RESEARCH PAPER

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Comparative efficacy of cocultured inoculants (Azophosmet) over individual inoculants of cotton under *in vitro* and *in vivo* conditions

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The effect of cocultured inoculant (Azophosmet) was studied under *in vitro* and *in vivo* conditions compared with other individual inoculants was investigated. The cotton seeds were treated with the Azophosmet, revealed the surviving ability of *Azospirillum* Sp7, PSB PB1 and PPFM CO 47 noticed up to 24 h on the seeds. Inoculation of cocultured inoculants recorded the maximum increase in root length; shoot length, germination per cent and vigour index of cotton plants followed by CO 47 under *in vitro* conditions. Cocultured inoculants (Azophosmet) was effective in enhancing rhizosphere population of individual bioinoculants, plant growth and seed cotton yield under pot culture conditions.

Key words : Azophosmet, Cotton, Coculturing and PPFM.

INTRODUCTION

Microorganisms play a vital role in sustainable agriculture and are used in maintaining soil texture, health and fertility. To have sustainability in agriculture, it is necessary to establish a production system, which is efficient, profitable, eco-friendly, conserving or enhancing renewable sources. Biofertilizers are now well recognized as important component of sustainable agriculture. Strategies to meet the growing nutrient demands for a sustainable production of cotton in cotton based cropping systems is needed (Kairon and Venugopalon, 2000). The results available suggested that the plant growth can be optimized to obtain maximum yields using specific combinations of selected microorganisms.

Nitrogen is an essential plant nutrient that is most commonly deficient, contributing to reduced agriculture yields. Atmosphere contains about 78% nitrogen, but crop plants can not take this directly. Some crop plants have an association with nitrogen fixing microorganism. *Azospirillum* is associative nitrogen fixing bacteria make contribution of nitrogen fixation.

Phosphorus is another major plant nutrient required in optimum for proper plant growth. About 98% of the soils in india have inadequate supply of available phosphorus. The role of Phosphobacteria particularly *Bacillus* and *Pseudomonas* insoluble phosphate in soil make it available to the plant is well known.

The introduction of efficient PGPR belong to the genus *Methylobacterium* ubiquitous in the phyllosphere and rhizosphere of the plants.It is well documented that bacteria have theability to synthesize phytohormones such as Indole acetic acid, cytokinin and gibberlic acid and this organism utilize methanol as a source of carbon and energy source and promote plant growth, it also induce systemic resistance against fungal plant pathogen.

However, reports are scanty on the use of mixed inoculants. Hence there were previous attempts in our laboratory to study the feasibility of using the ‘N’ fixers, phosphate solubilizers and a plant growth hormone synthesizer as single and combined inoculations using three different method of application to cotton crop (Senthil Kumar and Sundaram, 2005). The possibilities are still to be explored to coculture all beneficial microorganisms in a suitable single medium for easy application at a reduced input cost. In the present study, it was attempted to coculture three bioinoculants in a single medium and using as a single bioinoculant for cotton is explored.

MATERIALS AND METHODS

Comparative efficacy of Azophosmet over individual inoculations on seed germination and seedling vigour of cotton under in vitro conditions:

The cottonseeds were surface sterilized and were treated with the pure cultures and cocultured inoculants. The seedling vigour of the treated seeds was calculated by the standard germination paper method (ISTA, 1993).

The treatment details of this experiment are as : T_1- Control, T_2- Seed treatment with *Azospirillum* Sp7, T_3- Seed treatment with phosphate solubilizing bacteria PB1, T_4-Seed treatment with PPFM CO 47, T_5 - Seed treatment with Azophosmet.

Germination (%) (ISTA, 1999):

Germination test in quadruplicate of 10 seeds, were carried out in paper media in a germination room maintained at a temperature of 25 ±1°C and RH 96 ± 2% with diffused light. Germination per cent of cottonseeds were recorded on 15 day after inoculation.

The root length between collar and tip of the primary root was measured in cm and mean value was recorded as root length.

The shoot length between collar and tip of the primary shoot was measured in cm and mean value was recorded as shoot length.

Vigour index value was computed using the formula suggested by Abdul-Baki and Anderson (1973) and was expressed as whole number.

Vigour index = Germination per cent x (Root length + Shoot length)

Comparative efficacy of Azophosmet over individual inoculation and combined inoculations on growth and yield of cotton under in vivo (pot culture) conditions:

General:

To know the comparative effect of Azophosmet with Azophos and PPFM alone on cotton plant growth and yield parameters under pot culture conditions in RBD design, with 4 treatments and 3 replications is described below.

