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Research Article
Bio-filmed Bio-fertilizers and Organic Manure Application for Reducing Chemical Fertilizer in Sustainable Okra production

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Abstract
Background and Objective: The search for sustainable means of reducing deleterious effects of chemical fertilizers in crop productivity is a global concern. Thus, the present study was carried out from 2015-2017 to investigate the potentials of *in vitro* developed bio-film bio-fertilizers and organic manure for reducing chemical fertilizer in okra (*Abelmoschus esculentus* (L.) Moench) production at Tala village (Jaipur district), Rajasthan state, India. **Materials and Methods:** Treatments were prepared from organic manure (OMT), arbuscular mycorrhizal fungi-*Azotobacter* bio-filmed bio-fertilizers (BFT), bio-filmed bio-fertilizers+organic manure (OBT), 65% recommended rate chemical fertilizer (CHT) and an untreated (UT) control in three replicates on randomized block design layout. **Results:** The cumulative effect of OBT treatment produced the highest values for all the parameters examined. Followed by the BFT and OMT treatments which were similar. The CHT treatment was next and the untreated control (UT) treatment had the lowest values for all parameters analyzed. **Conclusion:** There is the possibility to customize combinations of bio-filmed bio-fertilizers and organic manure for optimizing economics of production by reducing chemical fertilizer application.

Key words: *Abelmoschus esculentus*, organic manure (OMT), bio-filmed bio-fertilizers, chemical fertilizer application, economics of production

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Okra (*Abelmoschus esculentus* (L.) Moench) production has become a major enterprise in horticulture. Its consumption and production has gained immense popularity globally due to the greater appreciation of its food values, contains vitamins, proteins, oils and minerals such as calcium, iron magnesium and phosphorus[1]. The use of many improved okra varieties coupled with extensive use of synthetic fertilizers[2] has increased yield.

However, overuse and misuse of synthetic fertilization in food production has begun to exhibit diminishing dividends, resulting in rising levels of uncertainty in its suitability for future food production[3]. Continuous dependence may result in reduced food quality and increased cultivation cost[4]. Hence, the deleterious effects in the use of chemical fertilizers have led to a search for other ways to better crop productivity. Organic farming with application of bio-fertilizers and organic manure has been used to improve environmental safety and sustainable agricultural practice[5]. Advancement in bio-fertilizers research has led to development of bio-filmed bio-fertilizers (BFBFs) with abilities to overcome reduced persistence and inability to successfully compete with indigenous soil micro-flora as is the case with conventional bio-fertilizers[6].

Bio-filmed bio-fertilizers are *in vitro* developed surface-attached mass of single colonies or multi species of plant growth promoting microbial cells in intimate contact with each other, encased in a self-produced matrix of extracellular polymeric substances (EPS). Application of bio-filmed bio-fertilizers protects inoculants against adverse environmental conditions, competition by native soil populations[7] and resistance to protozoan grazing[8]. Bio-film formation has been reported to enhance effective establishment of inoculants in plant roots and rhizosphere, where they can compete well with indigenous micro-flora and provide improved plant growth promotion, resulting in increased agricultural productivity[9].

Organic manures help improve nutrient status, water holding capacity, structure and soil aeration and provides better establishment of inoculated micro-organisms along with accumulation of excess humus in the soil[4]. As a novel approach to sustainable food production, application of bio-filmed bio-fertilizers and organic manure either alone or in combination was capable of replacing at least 50% of all 3 major synthetic fertilizers when applied to several non-leguminous crops[8] and tomato, chili pepper, cabbage[9]. Long term use of these organic inputs is economical, eco-friendly and increases crop productivity through enhancement of plant-associated nitrogenase activity, improvement of soil carbon sequestration[10], increment of plant growth promoting activities and efficient nutrient recycling compared to their conventional counterparts[11].

Previous studies justifying the effects of application of *in vitro* developed bio-film bio-fertilizers (BFBFs) and organic manure on growth and yield of okra are non-existent. Thus, the present study investigated the potentials of application of *in vitro* developed fungal-bacterial bio-film bio-fertilizers (FBBs) and organic manure on the growth and yield of okra.

