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EVALUATION OF NEW MICROBIAL CONSORTIUM THROUGH BIOFERTIGATION FOR PRECISION FARMING OF BHENDI (COBH 1)

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ABSTRACT

Use of carrier based inoculum for crop growth and yield is wide practice among the conventional agriculture. 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 protectants of 15% glycerol. We conducted a field trail 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* (7.00 ± 0.12 ml log cells/ml) and *Pseudomonas (*7.30 ± 0.02 log cells/ml), plant growth and 10% increased yield over conventional method

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

Abbreviations

RDF: Recommended dose of NPK fertilizers

Introduction

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). Precision agriculture mergers the new technologies borne of information age with a mature agricultural industry. It is an integrated crop management system that attempts to match the kind and amount of inputs with the actual crop needs for small area within a farm field (Ahlwaalia et al., 1993). It is based on soil, weather and crop requirement to maximize sustainable productivity, quality and profitability. 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).

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). The potential of precision farming for economical and environmental benefits could be visualized through reduced use of water, fertilizers, herbicides and pesticides besides the farm equipments (Anil Kumar Singh, 2009). Instead of managing an entire field based upon some hypothetical average condition, which may not exist anywhere in the field, a precision farming approach recognizes site specific differences within fields and adjusts management actions accordingly. Farmers usually aware that their fields have variable yields across the landscape. This variation can be traced to management practices, soil properties and/or environmental characteristics. Soil characteristics that affect yields include texture, structure, moisture, organic matter, nutrient status, landscape position and environmental characteristics include weather, weeds, insects and diseases (Anil Kumar Singh, 2009). Consideration of all the above factors

each input *viz.*, nutrients, herbicides, pesticides, biocontrol agents, growth hormones and biofertilizers should be taken into account for integrated and precision approaches in which to get maximum yield with ecofriendly. Introducing beneficial microorganisms to the crop plants, referring inoculation is a good practice being popularized among the farmers for most of the field crops. The nitrogen fixing, P solubilizing and plant growth promoting effective bacteria, isolated from different ecological niches will be introduced to crop through seed, seedling or soil in order to colonize and help for nutrient supplementation (Prabhakara and Ravi, 1991; and Tilak and Singh, 1994). Present microbial inoculant technology ensures 25 per cent reduction of chemical N and P fertilizers in India (Vijaya Nirmala and Sundaram, 1996). Rana et al. (1975) reported increased in the wheat yield, P uptake and the available P in neutral soil on inoculation of wheat seeds with *Bacillus polymyxa*, *B. circulans*, *Pseudomonas striata* and *Aspergillus awamorii.* Among the inoculants, *Azospirillum* (nitrogen fixing organism), *Bacillus megaterium* (P solubilizing bacteria) and *Pseudomonas fluorescens* (Plant Growth Promoting Rhizobacteria, PGPR) are the potential candidates used in India as well as worldwide (Tiwari et al., 1993). They colonize the roots of crop plants, provide nutrients and protect crop plants from pathogens. The packages of practice to use these inoculants are well studied and formulated for conventional agriculture. In conventional agriculture, there will be spacial and temporal differentiation between biological inputs such as microbial inoculants and chemical fertilizers, as most of the inputs are harmful to microbial activities. Under precision farming, all the inputs are in liquid form and they were fed through lateral tubes from the source tank directly to the crop root zone (Bafna et al., 1993; and Ahlwaalia et al., 1993). The liquid fertilizers, herbicides, pesticides and growth harmones were used to fed with regular intervals under precision farming system. When microbial resources are used, their feeding schedule along with other chemical inputs should be standardized.

Further, the fate of these inoculants passing through the lateral tubes and how best they reached the root zone of crops are to be studied and optimized. Hence, the present investigation focused to standardize the microbial inoculant's schedule for precision farming and to evaluate the feasibility of the delivery system to effectively introduce the microbial inoculants to the rhizosphere of crop plant.

Materials and Methods

Microbial inoculant strains and growth media

Associative nitrogen fixing bacterial strain, *Azospirillum brasilense* strain sp7 and free living P solubilizer, *Bacillus megaterium.* var. *Phosphaticum* strain Pb 1 obtained from Department of Agricultural Microbiology, Tamil Nadu Agricultural University, Coimbatore, India and Plant Growth Promoting Rhizobacterium, *Pseudomonas fluorescens* strain Pf 1 from 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* was grown in King's B medium **(**King et al., 1954).