Preparation of pot mixture:

Red soil having the chemical properties of pH 7.4, EC 0.4 dSm^{-1}, available nitrogen 230 kg ha^{-1}, available P_2O_5 10 kg ha^{-1} and available K_2O 250 kg ha^{-1} was passed through a 4mm sieve and mixed along with farmyard manure in 2:1 proportion and filled in pot of 47 x 30 cm size at the rate of 4 kg pot^{-1}.

Treatment details:

T_1 – control

T_2 – Soil application and seed treatment with Azophosmet

T_3 – Soil application and seed treatment with Azophos

T_4 – Soil application and seed treatment with *Methylobacterium* sp PPFMs-CO-47

Observations recorded:

The following observations *viz*., microbial population, plant growth parameters were recorded at 30 DAS, 60 DAS and 90 DAS. The yield parameters were recorded at harvest.

Survival of bioinoculants in the rhizosphere soil:

Rhizosphere soil samples from different treatments were collected in three intervals (30,60 and 90 DAS) for testing the survival of, *Azospirillum*, PSB and PPFM. The population dynamics of *Azospirillum* Sp7, PSB PB1 and PPFM CO 47 in the rhizosphere region was studied by spreading 1g of serially diluted samples on Pikovskya's agar (Pikovskya's, 1948) and ammonium mineral salt agar (Whittenbury *et al*., 1970), respectively in weekly intervals upto 90 days for PSB and PPFM. The growth characteristic of PSB PB1 and PPFM CO 47 on their respective media were taken care of and colony forming units per ml was estimated. The population of *Azospirillum* was assessed by MPN technique on Nfb semisolid medium (Dobereiner, 1980).

Plant growth:

The height of the plant from the bottom to the tip of the main stem was measured in a plant in each replication and the means were worked out and expressed in cm during pre-flowering stage, flowering stage and boll forming stage.

Plant dry matter production:

Shoot, root and leaves were dried in hot air oven at 60 – 70°C till the constant dry weight was obtained and expressed as g $plant^{-1}$.

Yield components:

No. of sympodial branches:

No. of reproductive branches arising from the extra auxiliary buds were counted on 120 DAS from 3 plants in each pot and mean values expressed as no per plant.

No. of bolls / plant

From three plants in each pot total numbers of bolls retained by each plant were counted on 120 DAS and mean no of bolls per plant was worked out. At each picking the no of bolls actually harvested were also counted from 3 plants in each pot and mean no of open bolls per plant were worked out.

Boll weight:

The kapas weight collected from 5 fully opened bolls at random from 3 marked plants per pot was recorded and the boll weight was expressed in kg / plant.

RESULTS AND DISCUSSION

Comparative efficacy of Azophosmet over individual inoculation and combined inoculations under in vitro conditions:

In general bioinoculants have considerably increased the root length, shoot length and vigour index of the cotton seedlings. The maximum root length (22.42 cm), shoot length (20.78 cm), germination per cent (93) and vigour index (4017.6) was recorded in cotton seedlings inoculated with Azophosmet followed by PPFM CO 47 which recorded the root length of 18.45 cm shoot length15.42 cm, germination per cent 90 and vigour index 3048.6. Inoculation of Azophosmet and PPFM recorded significantly higher values than other inoculants and control. *Azospirillum* Sp7 and PSB PB1 recorded the at par results in all the parameters. Uninoculated control plants recorded the lowest of shoot length (12.82 cm), root length (10.67 cm), germination (80%) and vigour index (1879) (Table 1). In the present study, the cocultured Azophosmet bioinoculant recorded significant increase in germination percentage and seedling vigour of cotton. The increase in seedling vigour due to Azophosmet can be attributed to various beneficial effects of consortia of bioinoculants application as proposed by Watanabe and Lin (1984).

Comparative efficacy of Azophosmet over individual inoculation and combined inoculations under in vivo conditions:

The present study showed increased rhizosphere population of *Azospirillum* (17.42 x 10^6 cfu ml^{-1}), PSB (5.66 x 10^3 cfu ml^{-1}) and PPFM (21.60 x 10^4 cfu ml^{-1}),at 90DAS due to the treatments with Azophosmet (Table 2). The rhizosphere microorganisms are under the constant influence of the host plant and favors the proliferation of numerous microorganisms and this region is considered important as it influences crop growth (Rovira, 1965).