MATERIALS AND METHODS

Site of the experiment: The field experiment was conducted at Tala village in Jaipur district of Rajasthan during the 2015-2017 okra planting seasons. Tala village is located at latitude 27°35 North and longitude 76° 07 East at an elevation of 351 m above sea level. The area is in the semi-arid agro-ecological zone of India with alluvial soil type[12]. Mean monthly values of temperature and rainfall during the okra cropping seasons varied (Table 1).

Soil physico-chemical parameters: Soil samples were collected for physicochemical analysis at a depth of 0-30 cm using soil auger before commencement of the experiment in 2015. Soil pH, EC, CEC, available phosphorus, total nitrogen, exchangeable Ca, Mg, K, Na, soil OC, particle content and texture (Table 2) were determined according to standard methods[13].

Screening of inoculants: Azotobacter cells and Arbuscular mycorrhizal fungi (AMF) spores were procured from Microbial Type Culture Collection and Gene Bank, Institute of Microbial Technology, CSIR, Chandigarh, India. The Azotobacter and AMF spores were screened for potency for bio-film formation and tolerance abilities using the method described by Christenson[14], while quantity of bio-film formed was determined by measuring absorbance at 570 nm in a micro-liter plate reader[15].

Development of FBBs: The FBBs were developed using the method of Senevirante *et al.*[7]. Bio-film maturity was determined microscopically at different stages. Nitrogenase activity was determined using acetylene reduction assay method described by Zuberer and Silver[16]. The culture was incubated at 27-30°C for 7 days in a shaker (80 rpm) to form mature bio-film.

Table 1: Mean values of temperature and rainfall during the planting seasons

	2015		2016		2017	
Months	Mean temperature (°C)	Mean rainfall (in)	Mean temperature (°C)	Mean rainfall (in)	Mean temperature (°C)	Mean rainfall (in)
May	32.4	0.8	31.2	0.9	31.9	0.8
June	32.3	2.7	33.8	2.1	32.8	2.4
July	31.6	9.5	32.6	9.2	30.5	9.7

Table 2: Some soil physico-chemical parameters of the study site

Parameters	Unit	Values
pH	-	8.32
EC	dS m^{-1}	0.35
Organic carbon	%	0.23
Nitrogen (N)	mg kg^{-1}	28.30
Phosphorous (P)	mg kg^{-1}	21.70
Calcium (Ca)	cmol kg^{-1}	1.23
Magnesium (Mg)	cmol kg^{-1}	1.25
Potassium (K)	cmol kg^{-1}	2.51
Sodium (Na)	cmol kg^{-1}	1.74
CEC	cmol kg^{-1}	33.31
Sand	%	56.50
Silt	%	31.70
Clay	%	13.30
Textural class	Sandy loam	

EC: Electrical conductivity, CEC: Cation exchangeable capacity

Experimental field design and operation: Before commencement of the 2015 planting, experimental field was deep plowed by tractor and leveler was used to break up soil clumps. The experiment was laid out in a randomized block design with 3 replicates consisting of 2 blocks, each comprising 18 plots to give a total of 36 plots. Each plot was manually tilled with hoe before planting in subsequent cropping seasons.

Treatment design and application: Fertilizers were prepared as treatments and applied at different rates (Table 3) viz: 10 t ha^{-1} of sun-dried and pulverized, goat droppings (OMT), 25 mL of bio-filmed bio-fertilizers (BFT), 100 kg ha^{-1} of 65% RDF (urea, superphosphate and muriate of potash at 100:60:30) chemical fertilizer treatment (CHT), 25 mL bio-filmed bio-fertilizers (BFT) plus 5 t ha^{-1} organic manure (OBT) and an untreated control (UT). All organic manure treatments were applied a week before sowing to allow for proper mixing and decomposition with the soil. A 100 kg ha^{-1} of 65% RDF was applied to the rhizosphere of plants in the CHT treatment designated plots 2 weeks after sowing. Okra seeds were coated with 20 mL of developed FBBs loaded with vermiculite carrier and Arabian gum was added to the mix and left to dry for 2 h to improve adhesion of carrier particles to seeds[11]. A second dose of 5 mL of fresh liquid culture of FBBs was mixed with irrigation water and added to the rhizosphere of plants 2 weeks after germination.

