.00	0.81	2.03	4.17	18.60
T_8	50% RDF	0.00	0.00	0.00	0.00	0.00	0.00	0.81	2.02	3.92	17.38
T_9	25% RDF	0.00	0.00	0.00	0.00	0.00	0.00	0.80	2.00	3.17	16.74
T_{10}	Molybdenum	0.00	0.00	0.00	0.00	0.00	0.00	0.72	1.49	2.92	9.15
	F Test	Sig	Sig	Sig	Sig	Sig	Sig	Sig	Sig	Sig	Sig
	SE (m)±	0.27	0.47	0.57	0.33	0.52	0.42	0.014	0.016	0.016	0.36
	CD P=0.01	0.79**	1.40**	1.69**	0.97**	1.53**	1.24**	0.034**	0.047**	0.041**	1.06

Table.4 Residual effect of composite *Rhizobium* inoculation on nodulation, nodule and plant dry weight and N uptake in chickpea

Treat no	Treatment Details	Nodule number			Nodule dry weight(mg/plant)			Plant dry weight(g/plant)			N uptake by plant(kg/ha)
		30 DAS	45 DAS	60 DAS	30 DAS	45 DAS	60 DAS	30 DAS	45 DAS	60 DAS	
T_1	Control	0.00	0.00	0.00	0.00	0.00	0.00	0.61	1.09	1.66	6.30
T_2	*B.japonicum*	1.00	6.17	9.17	1.67	8.33	12.50	0.86	1.59	2.18	16.71
T_3	*B.japonicum + R.leguminosarum*	0.67	2.83	6.00	0.89	3.33	8.33	0.72	1.22	1.92	11.44
T_4	*B.japonicum + Mo*	1.67	8.17	13.17	1.83	10.50	14.67	0.92	1.75	2.35	15.71
T_5	*B.japonicum + R.leguminosarum* + Mo	0.83	3.33	8.50	1.00	4.17	10.50	0.74	1.27	2.00	13.00
T_6	RDF	0.00	0.00	0.00	0.00	0.00	0.00	1.06	1.92	2.48	22.77
T_7	75% RDF	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	19.66
T_8	50% RDF	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	18.40
T_9	25% RDF	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	12.29
T_{10}	Molybdenum	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	8.99
	F Test	Sig	Sig	Sig	Sig	Sig	Sig	Sig	Sig	Sig	Sig
	SE (m)±	0.16	0.25	0.42	0.06	0.31	0.28	0.04	0.05	0.02	0.71
	CD P=0.01	0.47*	0.73**	1.25**	0.19**	0.92**	0.84**	0.12**	0.16**	0.06**	2.11

Table.3 Residual effect of composite *Rhizobium* inoculation on seedling vigour index in chickpea

Treat No	Treatment Details	Germination (%)	Shoot length (cm)	Root length(cm)	Seedling Vigour Index
T_1	Control	73.33 (8.56)	6.3	4.14	765.60
T_2	*B.japonicum*	77.67 (8.81)	8.4	6.5	1157.23
T_3	*B.japonicum + R.leguminosarum*	75.33 (8.86)	6.51	3.53	783.47
T_4	*B.japonicum + Mo*	79.00 (8.89)	8.57	6.85	1218.18
T_5	*B.japonicum + R.leguminosarum* + Mo	76.67 (8.76)	7.45	4.12	887.03
T_6	RDF	84.33 (9.18)	8.58	7.15	1326.56
T_7	75% RDF	83.00 (9.11)	8.31	7.06	1275.71
T_8	50% RDF	81.67 (9.04)	8.05	6.85	1216.83
T_9	25% RDF	77.33 (8.79)	7.87	6.79	1133.71
T_{10}	Molybdenum	74.00 (8.60)	6.11	5.86	885.78
	F Test	Sig			Sig
	SE (m)±	0.069			15.84
	CD P=0.01	0.203			46.72

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