Microbial consortium preparation

The three bacterial strains, grown in 250 mL of respective broth at 30ºC upto late log phase were centrifuged at 10,000 rpm for 15 min. at room temperature using high volume centrifuge (Hitachi, Japan). The cell pellets were washed with sterile distilled water twice, resuspended in sterile distilled water with 15 per cent glycerol and stored at -20ºC 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). After enumerating each inoculant, equal ratio of *Azospirillum*, phosphobacteria and *Pseudomonas* were mixed based on their population density in a sterile plastic container and stored at room temperature.

Field experiment

To assess the feasibility of fertigation system of precision farming for the delivery and dosage standardization of microbial inoculant, field experiment was carried out in Bhendi (Cultivar. COBH 1) at experimental station of Tamil Nadu Agricultural University, Coimbatore, India during October, 2008. The physico chemical and microbiological populations of the experimental field soil were presented as Table 1. Experiment was carried out in a randomized block design with three replications with a plot size of 40 sq.m. each. Fertilizers in liquid form were applied at 200:100:100 Kg N: P: K per ha through fertigation tank. When microbial inoculants are included as treatment, 75 per cent of N and P fertilizers were applied. The mixed microbial inoculant was applied as single dose, two split and three split dose applications along with 75% N and P fertilizers. Microbial inoculants were diluted at 60 mL/ha in fertigation tank (60 lit capacity) and time intervals of 7 days were maintained between microbial inoculant and liquid fertilizer application. Single dose of microbial inoculant was applied at 30 days after sowing; two splits at 30 and 45 days and three splits at 30, 45 and 60 days after sowing. Inorder 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 collected at 30, 60 and 90 days after sowing were quantified for population of *Azospirillum*, phosphobacteria and *Pseudomonas*. The plant samples collected at 30, 60 and 90 days old crops were analysed for total N (Humphries, 1956); P and K (Jackson, 1973) and with the help of dry matter content, uptake of nutrients were calculated as Kg per ha.

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

Values are mean ± SE of three replications
a Total bacteria were enumerated by serial dilution plating method on soil extract agar medium (James, 1958).
B Total culturable fungi were enumerated by serial dilution plating method as described by Parkinson et al. (1971)
C Total diazotrophs were enumerated by the procedure as described by Rennie (1981).

Result

Microbial consortium for biofertigation

A liquid formulation of microbial consortium containing *Azospirillum*, phosphobacteria and *Pseudomonas* was developed exclusively for this study. The microbial consortium in presence of 15% glycerol stored at room temperature in sterile plastic container had high shelf life of more than 12 months than other preservatives. The population of 19 ± 1.15 x 1017 cells/ml, 4 ± 0.16 x 1017 and 46 ± 1.15 x 1017 cfu per ml of *Azospirillum*, phosphobacteria and *Pseudomonas* respectively were recorded in the microbial consortium just before used for field evaluation.

Delivery and distribution of microbial inoculants

To evaluate the efficiency of fertigation system for effective delivery of microbial consortium, water samples at different distance from fertigation tank were collected throughout the field and the populations were assessed. The 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 2).

Influence of biofertigation on rhizosphere colonization of beneficial bacteria

The field experiment to study the fate of microbial inoculants in rhizosphere of Bhendi crop when applied through biofertigation revealed that single dose of microbial consortium at 30 days old crop is sufficient to keep the populations at higher levels in the roots. The treatment, 75 per cent NPK as fertilizers and single dose of microbial consortium (T4) reported maximum *Pseudomonas* (Fig. 1c) and phosphobacteria (Fig. 1b) populations in the rhizosphere of bhendi, while maximum *Azospirillum* populations was recorded in two split dose application (T6) (Fig. 1a). In general, the split dose application of microbial consortium as 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.

Influence of biofertigation on plant growth and nutrient uptake of bhendi (COBH 1)

To assess the impact of biofertigation of bioinoculants, the nutrient uptake of bhendi was estimated in three different stages of the crop and results revealed that all the treatments involving microbial consortium evaluated the root and shoot length and dry weight of bhendi than the controls (Table 3). Among the three different split dose applications of microbial consortium, single dose of 300 ml of consortium application at 30 days after sowing influenced maximum plant growth than other two split doses.