Sowing and cultural operations: The FBBs coated and uncoated seeds were sown in the middle of May in each planting season in rows at a depth of 2 cm with each plot consisting 10 plants. Cultural activities started 6 DAS (days after sowing) with thinning, weeding and water application at appropriate times. Five plants per plots were randomly selected at vegetative growth stage and analyzed for plant height, shoot diameter, root length and number of leaves[17]. Leave area index (LAI) was determined using methods of Kamara *et al.*[18]. Analysis of yield attributes commenced at fruit production stage by harvest at a regular basis to determine fruit weight, fruit girth, fruit length, number of fruit per plant, while total yield ha^{-1} was determined after harvest based on cumulative records of number of fruit per plant.

Data analysis: Means and analysis of variance (one-way ANOVA) were calculated for all treatment. Precision of the experiment was determined by calculating coefficient of variance (CV) while Duncan multiple range test (DMRT) was used to evaluate level of significance ($p<0.05$ and $p<0.01$) between pairs of treatment means using the IBM® SPSS® Statistics V21×86 model Software Application (IBM, NY, USA).

RESULTS

Analysis of growth and yield performances in the first planting season (2015): Data obtained on growth performances of okra during the initial planting season (Table 4) revealed that application of CHT treatment recorded significantly ($p<0.05$) highest shoot diameter, number of leaf and leaf area index values while highest values of root length and plant height were recorded with OBT treatment application. Both BFT and OMT treatments gave growth parameter values that were statistically at par.

Table 5 showed data obtained for yield characters and yield outputs performances in the 2015 okra cropping season. Result showed that all yield character and yield output values recorded were highest for CHT treatment. The values obtained for the organic nutrient sources (OBT, BFT and OMT treatments) were statistically similar.

Table 3: Treatment design and application

Treatments	Components	Application rate
UT	No treatment	Control
OMT	Sundried and pulverized goat droppings	10 t ha^{-1} OM
BFT	*In vitro* developed fungal-bacterial biofilm culture (FBBs)	25 mL FBBs
CHT	Urea, Superphosphate and Muriate of potash at 100:60:30	100 kg ha^{-1} (65%)
OBT	Sundried and pulverized goat droppings+*In vitro* developed fungal-bacterial biofilm culture (FBBs)	5 t ha^{-1} OM+25 mL FBBs

Table 4: Effect of treatment on growth characters in the first okra planting season (2015)

Treatments	Plant height (cm)	Shoot diameter (cm)	Root length (cm)	Number of leaves	Leaf area index
UT	40.81^{c}	0.63^{c}	10.02^{c}	20.09^{c}	1.04^{c}
OMT	62.20^{b}	0.70^{b}	11.14^{b}	24.43^{b}	1.54^{b}
BFT	64.93^{bb}	0.73^{b}	11.33^{b}	24.59^{b}	1.67^{b}
CHT	$70. 57^{ab}$	1.04^{a}	11.85^{b}	25.08^{a}	2.01^{a}
OBT	85.62^{a}	0.82^{b}	12.06^{a}	24.33^{b}	1.72^{b}
LS	**	**	**	**	**
CV (%)	23.65	29.34	18.04	18.41	53.15

**Significant at 1% level, same letter do not differ significantly at 5% level of probability. UT: Untreated control, OMT (10 t ha^{-1} OM), BFT (25 mL FBBs), CHT (100 kg ha^{-1} 65% RDF) and OBT (5 t ha^{-1} OM+25 mL FBBs), LS: Level of significance, CV: Co-efficient of variation

Table 5: Effect of treatment on yield in the first okra planting season (2015)

