Vegetable Science (2017) 44 (2) : 26-33

Performance of liquid microbial consortium on bhendi (COBH -1) under precision farming system

Jeya M. Bharathi* **and D Balachander**

Received: July 2017 / Accepted: November 2017

Abstract

Carrier based microbial inoculants are widely used in conventional agriculture to improve crop growth and yield. Precision farming is a site specific management approach whereas the conventional practice is a uniform fertilizer application. Carrier based inoculum is not suitable for precision farming system due to clogging effect. Hence the liquid microbial consortium was developed using three inoculants *viz*., *Azospirillum brasilense sp 7*, *Bacillus megaterium var. phosphaticum* and *Pseudomonas fluorescens* with suitable cell protectant i.e. 10mM of phosphate buffer with 10% glycerol. We conducted a field experiment to study the effect of biofertigation on plant growth under precision farming system in bhendi (COBH 1). The result suggested the positive influence of 75% RDF of NPK + Microbial consortium application with single time (60 lit/ha) on delivery of inoculants viz., *Azospirillum* (5.96 ± 0.12 log cells/ml), *Bacillus megaterium var. phosphoticum*(7.00 ± 0.12 ml log cells/ml) and *Pseudomonas* (7.30 ± 0.02 log cells/ml) on plant growth and 10% increase in yield over conventional method.

Keywords: Microbial consortium, biofertigation, inoculants, precision farming system

Introduction

The global necessity to increase agricultural production from a steadily decreasing and degrading land resource base has placed considerable strain on the fragile agro ecosystems. Current strategies to maintain and improve agricultural productivity *via* high input practices place considerable emphasis on 'failsafe' techniques for each component of the production sequence with little consideration to the integration of these components in a holistic, systems approach. While the use of mineral fertilizers is considered the quickest and surest way of boosting crop production, their cost and other constraints deter farmers from using them in recommended quantities (Tilak et al. 2005). It is widely accepted that agricultural intensification has greatly increased the productive capacity of agroecosystems, but had unintended environmental consequences including degradation of soil and water resources and alteration of biogeochemical cycles (Drinkwater and Snapp 2007). In recent years, concepts of integrated plant nutrient management (IPNM) have been developed, which emphasize on maintaining and increasing soil fertility by optimizing all possible sources (organic and inorganic) of plant nutrients required for crop growth and quality. This is done in an integrated manner appropriate to each cropping system and farming situation. Improvement in agricultural sustainability requires optimal use and management of soil fertility and soil physical properties, both of which rely on soil biological processes (Saxena and Tilak 1998).

Precision farming is an integrated plant nutrient, pest and disease management which emphasize on maintaining and increasing soil fertility by optimizing all possible sources (organic and inorganic) of plant nutrients required for crop growth and quality (Tilak 1993). Improvement in agricultural sustainability requires optimal use and management of soil fertility, soil physical properties, both of which rely on soil biological processes (Saxena and Tilak 1998). Soil microorganisms play an important role in soil processes that determine plant productivity. For successful functioning of introduced microbial bioinoculants and their influence on soil health, exhaustive efforts have been made to explore soil microbial diversity of indigenous community, their distribution and behaviour in soil habitats (Hill 2000). The function of soil biota is central to decomposition processes and nutrient cycling. Microbes are the potential indicators of soil health and its biological properties. Microbial ecologists have, in particular, studied microbial community composition, since it exerts important control over soil processes (Bakker et al. 2001).

Microbiology, Tamil Nadu Agricultural University,
Coimbatore-3, Tamil Nadu
Corresponding author, Email: jbharathi86@ gmail.com

Biofertigation is recently developed technology, in which the liquid microbial inoculants are delivered through drip irrigation system. It has been reported that inoculation of liquid formulation of phosphate solubilizing *Bacillus megaterium* var. *phosphaticum* recorded higher root length, shoot length and cob yield in maize. Formulation is a crucial aspect for producing inoculants containing an effective bacterial strain and can determine the success or failure of a biological agent (Bashan 1998). Good quality formulation should promote the survival of bacteria preserving the beneficial traits and when applied should protect the cells from various biotic and abiotic stresses (Bashan *et al.* 1986). The liquid inoculum was as effective as peat based inoculum when the number of rhizobia provides higher cell load in the rhizosphere (Hely *et al.* 1976). Though the focus thus far in biofertigation on yield increases and soil microbial population has not yet been studied. Hence, the present study was focused to standardize the microbial inoculants schedule for precision farming system and evaluate the feasibility of the delivery system of inoculants to the rhizosphere of crop plant.