This was also reflected in the nutrient uptake of bhendi (Fig. 2). The 75% RDF of NPK fertilizer along with single dose of microbial consortium applied at 30 days after sowing reported to be the maximum uptake of nitrogen, phosphorus and potassium (Fig. 2). The split doses namely double and triple split of bioinoculants at 30 x 45 and 30, 45 and 60 days could not enhance the nutrient uptake of the crop. This trend was also reflected in the overall fruit yield increase was recorded in single dose of bioinoculants with 75 per cent chemical fertilizer application followed by farm yard manure applications.

Discussion

Soil is a natural ecosystem in which large variety and number of microorganisms proliferate. Bacteria present in the rhizosphere called rhizobacteria have the ability to colonize the plant roots and/or their immediate environment, in many species. These rhizobacteria help the plant directly by promoting plant growth through physiological and biochemical mechanisms and indirectly through disease control by competition (Bashan and Holguin, 1998). Identification, possible manipulation of relationship between rhizobacteria and crop plant and effective delivery to the rhizosphere are considered as basic strategy of modern agriculture in developing countries. Many successful microbial inoculant technologies have been developed and practiced for modern agriculture (Rengel and Marschner, 2005). Precision farming is one among the integrated management approaches of agriculture, which include fertigation and combined practice of organic and inorganic farming to get highest yield and to minimize the cost of farming.

Fertigation system of precision farming is considered as effective delivery of nutrients exactly at the root zone of crop, which minimize the loss as well as reduce the environmental hazards caused by the chemicals. This technology ensures the fertilizer use effectively to 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).

Liquid formulations of microbial inoculants have advantages such as zero contaminations, longer shelf life, higher efficiency, low quantity requirement for applications (Singleton et al., 2002). These 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.

Inorganic inputs have been already well standardized for most of the crops and no such investigation were made for microbial inputs. Further, it is also essential to develop a consortium of microbial inputs exclusively for precision farming, as the delivery system and method are different than the conventional agriculture. To fulfill this objective, the present work was conducted and the resultant microbial consortium obtained has high number of population per ml and almost equal number of cells of individual organisms. Among the different cell protectants used to keep cells in viable farm in liquid culture, glycerol (10%) had performed well. 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; Tamil vendan and Thangaraju, 2006).
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 dose is sufficient to colonize maximum rhizosphere colonization of these rhizobacteria. Further, the inoculants along with 75% recommended liquid fertilizers performed better in terms of nutrient uptake, plant growth and fruit yield of bhendi. The temporal separation of organic inputs and inorganic inputs through feeding schedule make it more viable technology for modern agriculture.

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Appendix:

Table 2. Distribution pattern of microbial inoculants through biofertigation for bendhi (Cultivar COBH 1)

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

Distance from biofertigation tank [b]	Ez;qaph bry;fs; / kpyp		
	Azospirillum	Phosphobacteria	***Pseudomonas***
7 meters	5.54	6.80	7.25
14 meters	5.23	6.62	7.10
20 meters	4.48	6.35	6.95
27 meters	4.24	6.10	6.87

Table 3. Effect of biofertigation with microbial consortium on plant growth at 45 DAS and yield of bhendi (COBH 1) under precision farming system

Treatments	Root length (cm/plant)	Shoot length (cm/plant)	Dry weight (g/plant)	Yield (Kg/ha)
T_1 Uninoculated and unfertilized control	20 ± 1.15	33.5 ± 1.73	25.0 ± 0.58	5352.5 ± 17.41
T_2 .75% RDF (recommended dose of fertilizers) of NPK	25 ± 1.58	35.0 ± 0.58	40.9 ± 1.15	6050.0 ± 16.16
T_3 . 75% RDF of NPK + FYM	38 ± 1.73	40.0 ± 1.15	43.0 ± 1.73	6850.0 ± 18.47
T_4 . 75% RDF of NPK+ One time microbial consortium application (30 DAS)	32 ± 1.15	45.0 ± 0.58	45.0 ± 1.73	6860.0 ± 16.75
T_5 . 75% RDF of NPK + Microbial consortium application at two intervals (30 and 45 DAS)	28 ± 0.58	38.3 ± 1.15	28.0 ± 0.58	6425.0 ± 15.01
T_6 . 75% RDF of NPK + Microbial consortium application at three intervals (30, 45 and 60 DAS)	26 ± 2.31	32.0 ± 1.15	25.4 ± 0.58	6550.0 ± 17.89
T_7 .100% RDF of NPK	24 ± 0.58	35.0 ± 0.58	35.6 ± 1.45	5850.0 ± 19.63
T_8 . Microbial consortium application alone	32 ± 1.15	32.0 ± 0.58	37.0 ± 0.58	4855.1± 16.17