Treatments	Fruit weight (g)	Fruit length (cm)	Fruit girth (cm)	Number of fruit/plant	Fruit yield/plot (g)	Total fruit yield (t ha^{-1})
UT	10.21	5.24^{c}	3.12^{c}	12.44^{c}	200.14^{c}	8.47^{c}
OMT	13.24^{b}	6.43^{b}	4.37^{b}	17.02^{b}	219.42^{b}	9.41^{b}
BFT	13.47^{b}	6.62^{b}	4.57^{b}	17.24^{b}	219.77^{b}	9.67^{b}
CHT	14.12^{a}	7.44^{a}	5.62^{a}	19.14^{a}	243.62^{a}	10.89^{a}
OBT	13.69^{b}	6.91^{b}	4.81^{b}	17.58^{b}	220.49^{b}	9.84^{b}
LS	**	**	**	**	**	**
CV (%)	12.85	21.20	22.28	14.10	21.54	22.24

**Significant at 1% level, Same letter do not differ significantly at 5% level of probability. UT: Untreated control, OMT: 10 t ha^{-1} OM, BFT: 25 mL FBBs, CHT: 100 kg ha^{-1} 65% RDF and OBT: 5 t ha^{-1} OM+25 mL FBBs, LS: Level of significance, CV: Co-efficient of variation

Table 6: Effect of treatment on growth characters in the second okra planting season (2016)

Treatments	Plant height (cm)	Shoot diameter (cm)	Root length (cm)	Number of leaves	Leaf area index
UT	39.01^{c}	0.57^{c}	9.27^{c}	17.04^{c}	0.92^{c}
OMT	90.45^{b}	0.84^{b}	12.61^{b}	24.46^{b}	2.01^{b}
BFT	90.89^{b}	0.96^{b}	12.99^{b}	24.82^{b}	2.19^{b}
CHT	100.11^{a}	1.12^{a}	13.43^{a}	25.48^{a}	2.42^{a}
OBT	101.27^{a}	1.29^{a}	13.88^{a}	25.89^{a}	2.63^{a}
LS	**	**	**	**	**
CV (%)	22.63	27.42	17.09	16.24	51.22

**Significant at 1% level, Same letter do not differ significantly at 5% level of probability. UT: Untreated control, OMT: (10 t ha^{-1} OM, BFT: 25 mL FBBs, CHT: 100 kg ha^{-1} 65% RDF and OBT: 5 t ha^{-1} OM+25 mL FBBs, LS: Level of significance, CV: Co-efficient of variation

Result of growth and yield performances in the second planting season (2016): The growth parameter data from second cropping season in 2016 as depicted in Table 6. All organic treatments (OMT, BFT and OBT) recorded significantly improved growth parameter values over the values recorded in the previous cropping season. However, highest growth performances were observed with the application of OBT treatment over other treatments, while the control (UT) treatment recorded lowest growth parameter values were recorded.

Results of yield and yield output performances in the second planting season were presented in Table 7. Data obtained revealed significantly ($p<0.05$) highest yield values with OBT treatment application while BFT and OMT treatments recorded yield output values that were slightly higher than values recorded by application of CHT treatment.

Result of growth and yield performances in the final planting season (2017): Data on growth characters examined during the 2017 planting season as shown in Table 8, revealed significantly ($p<0.05$) highest growth parameter values for plots receiving OBT treatment over other treatments, whilst both BFT and OMT treatments gave growth parameter values that were significantly ($p<0.05$) higher than CHT treatment.

All organic nutrient treatments (OBT, BFT and OMT) recorded significantly higher yield values than CHT treatment in the 2017 cropping season (Table 9). However, OBT treatment gave significantly ($p<0.05$) highest yield values. The

Table 7: Effect of treatment on yield in the second okra planting season (2016)

Treatments	Fruit weight (g)	Fruit length (cm)	Fruit girth (cm)	Number of fruit/plant	Fruit yield/plot (g)	Total fruit yield (t ha^{-1})
UT	10.17[c]	5.16[c]	3.07[c]	12.31[c]	200.07[c]	8.29[c]
OMT	14.62[b]	8.41[b]	4.69[b]	18.19[b]	224.11[b]	10.48[b]
BFT	14.79[b]	8.67[b]	4.87[b]	18.27[b]	233.54[b]	10.76[b]
CHT	14.32[b]	8.01[b]	4.36[b]	18.06[b]	215.69[b]	10.07[b]
OBT	15.74[a]	11.96[a]	6.73[a]	19.66[a]	264.36[a]	11.66[a]
LS	**	**	**	**	**	**
CV (%)	12.85	21.20	22.28	14.10	21.54	22.24