Materials and Methods

Microbial inoculants and growth media: Associative nitrogen fixing bacteria *Azospirillum brasilense* strain sp7 and P solubilizer of *Bacillus megaterium.* var. *phosphaticum* strain Pb 1 were obtained from the Department of Agricultural Microbiology, Tamil Nadu Agricultural University, Coimbatore, India and Plant Growth Promoting Rhizobacterium *Pseudomonas fluorescens* strain Pf 1 was obtained from the Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore were used for this study. *Azospirillum* was grown and maintained in N free malic acid medium (Okon *et al.* 1977), phosphobacteria in nutrient agar medium (Parkinson *et al.* 1971) and *Pseudomonas* in King's B medium (King *et al.* 1954).

Standardization of incubation time and population load: The *Azospirillum* culture was grown in sodium malate broth (Okon *et al.* 1977), phosphobacterial and *Pseudomonas* cultures were grown in nutrient broth (Rangaswamy and Bagyaraj 1966) and King's B broth (King *et al.* 1954) respectively of 250 mL in 500 mL conical flasks and incubated up to 5 days. The broths were centrifuged @ 10, 000 rpm for 10 min. in high volume centrifuge (Hitachi Japan). The cell pellets were washed with sterile distilled water twice, resuspended in sterile distilled water with 10% per cent glycerol and stored at -20°C for further use. The population of microbial inoculant suspended per ml of sterile water was enumerated by standard methods (*Azospirillum* by MPN, Okon et al. 1977), phosphobacteria and *Pseudomonas* by drop plate techniques (Somasegaran and Hoben 1988) to decide the quantity of broth to be used for mixing the cultures to prepare the liquid inoculum containing the microbial consortium for application through drip irrigation under precision farming system. The concentrated cell pellets were collected in 5 ml of the distilled water for *Azospirillum* and *Bacillus*; whereas 8 mL was used for *Pseudomonas* due to high concentration of cell pellets in *Pseudomonas*.

Survival of inoculant cells in microbial consortium with various cell protectants: The standardized microbial consortium were added with different cell protectants immediately after centrifugation to study the maximum survival capacity as T_1 (Microbial consortium + water); T_2 (Microbial consortium + 10 mM of glycerol), T_3 (Microbial consortium + 10 mM of polyvinylpyrrolidone), T_4 (Microbial consortium + 10 mM of trehalose), and T_5 (Microbial consortium + 10 mM of phosphate buffer, pH 7 with 10% glycerol). Two hundred ml of concentrated inoculants of three cultures supplied with various cell protectants were maintained in two sets, one set was incubated in refrigerator and another set at room temperature separately for assessing the survival of microbial consortium.

Drop plate method: The Sperber's hydroxy apatite agar and King's B agar medium were prepared, sterilized and plated in sterile Petri plates. The plates were kept at room temperature for 48 hrs (Somasegaran and Hoben 1988). The agar surface was divided into 6 quadrants. A quantity of 10 ìl from each dilution of 10^{-19}, 10^{-20}, 10^{-21}, 10^{-22}, 10^{-23} and 10^{-24} of the above microbial consortium with five treatments was placed in a quadrant with three replications. The plates were incubated without any disturbance and individual colonies were counted. The population of phosphobacteria was assessed after 5 days. In case of *Pseudomonas* the population was assessed after 3 days to study the maximum survival capacity of the inoculant present in the microbial consortium

Field experiment: To assess the feasibility of fertigation system of precision farming for the delivery of microbial inoculants, field experiment were carried out in Bhendi (Cultivar. COBH 1) at experimental station of TNAU, Coimbatore during July to December 2008. The chemical and microbiological properties of the experimental field were presented in Table 1. Experiment was carried out in a randomized block design with three replications having plot size of 40 sq.m. The mixed microbial inoculant was applied as single time, two split and three split times applications along with 75% N and P fertilizers. Concentrated liquid Microbial consortia (60

ml) were diluted with water (60 lit/ha) and fed through fertigation tank (60 lit capacity). In general the fertigation tank connected with drip lines for dropping the water and other nutrients nearer to the plant root zone. Seven days time intervals were maintained between microbial inoculant and liquid fertilizer application. Single time (60 ml) of microbial inoculant was applied at 30 days after sowing; two splits (of 30 ml each time) at 30 and 45 days and three splits (of 20 ml each time) at 30, 45 and 60 days after sowing. In order to evaluate the distribution pattern of bioinoculant's spread through biofertigation, four different samplings were made to collect the water samples from laterals each time, when microbial inoculants were applied. From a total 27 m length of laterals tubes of biofertigation, samples at 7 m, 14 m, 20 m and 27 m were collected in sterile containers and population of *Azospirillum*, phosphobacteria and *Pseudomonas* were enumerated by following standard procedure as described above. The rhizosphere soil samples were collected for enumeration of *Azospirillum*, phosphobacteria and *Pseudomonas* population. The plant samples were collected for total N (Humphries 1956); P and K (Jackson 1973) content analysis and yield was calculated and expressed as kg per ha.