Values are mean ± SE of three replicates

Table 4. Effect of biofertigation with microbial consortium on plant growth and yield of bhendi (COBH 1) under precision farming system

ஆய்வுகள்	Root length (cm/plant)	Shoot length (cm/plant)	Dry weight (g/plant)	Yield (Kg/ha)
Uninoculated and unfertilized control	20	33.5	25.0	5352.5
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

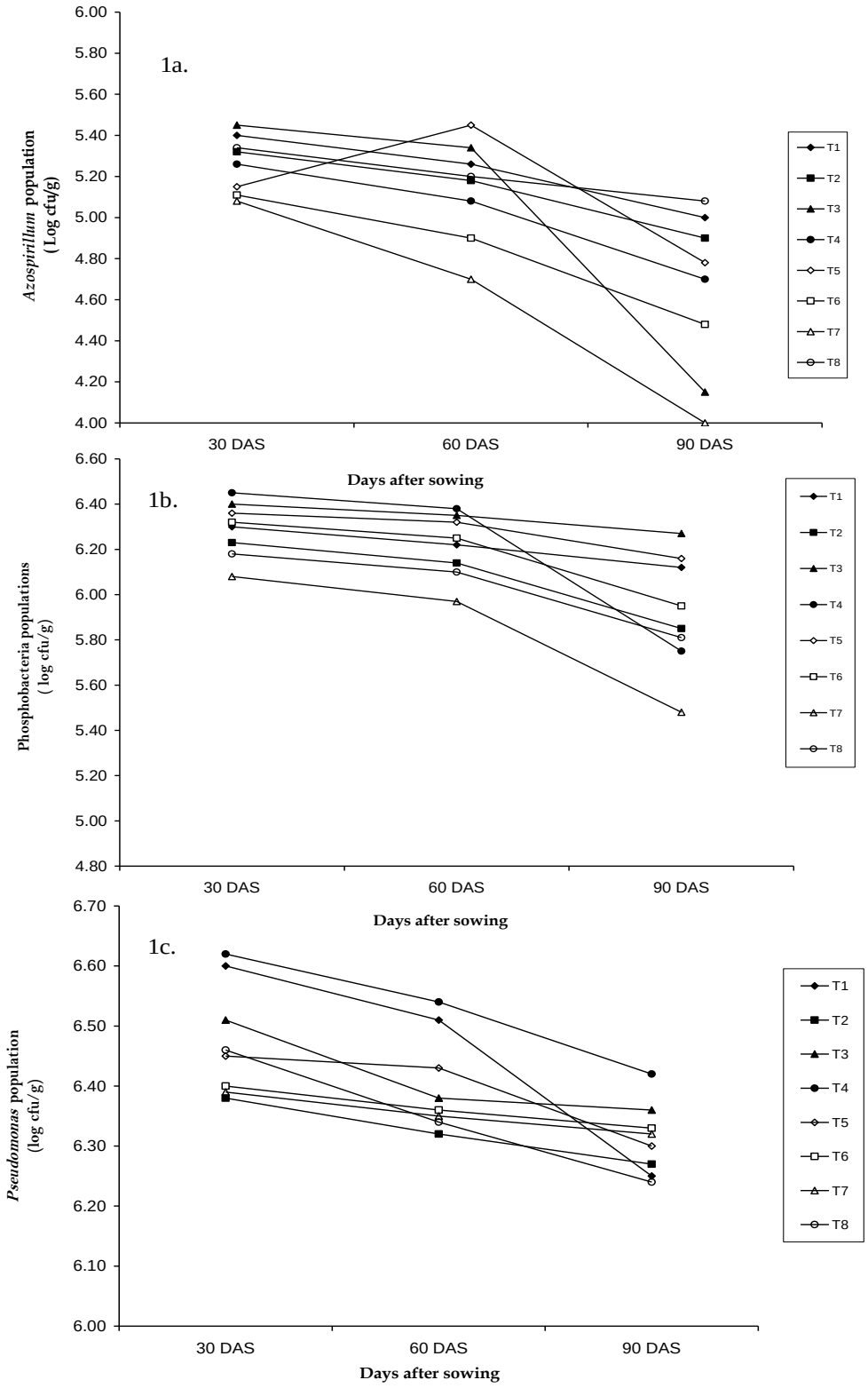

Fig 1. Biofertigation with microbial consortium on survival of inoculants in the soil cropped with bhendi (COBH 1) under precision farming system