**Significant at 1% level, Same letter do not differ significantly at 5% level of probability. UT: Untreated control, OMT: 10 t ha^{-1} OM, BFT: 25 mL FBBs, CHT: 100 kg ha^{-1} 65% RDF and OBT: 5 t ha^{-1} OM+25 mL FBBs, LS: Level of significance, CV: Co-efficient of variation

Table 8: Effect of treatment on growth characters in the third okra planting season (2017)

Treatments	Plant height (cm)	Shoot diameter (cm)	Root length (cm)	Number of leaves	Leaf area index
UT	38.73[c]	0.51[c]	7.21[c]	15.09[c]	0.84[c]
OMT	111.55[ab]	1.27[ab]	13.40[ab]	27.18[ab]	2.32[ab]
BFT	112.31[ab]	1.38[ab]	13.54[ab]	27.45[ab]	2.44[ab]
CHT	102.83[b]	0.98[b]	12.83[b]	26.88[b]	2.04[b]
OBT	135.42[a]	1.95[a]	14.09[a]	28.22[a]	3.07[a]
LS	**	**	**	**	**
CV (%)	24.55	31.39	14.10	13.78	36.79

**Significant at 1% level, Same letter do not differ significantly at 5% level of probability. UT: Untreated control, OMT: 10 t ha^{-1} OM, BFT: 25 mL FBBs, CHT: 100 kg ha^{-1} 65% RDF and OBT: 5 t ha^{-1} OM+25 mL FBBs, LS: Level of significance, CV: Co-efficient of variation

Table 9: Effect of treatment on yield in the last okra planting season (2017)

Treatments	Fruit weight (g)	Fruit length (cm)	Fruit girth (cm)	Number of fruit/plant	Fruit yield/plot (g)	Total fruit yield (t ha^{-1})
UT	10.10[c]	5.10[c]	2.90[c]	12.06[c]	199.15[c]	7.78[c]
OMT	15.13[ab]	9.09[ab]	5.29[ab]	18.26[ab]	227.60[ab]	11.66[ab]
BFT	15.58[ab]	9.13[ab]	5.40[ab]	18.42[ab]	240.16[ab]	11.71[ab]
CHT	14.95[b]	8.80[b]	4.76[b]	17.87[b]	216.14[b]	10.97[b]
OBT	17.18[a]	13.13[a]	7.24[a]	21.84[a]	272.73[a]	13.87[a]
LS	**	**	**	**	**	**
CV (%)	13.27	22.49	21.64	15.27	22.17	21.26

**Significant at 1% level, Same letter do not differ significantly at 5% level of probability. UT: Untreated control, OMT: 10 t ha^{-1} OM, BFT: 25 mL FBBs, CHT: 100 kg ha^{-1} 65% RDF and OBT: 5 t ha^{-1} OM+25 mL FBBs, LS: Level of significance, CV: Co-efficient of variation

control treatment (UT) plot showed progressive reduction in growth and yield values with successive cropping season, thus lowest values were recorded in the 2017 cropping season.

DISCUSSION

In this study, results showed progressive increase and decrease in growth and yield performances for organic treatments (BFT, OMT and OBT) and chemical treatment (CHT), respectively with successive planting seasons. Thus, significantly ($p<0.05$) higher values of shoot diameter, number of leaf, leaf area index values recorded with application of CHT treatment at first cropping season in 2015 could be attributed to the reported potentials of chemical fertilizers to solubilize and immediately release nutrients for plants uptake[1,2,19]. The highest root length and plant height values recorded with application of OBT treatment could be attributed to improved soil aggregate, enabled by the organic manure component in the treatment for fast sprouting of root and shoot[20] and the unique ability of FBBs to regulate production of hormone like Indole acetic acid (IAA) for rapid seed germination and vigor through breaking of dormancy[8,9].