Results

Effect of cell protectants on *Azospirillum* population: The maximum *Azospirillum* population was observed in microbial consortium + water (9.20 x 10^{16} cells / ml) followed by microbial consortium + 10 mM of phosphate buffer (pH 7) with 10% glycerol (5.40 x 10^{16} cells / ml). Whereas, the *Azospirillum* population of microbial consortium + 10 mM of polyvinylpyrrolidone (2.50 x 10^{16} cells / ml) was close to microbial consortium + 10 mM of trehalose (2.40 x 10^{16} cells / ml). But the treatment of microbial consortium + water (0.58 x 10^{21} cells / ml) produced low level of *Azospirillum* population compared to microbial consortium + 10 mM of phosphate buffer (pH 7) with 10% glycerol (0.76 x 10^{21} cells / ml) when increased dilution. The results suggested the positive effect of 10 mM of phosphate buffer (pH 7) with 10% glycerol on the protection of microbial cells during incubation period (3 days) than other cell protectants (Table 2).

Table 1: Physiochemical properties of experimental plot soil

Properties	Mean ± SE
pH	6.36 ± 0.09
EC (ds/m)	1.6 ± 0.15
Organic carbon (%)	0.26 ± 0.01
Available N (%)	310 ± 2.88
Available P (%)	44 ± 0.57
Available K (%)	935.3 ± 1.45
Total bacteria (cfu x 10^5 /gram dry weight of soil)[a]	19 ± 1.15
Fungi (cfu x 10^3 /gram dry weight of soil)[b]	4 ± 0.16
Diazotrophs (cfu x 10^4 /gram dry weight of soil)[c]	46 ± 1.15

Effect of cell protectants on phosphobacterial population: The data from the study revealed that the maximum phosphobacterial population was found in microbial consortium + 10 mM of phosphate buffer (pH 7) with 10% glycerol (250 x 10^{16} cfu / ml), followed by microbial consortium + 10 mM of glycerol (140 x 10^{16} cfu / ml). The data suggested that the survival of phosphobacteria was higher than *Azospirillum* and *Pseudomonas* inoculants, due to the ability of *Bacillus megaterium* var. *phosphoticum* to produce endospore, while the other two strains are not capable of producing the spores (Table 2).

Effect of cell protectants on *Pseudomonas* population: Among the treatments, the highest population of *Pseudomonas* was recorded in the treatment, microbial consortium + 10 mM of phosphate buffer (pH 7) with 10% glycerol (140 x 10^{16} cfu / ml), followed by microbial consortium + 10 mM of glycerol (30 x 10^{16} cfu / ml). Whereas, the treatment *viz*., microbial consortium + 10 mM of polyvinylpyrrolidone was found to be on par with microbial consortium +

Table 2: Effect of addition of cell protectants on survival of *Azospirillum, Phosphobateria* and *Pseudomonas* population in the microbial consortium 3 days after incubation under room temperature

	Treatments	*Azospirillum* (x 10^{16} cells/ml)	Phosphobacteria (x 10^{16}cfu / ml)	*Pseudomonas* (x 10^{16}cfu / ml)
T_1	Microbial consortium + water	9.20 (16.96) *	30 (17.48) *	20 (17.30) *
T_2	Microbial consortium + 10 mM of glycerol	3.50 (19.54) *	140 (18.15) *	30 (17.48) *
T_3	Microbial consortium + 10 mM of polyvinylpyrrolidone	2.50 (16.40) *	70 (17.85) *	20 (17.30) *
T_4	Microbial consortium + 10 mM of trehalose	2.40 (16.38) *	50 (17.70) *	00 (17.00) *
T_5	Microbial consortium + 10 mM of phosphate buffer (pH 7) with 10% glycerol	5.40 (16.73) *	250 (18.40) *	140 (18.15) *
	SE_d	1.14	1.23	1.19
	CD (P: 0.05)	2.62	2.83	2.75

* Transformed values given in parentheses

water (20 x 10^{16} cfu / ml) for *Pseudomonas* population. No growth was observed in microbial consortium + 10 mM of trehalose at 10^{-16} dilution. The results showed that the survival of *Pseudomonas* was lower than phosphobacteria and *Azospirillum.* Therefore, more amount of *Pseudomonas* inoculum than other two cultures has to be added in microbial consortium for maintaining uniform population of all the three cultures (Table 2).