Plant growth:

The effect of Azophosmet inoculation, combined inoculation of *Azospirillum*, PSB and PPFM and individual inoculations on plant height was studied at three stages of plant growth vegetative phase (30 DAS), flowering phase (60DAS) and Boll formation stage (90DAS). The results are presented in Table 3

At 30 DAS maximum plant growth was recorded (28.58 cm $plant^{-1}$) in the seed treatment and soil application with Azophosmet while the plant growth was 27.36 cm $plant^{-1}$ due to PPFM. The least plant growth (17.60 cm $plant^{-1}$) was recorded in the uninoculated control.

At 60DAS maximum plant growth was recorded (59.40 cm $plant^{-1}$) in seed treatment and soil application with Azophosmet. While the plant growth was least (40.75 cm $plant^{-1}$) in control. How ever, it was at par with Azophos and PPFM (58.94 cm $plant^{-1}$ and 58.11 cm

Table 1 : Comparative efficacy of Azophosmet over individual inoculants on seed germination and seedling vigour of MCU-12 cotton under *in vitro* Conditions

Sr. No.	Treatments	Root length (cm)	Shoot length (cm)	Germination percentage	Vigour index
1.	Control	12.82	10.67	80	1879.2
2.	*A.brasilense* Sp7	15.90	13.60	86	2537.0
3.	*Bacillus megaterium* PB1	16.50	13.70	83	2506.6
4.	*Methylobacterium extroquens* CO 47	18.45	15.42	90	3048.6
5.	Azophosmet	22.42	20.78	93	4017.6
	S.E.±	0.052	0.027		475.96
	C.D. (P=0.05)	0.116	0.061		1060.51

*Average of 10 seedlings

Table 2 : Survival of *Azospirillum*, PSB and PPFM in MCU12 cotton *Rhizosphere* upon inoculation

Treatments	30 DAS			60 DAS			90 DAS		
	1	2	3	1	2	3	1	2	3
Control	6.06	0.33	6.32	6.76	1.76	8.1	7.27	2.30	9.23
	(6.782)	(5.518)	(6.800)	(6.829)	(6.245)	(5.908)	(6.861)	(5.361)	(6.965)
PPFM	6.87	0.92	16.16	9.34	1.29	18.3	10.3	2.16	19.03
	(6.836)	(5.963)	(7.208)	(7.208)	(6.110)	(6.262)	(6.012)	(6.334)	(7.279)
Azophos	13.99	4.02	7.55	16.90	4.33	11.2	18.4	4.79	13.16
	(7.145)	(6.604)	(6.877)	(6.877)	(6.636)	(6.049)	(6.264)	(6.680)	(7.119)
Azophosmet	13.68	4.743	17.56	16.48	4.86	20.51	17.42	5.66	21.60
(Co-cultured)	(7.136)	(6.675)	(7.244)	(7.244)	(6.680)	(7.311)	(7.241)	(6.752)	(7.334)
S.E.±	0.011	0.008	0.008	0.009	0.010	0.008	0.005	0.041	0.008
CD (P=0.05)	0.026	0.020	0.019	0.022	0.024	0.020	0.011	0.094	0.019

1- *Azospirillum* (10^6 cfu g^{-1} dry weight of soil) 2- PSB (10^3 cfu g^{-1} dry weight of soil) 3- PPFM (10^4 cfu g^{-1} dry weight of soil)

Table 3 : Comparative efficacy of Azophosmet, Azophos and PPFM on plant growth parameters of MCU-12 cotton under pot culture conditions

Sr. No	Treatments	Plant height (cm)			Biomass	
		30 DAS	60DAS	90DAS	Fresh weight (g)	Dry weight (g)
1.	Control	17.60	40.75	75.79	70.43	10.80
2.	PPFM	27.36	58.11	80.80	83.29	13.20
3.	Azophos	27.90	58.94	82.36	83.33	14.11
4.	Azophosmet	28.58	59.40	84.25	85.61	16.27
S.E.±		0.385	0.628	0.449	0.011	0.049
C.D. (P=0.05)		0.942	1.538	1.100	0.027	0.120

DAS: Days after sowing

plant^{-1}).

At 90 DAS maximum plant growth was recorded (84.25 cm plant^{-1}) seed treatment and soil application with Azophosmet. The seed treatment and soil application with Azophos recorded (82.36 cm plant^{-1}). The bioinoculant treatment increased plant height significantly over control, which recorded (75.79 cm plant^{-1}).

Plant biomass:

The maximum dry weight recorded in Azophosmet treatment (16.27g), which was significantly superior over the combined inoculation of Azophos (14.11g) and individual inoculation of PPFM (13.20g) and was 23.45 and 18.18 per cent respective increase over the control regarding 10.80g.