Adequate supply of the three primarily required mineral elements (NPK) and the quick uptake of these readily absorbable form of nutrients in the CHT treatment, could have enhanced the synthesis of photosynthates which might have been translocated and efficiently distributed in the reproductive part (pods) resulting in increased accumulated biomass[21] and the consequent significantly highest values for yield and yield output performances over other treatments. Lower growth and yield performances recorded by all organic treatments (OMT, BFT and OBT) in the first cropping season could be attributed to the initial slow release of nutrients from organic nutrient sources[22].

The 2016 cropping season revealed enhanced growth and yield values by all organic treatments (OMT, BFT and OBT) which could be accredited to residual effects of decomposed organic matter residues in the treatments from previous cropping seasons[4] and enhanced establishment and proliferations of various beneficial micro-organisms for immediate supply and uptake of nutrients by the plants[1] for increased accumulation of biomass. Reversely, reduced growth and yield values recorded by CHT treatment could be due to direct outcome of N and P content imbalance, reduced rate of nitrification, increased soil bulk density and inefficient water uptake[19] as a result of repeated application of mineral nutrient source.

However, the highest growth and yield values recorded with application of OBT treatment over other treatments in the 2016 cropping season could be ascribed to increased NH_4^+ availability in the soil solution (near root hairs) for absorption and improved production of high acidity and indole acetic-acid-like substances (IAAS) in microsites[7]. Also, improved soil structure provided by the organic manure component might have stimulated the diazotrophic component (*Azotobacter*) in the OBT treatment to produce exudates[8] for increasing the population of other diazotrophic soil bacteria that had been reported[2] to enhanced growth and persistence of effective community, through supply of biologically fixed nitrogen and production of plant growth promoting substances such as phytohormones, ACC deaminase and hydrolytic enzymes.

The overall highest growth and yield performances recorded with OBT treatment application in the last cropping season could be credited to the organic matter components of the treatment which might have ensured physiological and anatomical stability of the biofilm structure for increased persistence[9], formation of root-biofilm association for rapid uptake of nutrients and development of nodule-like structures or 'pseudo nodules' for more efficient nitrogen fixation[8].

Furthermore, attainment of complete decomposition and solubilization of accumulated organic matter from preceding cropping seasons, gradual release of nutrients and enhanced soil condition through improved soil structure, aeration, water retention and efficient mobilization of minerals for plant uptake[4] could have resulted in the significantly higher growth and yield performances recorded by BFT and OMT treatments over CHT treatment in the last planting season. On the contrary, intensive use of mineral fertilizer might have disrupted the biotic activities of the soil[19], resulting in reduced absorbable nutrients, stunted plant growth and limited crop yield that led to the comparatively lower yield values recorded by CHT treatment. Similarly, progressive depletion of available organic matter and other nutrients from subsequent cropping seasons[22] might be accountable for the eventual lowest growth characters and yield performances recorded in the untreated control (UT) plot during the last planting season.

However, in spite of the capabilities of these organic inputs to enhance growth promotion, sustain ability and eco-friendliness, efficient integrated nutrient management (INM) models would be needed to overcome some of the major constraints such as slow nutrient release, unknown nutrient ratios and potential source of pathogens associated with the sole use of organic nutrient sources in crop production.

CONCLUSION

It is concluded that application of fungal-bacterial bio-film bio-fertilizers (FBBs) and organic manure either alone or in combination over relatively long period of cultivation was capable of reducing chemical fertilizer application in sustainable production of okra.

SIGNIFICANCE STATEMENT

The present study justifies the potentials of fungal-bacterial biofilm bio-fertilizers (FBBs) and organic manure application as sustainable means of improving growth and yield of okra, optimizing economics of production by reducing amount of chemical fertilizer application and minimizing its deleterious effects on environment, soil and human health. Appropriate adoption of these findings by farmers and researchers would go a long way in enhancing sustainable production of okra in many agro-environments.

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