Effect of incubation time on survival of *Azospirillum*, phosphobacteria and *Pseudomonas*: The data showed that, the maximum population of *Azospirillum* was observed in microbial consortium + 10 mM of phosphate buffer (pH 7) with 10% glycerol (0.72 x 10^{19} cells / ml) followed by microbial consortium + water (0.64 x 10^{19} cells / ml). The population of *Azospirillum* in microbial consortium + 10 mM of glycerol (0.56 x 10^{19} cells / ml) were significantly higher than microbial consortium + 10 mM of polyvinylpyrrolidone (0.48 x 10^{19} cells / ml) at 10 days after incubation (DAI). It was also observed from the results that the maximum phosphobacterial population was found in microbial consortium+10 mM of phosphate buffer (pH7) with 10% glycerol (25 x 10^{19} cfu/ml), followed by microbial consortium + water (18 x 10^{19} cfu/ml). Whereas, the other treatments such as microbial consortium + 10 mM of glycerol (13x10^{19} cfu/ml), microbial consortium + 10 mM of trehalose (14x10^{19} cfu/ml) and microbial consortium + 10 mM of polyvinylpyrrolidone (12 x 10^{19} cfu/ml) were close to each other for phosphobacterial population at 10 DAI (Table 3).

Effect of incubation time on survival of microbial consortium with cell protectants under refrigerated condition: The experimental results showed that the *Azospirillum* population was maximum in microbial consortium + 10 mM of phosphate buffer (pH 7) with 10% glycerol (4.8 x 10^{19} cells / mL), which was close to microbial consortium + water (4.7 x 10^{19} cells / mL) at 10 DAI. This is due to the possible protection of microbial cells by phosphate buffer and glycerol. The maximum phosphobacterial population was recorded in microbial consortium + 10 mM of phosphate buffer (pH 7) with 10% glycerol (30 x 10^{19} cfu / mL) followed by microbial consortium + 10% glycerol (26 x 10^{19} cfu / mL) at 10 DAI Among all the treatments, the maximum population of *Pseudomonas* was registered in microbial consortium + water (33 x 10^{19} cfu / mL), which was close to microbial consortium + 10 mM of phosphate buffer (pH 7) with 10% glycerol (28 x 10^{19} cfu / mL) at 10 DAI. The results showed that the *Pseudomonas* cells were activated in sterile water than cell protectants compounds.

Screening of microbial consortium for biofertigation: A liquid formulation of microbial consortium containing *Azospirillum*, phosphobacteria and *Pseudomonas* was developed exclusively for this study. The microbial consortium stored at room temperature in sterile plastic container had high shelf life of more than 12 months than other preservatives *viz*., trehalose and polyvinylpyrrolidone. The population of 19 ± 1.15 x 10^{17} cells/ml, 4 ± 0.16 x 10^{17} and 46 ± 1.15 x 10^{17} cfu per ml of *Azospirillum*, phosphobacteria and *Pseudomonas* respectively were recorded in the microbial consortium supplemented with 10 mM of phosphate buffer (pH 7) with 10% glycerol when it was used for field evaluation.

Effect of microbial consortium on distribution of inoculums within the lateral tubes of drip biofertigation system under precision farming system: Effect of microbial consortium load on distribution of inoculants in the lateral tubes, and delivery at emitter level present in the drip biofertigation system was investigated. The samples were collected randomly from the emitter or dripper after biofertigation with microbial consortium at 4.5 meter intervals of the lateral tubes present in the drip biofertigation system. The

Table 3: Effect of incubation time on the survival of the inoculants of the microbial consortium under room temperature

Treatments	*Azospirillum* (x 10^{19} cells/ml)		Phosphobacteria (x 10^{19} cfu /ml)		*Pseudomonas* (x 10^{19} cfu /ml)	
T_1 –Bioinoculants + water	0.64 (18.81) *	0.44 (18.64) *	18 (20.26) *	2 (0.30) *	18 (20.25) *	10 (20.0) *
T_2 –Bioioculants + 10mM of glycerol	0.56 (18.75) *	0.42 (18.62) *	13 (20.11) *	2 (0.30) *	14 (20.15) *	4 (0.64) *
T_3 –Bioioculants + 10mM of polyvinlprrolidine	0.48 (18.68) *	0.38 (18.68) *	12 (20.08) *	0 (0.00) *	5 (19.70) *	0 (0.00) *
T_4 –Bioinoculants + 10mM of trehalsoe	0.47 (18.67) *	0.32 (18.51) *	14 (20.15) *	1 (0.00) *	6 (19.78) *	0 (0.00) *
T_5 – Bioinoculants + 10mM of phosphate buffer (pH 7) with 10% glycerol	0.72 (18.86) *	0.50 (18.60) *	25 (20.40) *	4 (0.60)	21 (20.3) *	7 (19.8) *
SEd	1.28	1.27	1.38	0.021	1.37	0.042
CD (P: 0.05)	2.96	2.94	3.19	0.058	3.16	0.097

