Indian Journal of Agronomy **55**(1): 64–67 (March 2010)

Bio inoculants for enhancing the productivity and nutrient uptake of winter irrigated cotton (*Gossypium hirsutum*) under graded levels of nitrogen and phosphatic fertilizers

P. NALAYINI* K. SANKARANARAYANAN AND R. ANANDHAM

Central Institute for Cotton Research, Regional Station, Coimbatore, Tamil Nadu 641 003

Received: July 2008

ABSTRACT

Field experiments were conducted during winter (August – February) seasons of 2003-04 and 2004-05 under irrigated condition at Coimbatore to study the usefulness of bio-inoculants in reducing the inorganic nitrogen and phosphatic fertilizers for cotton. Four graded levels of N and P (0,50,75 and 100 % recommended dose (RDNP) and four bio inoculants viz*.*, *Azospirillum* from, Haryana, Tamil Nadu and Surat were combined with Phosphorus Solubilizing Bacteria (PSB) + Pink Pigmented Facultative Methylotrophs (PPFM) along with uninoculated control. Cotton 'LRA 5166' responded significantly to graded levels of N and P and bio-inoculants. Application of 75% RDNP along with *Azospirillum* (HAU) + PSB + PPFM yielded 2.91 tonne/ha seed cotton with higher net return (Rs 40,553) and net B:C ratio value of 1.94 while, application of 100% RDNP without bio-inoculants yielded only 2.54 tonne/ha. Judicious combination of bio-inoculants along with moderate use of chemical fertilizers is recommended for cotton.

Key words: Inoculation methods, Nitrogen, Nutrient uptake, Phosphorus, Seed cotton yield

Nitrogen and phosphorus are the two major plant nutrients required for higher productivity in many field crops grown in our country as majority of Indian soils are either low or medium in status for these two nutrients. Although nutrients are supplied as fertilizers to soil, they get either fixed or lost by various processes. However, micro-organisms play a major role in nutrient recycling in soil for supplementing crop requirements of nutrients either by dissolution from the soil reserve or by fixation from the atmosphere. Pink pigmented facultative methylotrophs (PPFM) influences seed germination and seedling growth by producing plant growth regulators like zeatin and related cytokinins. The efficiency of conventional bio-inoculants like *Azospirillum* and phosphorus solubilizing bacterium (PSB) can be enhanced by co inoculating with PPFM (Nalayini *et al.,* 2004). The gradual reduction in the use of pesticides and fertilizers and greater use of the biological and genetic potential of plant and microbial species may alleviate the high demand of agro-chemicals in sustaining high production in agriculture. However, reports are scanty on the use of mixed bio-inoculants for reducing the inorganic fertilizers for cotton. Hence an attempt was made to study the feasibility of using the N fixers (*Azospirillum*), phosphate solubilizers and plant growth hormone synthesizers (PPFM) under graded levels of N and P fertilizers to enhance the yield at desired level of fertilizer input.