Yield parameters:

The maximum number of reproductive clusters was observed in the case of Azophosmet (15.00) followed by Azophos (14.30) and control recorded the lowest sympodial branches (4.30).

The cocultured inoculant Azophosmet recorded the 20.30 bolls per plant. Where as the combined inoculation of Azophos had 23.30 bolls and individual inoculation of PPFM had 21.33 bolls these are at par with each other. The control plant showed lower number of bolls (18.30). The results are presented in Table 4.

Table 4 : Comparative efficacy of Azophosmet, Azophos and PPFM on plant growth parameters of MCU-12 cotton under pot culture

Treatments	Sympodial Branches (No/plant)	No. of boll/Plant	Boll weight/ plant (kg)
Control	4.30	18.30	1.23
PPFM	13.00	21.33	1.39
Azophos	14.30	23.30	1.43
Azophosmet	15.0	24.66	1.52
S.E.±	0.827	0.707	0.065
C.D. (P=0.05)	2.025	1.730	0.160

The observations of recording the boll weight of cotton revealed that the Azophosmet recorded maximum boll weight (1.52 kg plant^{-1}). The PPFM (1.39 kg plant^{-1}) and Azophos (1.43 kg plant^{-1}) are at par with each other.

Anu Rajan (2003) indicated that increase in the population of PPFM till 60 DAS due to the treatment with the microbial consortia as well as individual inoculation of PPFM in tomato and further reported that the microbial consortia had increased plant growth and dry matter production compared to individual inoculant in tomato. Reddy (2002) also reported similar results in groundnut. The better performance of the combined inoculation of *Azospirillum Methylobacterium* and phosphobacteria in cotton especially in the later stages of crop growth (Senthilkumar and Sundaram, 2005). There was also early flowering and boll development due to combined inoculation. 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. Nalayini *et al*. (2005) confirmed the benefits of the combined application of *Azospirillum*, phosphobacteria and *Methylobacterium* in cotton.Though the present study also indicated that Azophosmet application had increased plant growth, biomass and number of bolls per plant compared to individual bioinoculant, it further reduced 2/3 in cost of bioinoculants and eased the technology of application with one bioinoculant 'Azophosmet' instead of mixing three bioinoculants to cotton.

REFERENCES

Abdul- Baki , A.A. and Anderson, J.D. (1973). Vigour determination in soybean seed by multiple criteria. *Crop Sci.*, **13** : 630-632.

Anu Rajan, S. (2003). Studies on the occurrence of pink pigmented facultative methylotrophs on vegetable crops. M.Sc. (Ag.) Thesis, Tamil Nadu Agricultural University, Coimbatore (T.N.)

Dobereiner, J. (1980). Forage grasses and grain crops. In : *Methods for Evaluating Biological Nitrogen fixation*. Bergerson, F.J., (ed.). John Willey and Sons, New York. pp. 535-555.

ISTA (1993). Proceeding of the International Seed Testing Association: International Rules for Seed Testing. *Seed Sci. Technol.*, **21**: 25-30.

ISTA (1999). International rules for seed testing. Supplement Rules, 27.

Kairon , M.S. and Venugopalan, M.V. (2000). Nutrient management in cotton and cotton based cropping systems. *Fertilizer News*, **45** (4): 51-56.

Nalayini, P., Anandham, R. and Chidambaram, P. (2005). Bioinoculants for sustainable cotton production National symposium on Microbial technology for productive agriculture, Tamil Nadu Agricultural University, Coimbatore. India. pp.110-111.

Reddy Suresh (2002). Studies on pink pigmented facultative methylotrophs as a new bioinoculant for groundnut (*Arachis hypogaea* L.), M.Sc. (Ag.) Thesis, Tamil Nadu Agricultural University, Coimbatore (T.N.).

Rovira, A.D. (1965). The interaction between plant roots and soil microorganisms. *Annu. Rev. Microbiol.*, **19**: 241-256.

Senthilkumar, M. and Sundaram, S.P. (2005). *In vitro* study of *Methylotrophs* with other bioinoculants on cotton. In: National symposium on Microbial technology for productive agriculture:Lead papers and research notes. 7-8,October 2005, Tamil Nadu Agricultural University, Coimbatore, India. pp. 44-45.

Watanabe, I. and Lin, C. (1984). Response of wetland rice to inoculation with *Azospirillum* lipoferum and *Pseudomonas* sp. *Soil Sci. Nutr.*, **30** : 117-124.

Whittenbury, R.S., Davies, L. and Wilkinson, J.F. (1970). Enrichment, isolation and some properties of methane-utilizing bacteria. *J. Gen. Microbiol.*, **61**: 205-218.