* Transformed values given in parentheses

Table 4: Effect of incubation time on the survival of the inoculants in the bioinoculants under refrigerated condition

Treatments	*Azospirillum* (x 10^{19} cells/mL)		Phosphobacteria x 10^{19} cfu /mL		*Pseudomonas* (x 10^{19} cfu /mL)	
	10 DAI	120 DAI	10 DAI	120 DAI	10 DAI	120 DAI
T_1 –Bioinoculants + water	4.7 (19.67) *	0.14 (18.15) *	20 (20.30) *	2 (0.30) *	33 (20.52) *	8 (0.90) *
T_2 –Bioioculants + 10mM of glycerol	0.40 (18.60) *	0.07 (17.85) *	26 (20.41) *	1 (0.00) *	22 (20.34) *	2 (0.30) *
T_3 –Bioioculants + 10mM of polyvinlprrolidine	0.38 (18.53) *	0.04 (17.61) *	14 (20.15) *	0 (0.00) *	10 (20.00) *	0 (0.00) *
T_4 –Bioinoculants + 10mM of trehalsoe	0.33 (18.58) *	0.02 (17.31) *	14 (20.15) *	0 (0.00) *	18 (20.26) *	0 (0.00) *
T_5 – Bioinoculants + 10mM of phosphate buffer (pH 7) with 10% glycerol	4.8 (19.68) *	0.17 (18.23) *	30 (20.48) *	2 (0.30) *	28 (20.45) *	3 (0.48) *
SEd	1.29	1.23	1.39	0.011	1.39	0.031
CD (P : 0.05)	2.99	2.81	3.20	0.025	3.21	0.071

results of precision farming could effectively deliver the microbial inoculants also. Uniform distribution of all the three microbial inoculants was recorded upto the full length of lateral pipes of fertigation (27 m). As the distance from biofertigation tank increased, there is no reduction in cell load per ml (Table 5).

Influence of biofertigation on rhizosphere colonization: The field experiment to study the fate of microbial inoculants in rhizosphere of Bhendi crop when applied through biofertigation revealed that 75% RDF of NPK+ One time microbial consortium application (30 DAS) reported maximum *Pseudomonas* (46.0 x 10^5 cfu/g) and phosphobacteria (33 x 10^5 cfu/g) populations in the rhizosphere of bhendi, while maximum *Azospirillum* populations was recorded in 75% RDF of NPK+ two split application of microbial consortium at 30 and 45 DAS (1.6 x 10^5 MPN/g). In general, the microbial consortium split dose application *viz*., double or triple did not show significant changes in the bacterial colonization at rhizosphere soils. The populations of all the three microbial inoculants declined gradually over the crop duration (Table 6).

Table 5: Distribution pattern of microbial inoculants through biofertigation

Distance from biofertigation tank [b]	Population (log cfu/ml)[a]		
	Azospirillum	Phosphobacteria	*Pseudomonas*
7 meter	5.96 ± 0.12	7.00 ± 0.12	7.30 ± 0.02
14 meter	5.73 ± 0.01	6.90 ± 0.09	7.20 ± 0.01
20 meter	5.54 ± 0.02	6.60 ± 0.01	7.08 ± 0.05
27 meter	5.34 ± 0.01	6.30 ± 0.01	6.90 ± 0.03

Values are pooled mean ± SE of three replicates of each sampling; [a] Water sample drops collected from emitters of laterals tubes were used for enumeration of bioinoculant organism; [b] Bioinoculants were applied through biofertigation tanks and samples were collected at different distances of laterals

Influence of biofertigation on plant growth and nutrient uptake: To assess the impact of biofertigation on the nutrient uptake of bhendi was estimated and the results revealed that all the treatments involving microbial consortium significantly increased root, shoot length

Table 6: Effect of biofertigation with microbial consortium on survival of *Azospirillum* phosphobacteria and *Pseudomonas* population