***Corresponding author**: (E mail: nalayiniganesh@gmail.com)

MATERIALS AND METHODS

Field experiments were conducted at Central Institute for Cotton Research, Regional Station, Coimbatore during winter seasons of 2003-04 and 2004-05 under irrigated condition. The design used was split plot with 3 replications. The main plot consisted of 4 fertilizer treatments *viz.,* 0, 50, 75 and 100% recommended dose of N and P fertilizers (60 and 13.2 kg/ha) and K is applied at recommended rate (25 kg/ha) uniformly to all the treatments. The sub-plot treatments are un-inoculated control, *Azospirillum* (TNAU) + PSB + PPFM, *Azospirillum* (HAU) + PSB + PPFM, *Azospirillum* (Surat) + PSB + PPFM. All the bio-inoculants culture were supplied by Department of Microbiology, Tamil Nadu Agricultural University (TNAU). The experimental soil was sandy clay loam with the pH (1: 2.5) of 8.35 with EC of 0.49 dS/m with low (165 kg/ha), medium (17.5 kg/ha) and high (580 kg/ha) in available N, P and K. The FYM @ 5 tonne/ha was applied common to all the treatments. The N, P and K content of FYM used was 0.52 ,0.49 and 0.61% respectively. Entire P as per treatment was applied as basal, while N and K were given in 2 equal splits at sowing and at 45 days later. The bio-inoculants were given as soil application as well as seed dressing. For seed dressings 20 g each bio-inoculants/kg of seed were used and the seed treatment was done one day prior to sowing. For soil application, 800 g of peat based culture of each /ha was mixed with 500 kg/ha of finely powdered FYM and spread uniformly over the ridges just before sowing. The sowing was done on 27 and 23 August in 2003 and 2004 cropping season respectively. Cotton 'LRA 5166' was sown at 75 cm x 30 cm. Besides, soil and seed treatments, PPFM was also given as foliar spray at 45 and 90 DAS. For foliar spraying, PPFM was mass multiplied in ammonium mineral salt medium until the population load of 10^9 cfu./ml in the broth and sprayed using knapsack sprayer. The plant biometric observations were recorded at 90 DAS and the yield attributes and yields at 150 DAS. Two seed cotton pickings were done at last week of January and third week of February. The plant samples collected for dry matter accumulation on 90 DAS were ground using wiley mill and analysed chemically for N and P. For arriving the nutrient uptake, the nutrient content was multiplied with dry matter production/ha and expressed in kg/ha. Enumeration of *Azospirillum* in rhizosphere soil was carried out in N-free semi-solid malic acid medium using most probable number technique (Dobereiner *et al*., 1976). PPFM and PSB were enumerated through standard dilution plate technique in ammonimum mineral salts medium and Pikovskaya's medium (Whittenbury *et al*., 1970 and Pikovskaya, 1948). The same trend was obtained in both years of experimentation and hence the data were pooled and analysed.

RESULTS AND DISCUSSION

Growth

The plant height was numerically enhanced due to the N and P levels and among the treatments, 100% RDNP produced 5.5 cm taller plants than control. The bio-inoculated plants were taller by 2.2–5 cm than uninoculated cotton. The dry matter accumulation followed the same trend as that of plant height (Table 1) and the enhancement in dry matter accumulation was 7.9, 12.8 and 15.7% at 50, 75 and 100% RDNP respectively over control. The bio-inoculants also enhanced the dry matter accumulation. The enhanced dry matter accumulation ranged from 4.9–15.2 % over uninoculated control. Higher dry matter accumulation with inoculation might be due to faster growth of inoculated plants due to enhanced uptake of nutrients. Dry matter enhancement due to inoculation was also reported by Anandham *et al.* (2006).

Boll number and boll weight

The production of bolls/plant was favourably influenced due to the application of 75 and 100% RDNP and was statistically on par and significantly higher than 50% RDNP and control. Among the bio-inoculated treatments, *Azospirillum* (HAU) + PSB + PPFM produced significantly higher number of bolls/plant (24.8 bolls/plant) than *Azospirillum* (TNAU) + PSB + PPFM and uninoculated control. The boll weight was not significantly affected due to the levels of N and P and bio-inoculants.

Seed cotton yield

The cotton crop responded significantly to the graded levels of N and P, bio-inoculants (Table 1) and their interaction (Table 2). The interaction effect interestingly reveals that when no bio-inoculants were given, the crop responded up to 100% RDNP and recorded significantly higher seed cotton yield (SCY) than 50% RDNP level. The lowest seed cotton was recorded under unfertilized control. The bio-inoculants influenced the SCY significantly and among the treatments, *Azospirillum* (HAU) + PSB + PPFM was on par with *Azospirillum* (TNAU) + PSB + PPFM. The interaction effect suggested that application of 75% RDNP with *Azospirillum* (HAU) + PSB + PPFM recorded the highest (2.91 t/ha) SCY and was on par with *Azospirillum* (TNAU) + PSB + PPFM at 75% RDNP and 100% RDNP alone. The beneficial effect of dual inoculation of *Azospirillum* with PSB was reported earlier on sorghum, bajra and cotton over single inoculation (Alaguwadi and Gaur,1992).