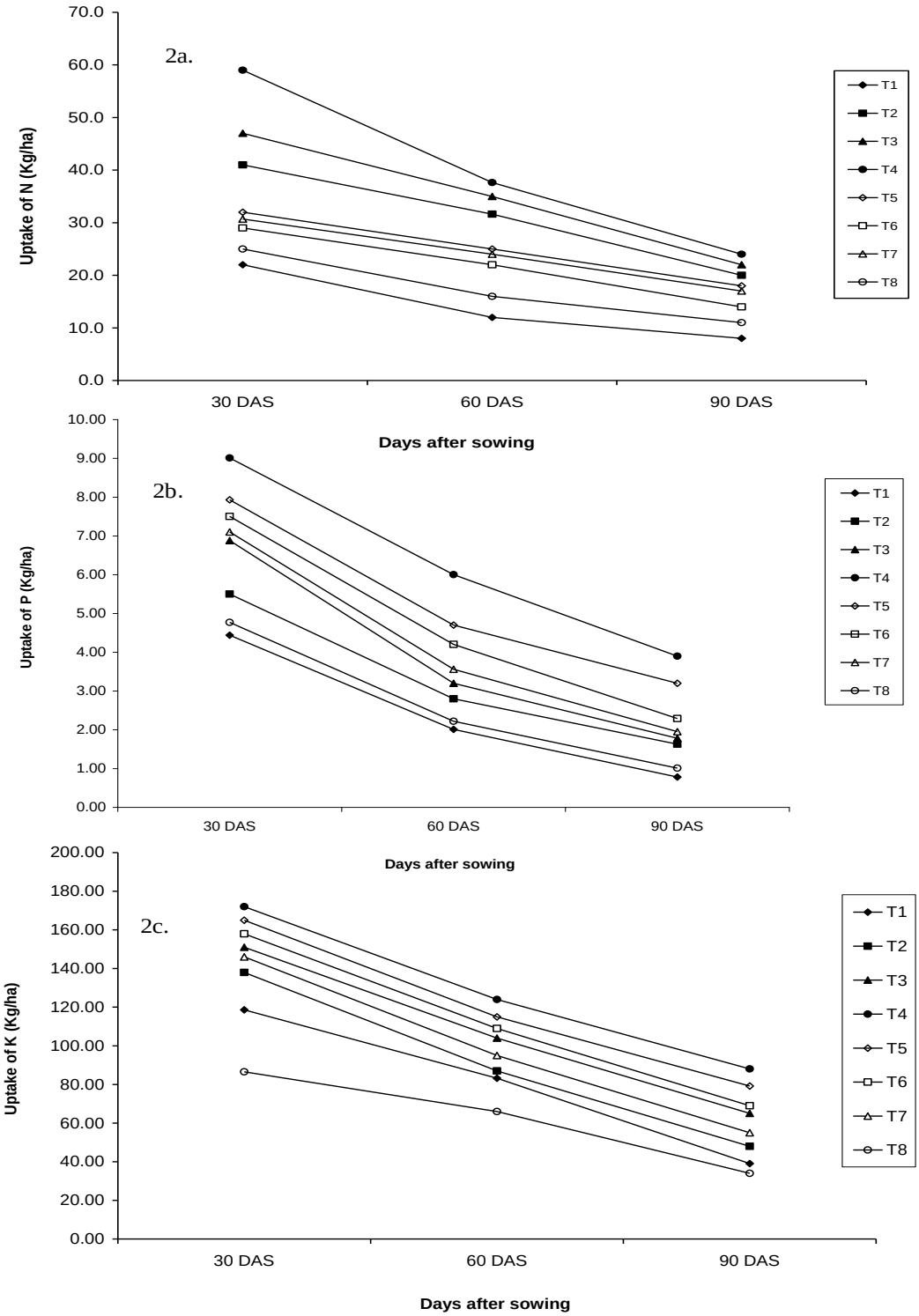

Fig 2. Biofertigation with microbial consortium on uptake of NPK of soil cropped with bhendi (COBH 1) under precision farming system