	Treatments	*Azospirillum* (x 10^5 MPN/ g)	Phosphobacteria (x 10^5cfu / g)	*Pseudomonas* (x 10^5cfu / g)
T_1	Uninoculated and unfertilized control	2.8 (5.45)*	25.0 (6.40)*	46.0 (6.66)*
T_2	75% RDF of NPK	2.5 (5.40)*	21.0 (6.32)*	32.0 (6.51)*
T_3	75% RDF of NPK + FYM	3.2 (5.51)*	26.0 (6.41)*	35.0 (6.54)*
T_4	75% RDF of NPK + one time microbial consortium application (30 DAS)	2.1 (5.32)*	33.0 (6.52)*	46.0 (6.66)*
T_5	75% RDF of NPK + Microbial consortium application at two intervals (30 and 40 DAS)	1.6 (5.20)*	26.0 (6.41)*	33.0 (6.52)*
T_6	75% RDF of NPK + Microbial consortium application at three intervals (30, 40 and 60 DAS)	1.5 (5.18)*	24.0 (6.38)*	29.0 (6.46)*
T_7	100% RDF of NPK	1.5 (5.18)*	15.0 (6.18)*	15.0 (6.18)*
T_8	Microbial consortium alone	2.7 (5.43)*	31.0 (6.52)*	43.0 (6.63)*
	SE_d	0.32	0.39	0.40
	CD (P: 0.05)	0.68	0.83	0.85

Values are pooled mean ± SE of three replicates of each sampling

Table 7: Effect of biofertigation with microbial consortium on plant growth and yield

	Treatments	Root length (cm/plant)	Shoot length (cm/plant)	Dry weight (g/plant)	Yield (Kg/ha)
T_1	Uninoculated and unfertilized control	20	33.5	25.0	5352.5
T_2	75% RDF of NPK	25	35.0	40.9	6050.0
T_3	75% RDF of NPK + FYM	38	40.0	43.0	6850.0
T_4	75% RDF of NPK + one time microbial consortium application (30 DAS)	32	45.0	45.0	6860.0
T_5	75% RDF of NPK + Microbial consortium application at two intervals (30 and 40 DAS)	28	38.3	28.0	6425.0
T_6	75% RDF of NPK + Microbial consortium application at three intervals (30, 40 and 60 DAS)	26	32.0	25.4	6550.0
T_7	100% RDF of NPK	24	35.0	35.6	5850.0
T_8	Microbial consortium alone	32	32.0	37.0	4855.0
	SE_d	1.81	2.30	2.25	376.92
	CD (P: 0.05)	3.87	4.92	4.82	808.57

Values are pooled mean ± SE of three replicates of each sampling

and dry weight of bhendi than the controls (Table 7). Among the three different split dose applications of microbial consortium, single time/single dose of 60 ml of concentrated microbial consortium application at 30 days after sowing influenced maximum plant growth than other two split time/doses. This was also reflected in the nutrient uptake of bhendi. The 75% RDF of NPK fertilizer along with single time/dose of microbial consortium applied at 30 days after sowing reported to be the maximum uptake of nitrogen, phosphorus and potassium. The split doses namely double and triple split of bioinoculants at 30, 45 and 30, 45 and 60 days could not enhance the nutrient uptake of the crop. This trend was also reflected in the yield. Overall fruit yield increases was recorded in single dose/time of bioinoculants with 75 per cent chemical fertilizer application followed by farm yard manure applications.

Discussion

Fertigation system of precision farming is help in effective delivery of nutrients exactly at the root zone of crop, which minimize the loss as well as the environmental hazards caused by the chemicals. This technology ensures the fertilizer use efficiency for a greater extent. Biofertigation can precisely deliver the bio inoculants in the root zone (Gomathy et al. 2008). It is an added advantage whereas microbial inoculants are supplied through biofertigation as it has more water use efficiency and fertilizer use efficiency, quality etc. Effective microorganisms can also applied in the field along with inorganic materials (Hussain et al. 1999). In the present study, the liquid microbial consortium was developed by mixing the cell pellets of *Azospirillum brasilense sp 7*, *Bacillus megaterium* var. *phosphaticum* and *Pseudomonas fluorescens* at 2:1:0.5 ratio after multiplication in the respective broth with different incubation time *viz.,* 20 days for *Azospirillum*, seven days for *Bacillus* and four days for *Pseudomonas*. Addition of the cell protectants along with microbial consortium favoured the survival of microbes up to 12 months. Maximum number of cells of the inoculants *viz*., *Bacillus* ($250x10^{16}$ cfu/ml) and *Pseudomonas* ($140x10^{16}$ cfu/ml) was observed in microbial consortium supplemented with cell 10mM of phosphate buffer with 10% glycerol than microbial consortium + water. Enhanced survival of inoculants in the microbial consortium may be due to the action of chemical amendments added in the liquid formulation. Among the cell protectants such as trehalose, polvinylpyrolidone and glycerol used, glycerol (10%) had performed well to keep cells in viable farm in liquid culture. Tamil vendan and Thangaraju (2006) reported that glycerol is an effective cell protectant enhances the cell viability and prevents the cells from desiccation by slow down the rate of drying (Lorda and Baltti 1996).

