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Impact of Biofertigation of Azophosmet on Cotton Yield under Dripirrigation

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Abstract: Field experiment was conducted at Tamil Nadu Agricultural University (TNAU) Tamil Nadu, to study the effect of biofertigation on cotton. Azophosmet a co cultured formulation of *Azospirillum*, phosphobacteria and methylobacterium was used as bioinoculants for biofertigation in cotton. Rhizosphere soil was analyzed at 15 days interval, plant height, dry matter production and yield parameters were determined at 30 days interval. Application of 150 per cent of recommended NPK as drip fertigation combined with biofertigation of liquid formulation of azophosmet was significantly better when compared to other treatments. Increasing levels of fertilizer application along with biofertigation favorably influenced the growth parameters and yield attributes of cotton.

Key words: Azospirillum,. Phosphobacteria, Methylobacterium, Liquid formulation

INTRODUCTION

Cotton is one of the most important commercial crops in India and occupies a major source of foreign exchange. It is an important raw material for textile industry and ginning industry. Water and nutrients are essential requirements for the crops to grow. Nutrients may be of organic or inorganic in nature. Inorganic fertilizers are costly and cause environmental problems. Use of organic fertilizers alone can not meet the nutritional requirement of the plant[1]. So combined application of organic and inorganic fertilizers are required which is called as integrated plant nutrient system to maintain and increase the soil fertility as well as to increase the crop production. Bioinoculants constitute an important component in integrated plant nutrient system. It is an added advantage when these microbial inoculants are supplied through biofertigation as it has more water use efficiency and fertilizer use efficiency, quality etc. Effective microorganisms can also be applied in the filed along with organic or inorganic materials[8]. The organic and biological sources of nutrients provide essential nutrients to the crop and also enhance the positive interaction with chemical fertilizers by increasing their efficiency[1]. Beneficial bacteria such as *Azospirillum*, phosphobacteria and methylotroph colonizing in the rhizosphere region and has the ability to fix nitrogen, solubilize phosphorus and stimulate plant growth. *Methylobacterium* sp. on coinoculation with *Rhizobium* sp. TNAU14 had significantly increased plant growth, nodulation and yield attributes in groundnut compared with individual inoculation[10,11]. The biofertigation can precisely deliver the bioinoculants in the root zone. However this technology needs to be standardized as the literature availability related to biofertigation is very scarce. The present study was undertaken to evaluate the performance of biofertigation under field condition along with fertigation of inorganic fertilizers on cotton. This will be the first report regarding biofertigation of Azophosmet in cotton.

MATERIALS AND METHODS

A field experiment was conducted at Tamil Nadu Agricultural University, Coimbatore during winter irrigated season of 2006-07 on drip irrigation in intraspecific cotton hybrid NCS145Bt (BunnyBt). The soil of the experimental field was sandy clay loam with low in available nitrogen (241.0 kg ha^{-1}), medium in available phosphorus (19.0 kg ha^{-1}) and high in available potassium (459.0 kg ha^{-1}). The experiment was laid out in a randomized block design and replicated thrice. The following treatments were used in this study.

T_1- Drip fertigation with 75% RDF of N & K
T_2- Drip fertigation with 75% RDF of N & K + biofertigation
T_3- Drip fertigation with 100% RDF of N & K

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T_4- Drip fertigation with 100% RDF of N & K+ biofertigation
T_5- Drip fertigation with 125% RDF of N & K
T_6- Drip fertigation with 125% RDF of N & K+ biofertigation
T_7- Drip fertigation with 150% RDF of N & K
T_8- Drip fertigation with 150% RDF of N & K+ biofertigation
T_9- Drip irrigation + soil application of 100% NPK fertilizer
T_{10}- Surface irrigation + soil application of 100% NPK fertilizer

Treatment of seeds: Seed treatment with Imidaglobrid @ 4 g kg^{-1} of seeds was done to control the sucking pests. Seeds were inoculated with *Azospirillum brasilense* sp 7, *Bacillus megaterium*, var *phosphaticum* (PB1) and *Methylobacterium* sp Co 47 @ 600 gram each per hectare by using rice gruel as the adhesive. The crop spacing of 75 x 60 cm was adopted for ridges and furrow method under surface irrigation. In drip irrigated plots, paired row method of planting was adopted with a row spacing of 60 x 90 cm under raised bed layout. Good viable seeds of hybrid NCS 145Bt @ 1 kg ha^{-1} as delinted seed were dibbled at one seed per hill.