Nutrient uptake

The uptake of nutrients were significantly enhanced due to the levels of N and P as evidenced from 10.9, 54.8 and 33.9% higher N uptake at 50, 75, and 100% RDNP level respectively over control. Among the fertilizer levels, 75% RDNP level was on par with RDNP for N uptake while for P uptake except control all other treatments were on par. This might be due to favourable growth at optimum available nutrients than above and below the optimum level. Among the bio-inoculants, TNAU and CCS HAU cultures of *Azospirillum* with PSB +PPFM recorded more N uptake that was significantly superior to Surat culture of *Azospirillum* with PSB + PPFM and un-inoculated control. The P uptake also followed the same trend as that of N uptake. The higher nutrient uptake due to bio-inoculants may also due to pectinolytic activity of *Azospirillum* cells (Okon, 1982). Enhanced mineral nutrient uptake due to *Azospirillum* was reported by Murty and Ladha (1988).

Rhizospheric population of Azospirillum, PSB and PPFM

The population of A*zospirillum* in the rhizosphere decreased with increasing level of fertilizers and among the

Table 1. Growth, yield attributes and seed cotton yield of cotton as influenced by graded levels of N and P fertilizers and bio-inoculants (pooled basis)

Treatment	Plant height (cm)	Dry matter (g/plant)	Bolls/plant	Boll weight(g)	Seed cotton yield (tonne/ha)
Recommended dose of N and P (%)					
0	79.9	96.2	17.8	3.86	1.84
50	82.6	103.8	21.4	4.03	2.24
75	84.8	108.5	24.6	4.04	2.53
100	85.4	111.3	24.5	4.01	2.45
SEm ±	2.6	5.4	1.0	0.11	0.12
CD (P= 0.05)	NS	13.2	2.4	NS	0.29
Bioinoculant					
Un inoculated	80.7	97.8	19.3	3.88	2.16
Azospirillum (TNAU) + PSB + PPFM	83.4	106.8	21.3	4.01	2.25
Azospirillum (CCS HAU) + PSB + PPFM	85.7	112.7	24.8	4.09	2.46
Azospirillum (Surat) + PSB + PPFM	82.9	102.6	22.9	3.92	2.20
SEm ±	2.9	6.9	1.2	0.08	0.08
CD (P= 0.05)	NS	14.3	2.5	NS	0.17

Table 2. Seed cotton yield (tonne/ha) as influenced by interaction of graded levels of N and P and bio-inoculants (pooled basis)

Bio-inoculant	% Recommend dose of N and P			
	0	50	75	100
Control	1.72	2.08	2.28	2.54
Azospirillum (TNAU) + PSB + PPFM	1.89	2.32	2.60	2.20
Azospirillum (CCS HAU) + PSB + PPFM	1.92	2.38	2.91	2.62
Azospirillum (Surat) + PSB + PPFM	1.83	2.19	2.32	2.46
			N, P levels at same Bio-inoculant	Bio-inoculants at same N, P level
SEm ±			0.18	0.18
CD (P=0.05)			0.44	0.37

Table 3. N and P uptake (kg/ha) and rhizospheric population of *Azospirillum*, PSB and PPFM and fibre quality of cotton as influenced by graded levels of N and P and bio-inoculants (pooled basis)