Since fertigation system ensures the effective delivery of inputs to the root zone of crop plants, the microbial consortium was evaluated for its effectiveness to spread throughout the field from fertigation tank to root zones through laterals. The total length of laterals for nutrient distribution was 27 meter and at 4 equal intervals samples from laterals were collected and enumerated the population of the three organisms. The results clearly confirmed that fertigation could be an effective system to deliver the microbial inoculants also. Effective number of cells (> 105 cells/ml) were observed at almost end of the lateral pipe, suggested that the lateral pipes of precision farming could deliver the microbial inoculants much more effectively than conventional farm methods. The experiments to find out the split dose of biofertigation (referring the fertigation of microbial inputs) revealed that single time/single dose is sufficient to maximum rhizosphere colonization of these rhizobacteria *viz., Pseudomonas* (46.0×10^5 cfu/g) and phosphobacteria (33×10^5 cfu/g) populations. Further, the inoculants along with 75% recommended liquid

fertilizers performed better in terms of root length (32 cm/plant), shoot length (45 cm/plant), dry weight (45 g/plant) and fruit yield (6860 Kg/ha) of bhendi. The temporal separation of organic inputs and inorganic inputs through feeding schedule make it more viable technology for modern agriculture.

Biofertigation with Azophosmet significantly enhanced boll number and seed cotton yield due to the effective translocation of nutrients to reproductive parts compared to conventional methods of soil application of nutrients (Gomathy *et al*. 2008). Better performance of the combined inoculation of *Azospirillum, Methylobacterium* and phosphobacteria in cotton especially in the later stages of crop growth was recorded (Senthilkumar and Sundaram, 2005). Plants grow well and the plant height was maximum, when the chemical fertilizers were combined with bioinoculants. Drip fertigation with 150% RDF of NPK and biofertigation produced significantly higher dry matter production of 2450.8, 5846.0 and 6875.5 kg/ha at 60, 90 and 120 DAS respectively (Gomathy *et al*. 2008). Liquid formulations of microbial inoculants have advantages such as less contaminations, longer shelf life, higher efficiency, low quantity requirement for applications (Singleton et al. 2002). The liquid formulations of microbial resources could be a potential organic input for precision farming, which can be easily delivered through fertigation system for effective colonization of root zone of crop plants. Tamil Vendan (2004) developed liquid microbial consortium with *Azospirillum* for standardization and evaluation of liquid formulation for field application. Manikantan (2008) developed liquid formulation of *Pseudomonas fluorescens* for management of tomato *Fusarium* wilt.

Acknowledgment

I would like to thank Dr. D. Balachander, Professor and K. Kumar, Retired Professor, Dept. of Agricultural Microbiology, Tamil Nadu Agricultural University, Coimbatore for the initiative spirit, assistantship and guidance to work in this area.

सारांश

भविष्य आधारित सूक्ष्म जीवी टीकाकरण (*माइक्रोबियल इनोक्यूलेट्स*) को व्यापक रूप से प्रचलित कृषि में फसल विकास और उपज में सुधार हेतु किया जाता है। तार्किक खेती एक क्षेत्र विशिष्ट प्रबंधन दृष्टिकोण है, जबकि परंपरागत पद्धति एक समान उर्वरक प्रयोग है। भविष्य आधारित टीकाकरण प्रभाव के कारण तार्किक खेती प्रणाली के लिए उपयुक्त नहीं है। इसलिए तरल माइक्रोबियल कंसोर्टियम का विकास तीन टीकाकरणों जैसे *एजोस्पीरील्लम ब्रासीलेन्स* प्रजाति–7, *बैसीलस मेगाटेरियम* वार. *फास्फैटिकम* एवं *स्यूडोमोनास फ्लोरोसेन्स* के साथ उचित कोशिका रक्षक, फॉस्फेटिकम और स्यूडोमोनस के साथ 10 एमएम फॉस्फेट बफर के साथ 10 प्रतिशत ग्लिसरॉल का उपयोग करके विकसित किया गया था। भिण्डी (सीओबीएच 1) में तार्किक खेती प्रणाली के तहत पौधों की वृद्धि पर बायोफर्टिशन के प्रभाव का अध्ययन करने के लिए एक प्रक्षेत्र प्रयोग किया। परिणाम से स्पष्ट हुआ कि नत्रजन, फास्फोरस, पोटाश का 75 प्रतिशत आर डी एफ + माइक्रोबियल कंसोर्टियम के (60 लीटर/हे.) के प्रयोग जैसे एजोस्पिरिलम (5.96 ± 0.12 लॉग कोशिका/मिली), *बेसिलस मेगाटेरियम* वार. । फॉस्फेटिकम (7.00 ± 0.12 मिली लाग कोशिका/मिली.), और स्यूडोमोनस 7.30 ± 0.12 लॉग कोशिका/मिली.) का प्रभाव उत्तम पाया गया जो परंपरागत पद्धति तुलना में उपज में 10 प्रतिशत ज्यादा वृद्धि थी।

References

Bakker PAHM (2001) Effects of *Pseudomonas putida* modified to produce phenazine 1 carboxylic acid and 2, 4 –diacetyl phloroglucinthe microflora of field grown wheat. Antonie van Leenwenhoek 81: 617-624.