Preparation of Standard Inoculum: The standard inoculum was prepared by inoculating log phase cultures of bioinoculants viz., *Azospirillum brasilense* in N-free malic acid broth, *Bacillus megaterium* var *phosphaticum* in pikovskya's broth and *Methylobacterium* sp. in AMS broth. After preparation of the standard inoculum, log phase culture of *Azospirillum brasilense, Bacillus megaterium* var *phosphaticum* and *Methylobacterium* sp was inoculated into YEM broth with 0.5% methanol. Then the flasks were kept in shaker @ 120rpm for five days to get 10^{12} cells/ml of liquid culture (Pooraniammal, 2006).

Biofertigation: Cultures of *Azospirillum brasilense*, *Bacillus megaterium* and *Methylobacterium* sp @ 500 ml (10^{12} cells ml^{-1}) was diluted in 500 litres of water ha^{-1} and given as biofertigation. Biofertilizer solution was prepared in a container from which it was sucked by venturi assembly and allowed through the irrigation system at an interval of 15 days commencing from 15 DAS up to 75 DAS (5 times). Biofertilizer application through drip was done 2 days after fertigation.

Survival Rate of Bioinoculants: Drip samples were collected at the time of biofertigation through drip from randomly selected drippers and rhizosphere soil samples were collected one day after biofertigation. The *Azospirillum*, *Bacillus megaterium* var *phosphaticum* and methylotroph population in liquid sample collected during biofertigation and rhizosphere soil sample were analyzed through serial dilution and plating technique on the respective agar media. Plates were incubated at 30° c for 48-96hrs and the population was estimated. Plant growth parameters such as plant height, dry matter production, boll number and seed cotton yield were also analyzed.

RESULTS AND DISCUSSION

Plant Growth Parameters: Combination of microbial inoculum and chemical fertilizers given through drip irrigation has shown variable results. Drip fertigation with 150 percent of recommended dose of NPK and biofertigation with azophosmet (T_8) significantly recorded the highest plant height of 79.13, 103.20 and 113.12 cm at 60, 90 and 120 DAS respectively (Table 1). The plant height of T_7 was 78.50, 102.70 and 112.87 cm at 60, 90 and 120 DAS and 77.40, 103.30 and 112.14 cm with T_6. Surface irrigation with soil application of 100 per cent NPK recorded the least plant height of 54.27, 75.50 and 79.41 cm at 60, 90 and 120 DAS respectively. The plants were grown well and the plant height was maximum when the chemical fertilizers were combined with bioinoculants. This may be due to the higher frequency of biofertigation with Azophosmet and the availability of soil moisture which leads to the effective absorption of nutrients and better proliferation of roots. This was supported by the researchers[10,12] who reported that methylobacterium involved in lowering ethylene levels and promoting

Table 1: Effect of fertigation and biofertigation on plant height (cm)

Treatments	30DAS	60DAS	90DAS	120DAS
T_1: DF with 75 per cent NPK	25.33	52.40	78.60	82.10
T_2: DF with 75 per cent NPK+biofertigation	25.68	63.07	86.10	92.07
T_3: DF with 100 per cent NPK	25.72	63.33	87.30	92.23
T_4: DF with 100 per cent NPK+biofertigation	25.59	69.80	94.10	101.51
T_5: DF with 125 per cent NPK	26.05	70.13	95.20	102.41
T_6: DF with 125 per cent NPK+biofertigation	26.33	77.40	103.30	112.14
T_7: DF with 150 per cent NPK	26.91	78.50	102.70	112.87
T_8: DF with 150 per cent NPK+biofertigation	26.61	79.13	103.20	113.12
T_9: DI + soil application of 100 percent NPK	26.16	54.97	77.90	81.47
T_{10}: SI + soil application of 100 per cent NPK	25.83	54.27	75.50	79.41
SEd 0.88	2.80	3.24	3.96	
CD (*P*= 0.05)	1.76	5.88	6.80	8.31

SEd, standard error. CD, critical difference.