Treatments	Nutrient uptake		Rhizospheric population (/g dry soil) at 45 DAS			Fibre quality		
	N	P	*Azospirillum* $\times 10^6$	PSB $\times 10^3$	PPFM $\times 10^4$	2.5% span length (mm)	Fibre strength (g/tex)	Micro-naire (mg/inch)
Recommended dose of N and P (%)								
0	73.5	10.7	14.40	34.9	83.3	28.44	22.39	3.79
50	81.5	17.2	9.05	47.4	46.8	28.70	22.36	3.80
75	113.8	18.4	4.05	28.8	67.9	28.52	21.99	3.76
100	98.4	16.6	2.31	29.6	58.5	28.75	22.37	3.94
SEm ±	7.5	1.5	0.90	0.3	0.3	0.39	0.67	0.11
CD (P=0.05)	18.4	3.6	2.22	0.8	0.7	NS	NS	NS
Bioinoculant								
Uninoculated	88.4	14.2	6.17	26.3	55.0	28.46	22.31	3.77
Azospirillum (TNAU) + PSB + PPFM	101.4	16.0	8.29	37.4	84.7	28.76	22.22	3.91
Azospirillum (CCS HAU) + PSB + PPFM	102.6	18.9	11.90	36.0	76.8	28.59	22.57	3.71
Azospirillum (Surat) + PSB + PPFM	82.4	14.8	3.51	41.0	43.0	28.59	21.92	3.90
SEm ±	4.2	1.0	1.07	0.6	0.3	0.39	0.67	0.11
CD (P=0.05)	8.6	2.1	2.20	1.2	0.6	NS	NS	NS

bio inoculants, TNAU and HAU culture of *Azospirillum* + PSB + PPFM recorded significantly higher population of *Azospirillum* than other two treatments The fertilizer levels influenced the PSB population significantly and higher population was maintained at 50% RDNP which recorded 47.4 $\times 10^3$ cfu/g of dry soil as against 29.6 x 10^3 cfu recorded in RDNP. All the bio-inoculated treatments recorded higher PSB population ranging from 36.9 to 55% than un-inoculated control. The population of PPFM also significantly higher under unfertilized control. Among the applied N and P levels, 75% level recorded significantly higher PPFM population than either 50 or 100% level. Among the bio-inoculants, TNAU and HAU culture of *Azospirillum* + PSB + PPFM recorded significantly higher population of PPFM than the un-inoculated control (Table 3).

Fibre quality

The fibre quality parameters (Table 4) were not significantly influenced due to graded levels of NP or bioinoculants.

Table 4. Economics (Rs/ha) of cotton cultivation as influenced by graded levels of N and P fertilizers and bio inoculants

Treatment	Cost of cultivation	Net return	B:C ratio
Recommended dose of NP (%)			
0	19,943	26,058	1.31
50	20,650	35,413	1.71
75	21,003	42,185	2.01
100	21,556	40,000	1.86
Bioinoculant			
Un inoculated	20,295	33,580	1.65
Azospirillum (TNAU) + PSB + PPFM	20,885	35,427	1.70
Azospirillum (HAU) + PSB + PPFM	20,885	40,553	1.94
Azospirillum (Surat) + PSB + PPFM	20,885	34,115	1.63

*Cost of bioinoculants @ Rs 6/pack of 200 g and for foliar PPFM the cost for mass multiplication of PPFM culture was not yet standardized and only spraying cost is included.

Cost of urea, superphosphate and muriate of potash respectively are Rs 5.10, 4.0 and 5.0 /kg and, cost of seed cotton is Rs 25/kg.

Economics

The economics of cotton cultivation under graded N and P fertilizers and bioinoculants (Table 5) revealed that the net returns and B: C values increased progressively up to 75% RDNP level and decreased at RDNP. Among the bio inoculants, *Azospirillum* (HAU) + PSB + PPFM recorded higher monetary benefit than other treatments.

Consequently bio-inoculants combined with 75% of fertilizers could yield 0.37 tonne/ha of additional seed cotton yield besides saving 25% of NP fertilizers thereby necessitating the potential role of bio-inoculants in sustainable cotton farming.

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