Bashan Y (1998) Inoculants of plant growth-promoting bacteria for use in agriculture. Biotech Adv 16:729-770.

Bashan Y Levanony H and Klein E (1986) Evidence for a weak active external adsorption of *Azospirillum brasilense* cd to wheat roots. J Gen Microbiol 132: 3069-3073.

Drinkwater LE and Snapp SS (2007) Nutrients in agroecosystems: Rethinking the management paradigm. Advan Agron 92: 163-186

Gomathy M Sathya Prakash D Thangaraju M Sundaram, SP and Manicka Sundaram P (2008) Impact of Biofertigation of Azophosmet on Cotton Yield under Drip irrigation. Agricul Biol Scien 4(6): 695-699.

Hely FW Hutchings RJ and Margaret Z (1976). Legume inoculation by spraying suspensions of nodule bacteria into soil beneath seed. J Aust Inst Agric Sci 42: 241-244.

Hill GT (2000) Methods for assessing the composition and diversity of soil microbial communities. Appl Soil Microbiol 15: 24- 6.

Humphries EC (1956) Mineral components and ash analysis. Modern methods of plant analysis. Springer –Verlag 1: 468-502.

Hussain T Javaid T Parr JF Jilani G and Haq MA (1999) Rice and wheat production in Pakistan with effective microorganism. Am. J. Alt. Agri., 14: 30-36.

Jackson ML (1973) Soil Chemical Analysis. Prentice Hall India Pvt. Ltd., New Delhi.

James N (1958) Soil extract in Soil Microbiology. Canad J Microbiol 4: 363

King KO Ward MK and Raney DE (1954) Two simple media for the demonstration of pyocyanin and fluorescein. J Lab Clin Med 44: 301-307.

Lorda G and Balatti A (1996) Designing media I and II. In: Legume Inoculants. Selection and Characterisation of Strains, Production, Use and Management (eds.) Balatti and Freire. Editorial Kingraf, Buenos Aires, pp148.

Lorda GS and Balatti A (1996) Designing media I. In: Legume inoculants selection and characterization of strains-Production, use and management. Editorial kingragf. Calle. Argentina,78-96.

Okon Y Albrect SL and Burris RH (1977) Carbon and ammonia

metabolism of *Spirillum lipoferum*. J. Bacteriol., 128: 592 - 597.

Parkinson D Gray JRG and Williams ST (1971) In: Methods for studying the Ecology of soil Microorganisms. Oxford Blackwell Scientific Publications. P. 116.

Rangaswami G and Bagyaraj DJ (1993) Agricultural Microbiology, Prentice Hall of India Pvt. Ltd., New Delhi.

Rennie RJ (1981) A Single medium for the isolation of acetylene reduction (nitrogen fixing) bacteria from soils. Can J Microbiol 27: 8-14

Saxena AK and Tilak KVBR (1998) Free living nitrogen fixers: Its role in crop production. In: microbes for Health, wealth and sustainable Environment (ed. Verma, AK.) Malhotra publ. Co, New Delhi.

Senthilkumar M and Sundaram SP (2005). *Invitro* 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.

Singleton PW Keyser HH and Sande ES (2002). Development and evaluation of liquid inoculants. In: Inoculants and nitrogen fixation of legumes in Vietnam. (ed.) D. Herridge, ACIAR Proceedings, 52-66.

Somasegaran PH Hoben J and Gurgun V(1988) Effects of inoculation rate, *Rhizobium* strain competition and nitrogen fixation in chickpea. Agron J 80: 68-73.

Tamil Vendan R and Thangaraju M (2006) Development and standardization of liquid formulation for *Azospirillum* bioinoculant. Indian J. of Microbiol., 64 : 379 - 387.

Tilak KVBR (1993) Bacterial Fertilizers- Indian Council of Agricultural Research. New Delhi.

Tilak KVBR, Ranganayaki N, Pal KK, De R, Saxena AK, Shekha C, Nautiyal S, Mital A, Tripath K and Johri BN (2005) Diversity of plant growth and soil supporting bacteria. Curr Sci 89 (1): 136-150.