Table 2: Effect of fertigation and biofertigation on dry matter production (kg ha^{-1})

Treatments	30 DAS	60 DAS	90DAS	120 DAS
T_1	190.7	1794.7	4016.1	4992.6
T_2	192.6	1937.3	4595.0	5555.5
T_3	192.6	1949.1	4635.1	5676.2
T_4	192.7	2154.2	5206.3	6248.1
T_5	195.3	2162.6	5211.4	6289.6
T_6	201.1	2414.7	5773.9	6876.2
T_7	211.8	2430.0	5810.7	6866.4
T_8	211.6	2450.8	5846.0	6875.5
T_9	191.5	1744.9	4000.1	4942.2
T_{10}	188.8	1740.4	3951.8	4933.3
SEd	14.8	46.5	274.8	249.8
CD(*P*=0.05)	29.6	97.8	577.5	524.8

SEd, standard error.
CD, critical difference.

Table 3: Influence of fertigation and biofertigation on number of bolls per plant and seed cotton yield

	Treatments	Kapas yield (kg ha^{-1})	No. of bolls $plant^{-1}$
T_1:	DF with 75 per cent NPK	2036.0	21.73
T_2:	DF with 75 per cent NPK+biofertigation	2308.3	22.92
T_3:	DF with 100 per cent NPK	2353.3	23.42
T_4:	DF with 100 per cent NPK + biofertigation	2744.0	25.30
T_5:	DF with 125 per cent NPK	2829.5	26.23
T_6:	DF with 125 per cent NPK + biofertigation	3217.3	28.90
T_7:	DF with 150 per cent NPK	3273.3	29.11
T_8:	DF with 150 per cent NPK + biofertigation	3395.3	29.47
T_9:	DI + soil application of 100 percent NPK	1993.5	21.33
T_{10}:	SI + soil application of 100 per cent NPK	1934.8	20.56
SEd		125.0	0.69
CD (P= 0.05)		262.7	1.46

SEd, standard error. CD, critical difference.

root elongation in canola. So the methylobacterium in the Azophosmet culture might have involved in the better growth of roots. Similarly, Azophosmet application has increased plant growth, biomass and number of bolls per plant compared to individual bioinoculant[16]. Root exudates influenced the plant growth and seed cotton yield[9]. Moreover in this study, microorganisms given through biofertigation may increase the production of root exudates which contains organic acids such as malic acid, formic acid, succinic acid etc., which might increase the plant growth and plant yield. Similar results were obtained when the crop was tested with *Azotobacter*, *Acetobacter*, *Azospirillum* and *Pseudomonas* on cotton crop under irrigated condition[14]. In this study chemical fertilizers along with microbial inoculants were given in equal amount in drip fertigation. So it was observed that plants grew well due to the application of microorganisms which stimulate the plant height and growth[15]. Similarly drip fertigation with 150 per cent recommended dose of NPK and biofertigation (T_8) produced significantly higher DMP of 2450.8, 5846.0 and 6875.5 kg ha^{-1} at 60, 90 and 120 DAS respectively (Table 2). Among the treatments T_1, T_9 and T_{10} recorded lower values compared to other treatments at all the growth stages and significantly inferior to the other fertigation treatments. The variation in DMP among the biofertigation treatments followed the similar trend as that of plant height. The nutrient was distributed equally in the plant system which may be resulted in higher dry matter production.

Yield Parameters: Similarly in this study, the boll number and seed cotton yield (Table 3) was higher due to the effective translocation of nutrients to reproductive parts compared to conventional methods of soil application of nutrients. Better performance of the combined inoculation of *Azospirillum*, *Methylobacterium* and phosphobacteria in cotton especially in the later stages of crop growth[18]. There was also early flowering and boll development due to combined inoculation. In this study when comparing all the treatments, maximum yield was recorded in drip fertigation with 150 per cent recommended dose of NPK and biofertigation (T_8). It produced the more number of bolls (29.47 bolls per plant). Drip fertigation with 150 per cent recommended dose of NPK (T_7) has 29.11 bolls per plant followed by drip fertigation with 125 per cent recommended NPK (T_6) (28.90 bolls per plant). The least number of bolls per plant was observed in the treatment which had surface irrigation and soil application of recommended NPK. Higher seed cotton yield, plant growth and survival of the bioinoculants may be attributed to the cumulative effect of nutrient transformation and plant growth promotion. Application of *Azospirillum surat*, *Bacillus* and PPFM giving maximum benefit in terms of cotton yield[5]. Significantly higher values of yield attributes were determined due to drip fertigation than conventional method of fertilizer application[6,17,19].

