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Comparison of a novel combination of bio-organic fertilizers *vis-à-vis* a chemical fertilizer

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Plant nutrients are essential for the production of healthy crops for the world's expanding population and thus, they are a vital component of sustainable agriculture. Increased crop production depends on the type of fertilizers used to supplement essential nutrients for plants. The nutrient level fertilizers provide depends on their nature- each type of fertilizer has its advantages and disadvantages concerning crop growth and soil fertility. The management of using fertilizer must aim to ensure both an enhanced and safeguarded environment. Thus, a balanced fertilization strategy must be implemented. An experiment was conducted under field conditions to assess the effects of combinations of bio-fertilizers on agronomic and quality criteria of *Brassica juncea* (brown mustard), *Basella alba* (climbing spinach), and *Amaranthus dubius* (red spinach). Randomized block design with three replicas were used for the study, one set with the application of fertilizers containing *Azotobacter*, *Rhizobium*, *Sesbania*, *Bacillus subtilis*, *Bacillus cereus*, *Bacillus megaterium*, *Pseudomonas fluorescens* and *Glomus (*Mycorrhizal inoculant)- under bio-fertilizer; another with a mixture of urea, Potassium Nitrate, Super Phosphate, Potassium Sulfate, and Maple EM solution as chemical fertilizer and a control (water). Results indicated that yield and other plant criteria like chlorophyll content and gel volume were enhanced in bio-fertilizer treated plants compared to the plants grown with chemical fertilizer and control. In general, the application of bio-fertilizer significantly increased leaf length by 16-50%, the total number of leaves by 50-80%, plant size 19.15-63.15%, and gel volume by 147% (approximately) in comparison with untreated plants.

Keywords: *Amaranthus dubius*, *Azotobactor*, *Bacillus cereus*, *Bacillus megaterium*, *Bacillus subtilis*, *Basella alba*, Biofertilizer, *Brassica juncea*, Chemical fertilizer, *Glomus*, *Pseudomonas fluorescens*, *Rhizobium*, *Sesbania*

Chemical fertilizers are produced synthetically from inorganic materials that are added to the soil to sustain plant growth. Though they are rich in the essential nutrients needed for plant growth that helps in meeting the food demand, they are proven to develop weak plant features, like nutritional characters, chlorophyll content *etc*[1,2]. On the other hand, they have disadvantageous effects on the soil composition, adversely affecting the chemical, physical, and biological properties of the soil, stunting the growth of microorganisms present naturally in the soil which are helpful for plant growth, and adversely affecting the environment[3-5].

Whereas, biofertilizers are mixtures of organic wastes, domestic sewage, animal manure, approved insects, and microorganisms- when applied to the host (plant), increases the availability of primary nutrients for the hosts, without harming the host, the soil fertility, the essential microorganisms residing in the soil, or the environment[5-9].

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Soil nutrients' deficiency is a prevalent problem among farmers, thus, in order to fill that void, most of the farmers have been using fertilizers, particularly chemical fertilizers. Three of the distinct advantages of chemical fertilizers over the biofertilizers that are currently prevalent in the market is their cost, nutritional value, and the preservation period. Though chemical fertilizers have adverse effects on plant growth and soil fertility, they are rich equally in all the essential nutrients, particularly, nitrogen, phosphorous and potassium, which has a great advantage in bulk food production. Whereas, most of the industrial biofertilizers have a deficit or may have low levels of all the essential nutrients[10]. Another key problem is the storage of bio-fertilizers, as these require special care for long term storage because these are alive. Thus, the current study aims to achieve a perfect balance in the mixture of different plant growth-promoting microorganisms, used as biofertilizers, so as to increase crop productivity which can compete with the nutrition value and cost of the prevailing chemical fertilizers without harming the environment.

Materials and Methods

An experiment was conducted under field condition to evaluate the effects of a novel combination of bio-fertilizer on agronomic and quality criteria of *Brassica juncea* (brown mustard), *Basella alba (*climbing spinach) and *Amaranthus dubius (*red spinach). The reason for using these plants in the experiment is that these plants are grown worldwide for their consumption; these plants don't grow more than a few feet, they are easy to be grown in block sets under field conditions even in house/home gardens, and the time-period of their growth were almost similar-ranging from 8 to 10 weeks. The final results were measured after 8 weeks of sowing the seeds for *Basella alba* and *Amaranthus dubius*. For *Brassica juncea*, the final results were estimated after 20 weeks of sowing the seeds.