Growth and yield was increased significantly with increase in fertilizer rates[2, 20]. Application of varying levels of fertilizer as fertigation in combination with biofertigation of azophosmet positively influenced the seed cotton yield. Drip fertigation with 150 per cent recommended dose of NPK and biofertigation (T_8) recorded the highest seed cotton yield of 3395.3 kg ha^{-1} (Fig. 1). Combination of organic amendments and biofertigation recorded maximum yield[2]. Similar results were observed by scientists who confirmed the benefits of the combined application of *Azospirillum*, phosphobacteria and *Methylobacterium*

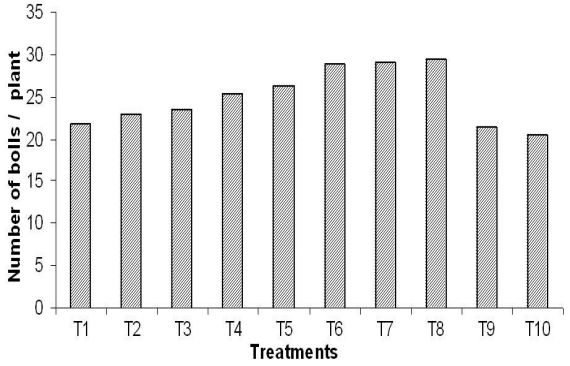

Fig. 1: Effect of biofertigation of Azophosmet on number of bolls of cotton

Table 4: Population of bioinoculants in rhizosphere soil sample

	Population (cfu/g)		
	Azospirillum	Phosphobacteria	PPFM
Initial	10×10^6	3.6×10^6	2.0×10^6
15 DAS			
T_2	1.5×10^9	1.0×10^9	1.0×10^9
T_4	2.5×10^9	1.5×10^9	3.0×10^9
T_6	29.5×10^8	27.5×10^8	33.5×10^8
T_8	17.5×10^7	19.5×10^7	25.0×10^7
Control	10×10^5	2.5×10^5	0.7×10^5
30 DAS			
T_2	1.5×10^9	6.5×10^9	2.0×10^9
T_4	5.5×10^9	4.0×10^9	1.5×10^9
T_6	5.5×10^8	9.0×10^8	3.5×10^8
T_8	21.0×10^7	1.0×10^7	11.0×10^7
Control	5.0×10^6	3.0×10^4	2.0×10^4
45 DAS			
T_2	1.5×10^9	7.5×10^8	1.5×10^8
T_4	1.5×10^8	3.0×10^8	3.5×10^8
T_6	0.5×10^8	1.5×10^7	1.5×10^6
T_8	2.5×10^7	1.5×10^6	1.5×10^6
Control	2.0×10^5	3.5×10^4	1.5×10^4
60 DAS			
T_2	5.0×10^8	6.5×10^8	3.0×10^8
T_4	1.5×10^8	7.0×10^8	1.5×10^8
T_6	6.0×10^7	1.5×10^8	6.0×10^7
T_8	4.0×10^7	7.5×10^7	6.5×10^7
Control	4.0×10^4	6.5×10^4	1.0×10^3
75 DAS			
T_2	1.0×10^8	6.5×10^7	3.0×10^7
T_4	2.5×10^7	5.0×10^7	8.5×10^6
T_6	4.0×10^6	1.0×10^6	2.5×10^6
T_8	2.5×10^6	5.5×10^6	2.5×10^5
Control	1.0×10^4	2.5×10^4	2.0×10^3

in cotton[13]. In this study as the water supply is continuous through drip, the plants tend to take more nutrients in the soil resulted in higher yield under higher available supply of fertilizer. Similar finding showed the enrichment of biofertigation using effective microorganisms increased 60% availability of major nutrients[4]. Plant nutrient availability get increased through microbial activity when the effective microorganisms were applied with organic amendments[7].

Survival of Microorganisms: Survival rate of *Azospirillum*, phosphobacteria, methylobacteria was determined on their respective media. It was found that the population was 4.0×10^7 for *Azospirillum* 7.5×10^7 for phosphobacteria and 6.5×10^7 for methylotrophs.in the treatment which received 150 per cent recommended dose of NPK and biofertigation with azophosmet on 60DAS (Table 4). In the liquid sample collected during biofertigation the population was found to be 10^7 cells throughout the sample collection. NPK fertilizer in combination with fermented water (contains EM) was equal to fermented water alone[2]. But the population was found to be low when compared to other treatments, but it was high when compared to control.

CONCLUSIONS

Even though the separate application of fertilizers found to increase the yield of cotton over the control, their combined application further increased the yield when compared to sole application of fertilizers which suggested that a balanced fertigation is required for optimum growth and yield of crops.

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