The bio-fertilizer includes *Azotobacter*, *Rhizobium*, *Sesbania*, *Bacillus subtilis*, *Bacillus cereus*, *Bacillus megaterium*, *Pseudomonas fluorescens*, and *Glomus* (Mycorrhizal inoculant) - mixed with industrial talc. These microorganisms have the potential to manage the fertility of the soil and the health of the crops by improving plant uptake of nutrients and thus increasing the efficiency of the applied manures[11-21]. Nitrogen is one of the vital components of Chlorophyll, and the largest contributor to the biological nitrogen fixation is *Rhizobium*. To delevop a Multilegume biofertilizer, rhizobia having broad host range have been used- it could help in increased nitrogen fixation, biomass yield, and rise in soil nitrate level. Choosing the right inoculant for the host plant can contribute to sustainable farming[16,22]. Fertilizers containing *Sesbania* proved to yield higher biomass and produce a higher number of nodules with increased nitrogenase activity, compared to plants treated with inorganic fertilizers[23]. Studies showed that incorporation of *B. subtilis* in fertilizer reduces the retention of $NH4^{+-}N$ in alkaline soil and thereby reduces NH3 volatilization compared to organic fertilizer, by up to 44%, thereby maintaining high crop yield through sustainable agriculture[17,27]. *B. megaterium* has a beneficiary role in developed plant features (increased root length, shoot length and plant weight) in mustard plants. Plants treated with fertilizer containing *B. megaterium* have higher levels of sucrose, glucose, fructose, and amino acids (Asp, Thr, Ser, Glu, Gly, Ala, Cys, Val, Met, Ilu, Leu, Tyr, Phe, Lys, His, Arg and Pro)[24]. Studies showed that the use of *B. cereus* as fertilizer increased the uptake of Nitrogen, Phosphorus, and Potassium by plants, availability of K in soil, and plant yield[25]. Specific strains of *Pseudomonas fluorescens* were proved to increase the yield of crops and promote plant growth under field contidions[26]. Application of bio-fertilizer containing *Glomus* inoculants results in enhanced physiological, biochemical process in plants with an increased levels of essential nutrient levels and total crop yield, as compared to plants treated with chemical fertilizer[27].

The chemical fertilizer used for the experiment contained Nitrogen, Phosphorus, Potassium, Calcium, Magnesium, and Sulphur, and Copper, Iron, Magnesium, Zinc, Chlorine, Boron, and Nickel as micronutrients.

Physical parameters, such as the size of the plant, diameter, and the number of leaves as well as the internal criteria such as the chlorophyll and gel content of the plants were measured. The condition of the plants was observed on a timely basis. The chlorophyll content of the plants was detected using "Arnon's method of chlorophyll estimation".

The bacterial cultures of the inoculums in the biofertilizer were tested for confirmation of the following:

a) Phosphate solubilization test of the inoculums was assayed using Pikovskaya's (PVK) agar containing TCP as the phosphate source. Plates were incubated at 30°C for 72 h and colonies with clear halo were marked for phosphate solubilization.
b) Bacterial isolates were assayed for Siderophores on the Chrome azural S agar medium. The plates containing this medium were inoculated with test organism and incubated at 28°C for 48 h. The development of a yellow–orange halo around the growth was considered positive for Siderophore production.
c) Bacterial isolates were tested for the production of ammonia in peptone water. Freshly grown cultures were inoculated in 10 mL peptone water in each tube and inoculated for 48 h at 28°C. 0.5 mL of Nessler's reagent was added to each tube. The development of brown to yellow colour was a positive test for ammonia production.

Nessler's reagent (for ammonia):

50 g of KI dissolved in 500 mL of cold water. A saturated solution of mercuric chloride (about 22 g in 350 mL of water) was added. Then 200 mL of 5N NaOH was added and diluted to 1 L.

d) Indole Acetic Acid: Bacterial cultures were grown for 72h (*Azotobacter*) and 48 h (*Pseudomonas* and

Bacillus) on their respective media at 28°C. Fully grown cultures were centrifuged at 3000 rpm for 30 min. The supernatant (2 mL) was mixed with two drops of Orthophosphoric acid and 4 mL of the Salkowski reagent (50 mL, 35% of Perchloric acid, 1 mL 0.5 M $feCl_3$ solution). Development of pink colour indicates IAA production.

Glomus (Mycorrhizal inoculant), the VAM culture was prepared in the laboratory. The culture was isolated from the soil by growing on a malt extract agar plate using the dilution plate technique. The isolated culture was then grown in malt extract media in a large conical flask. The culture was dried and mixed with industrial talc and charcoal powder. The mixed powder containing the inoculum has been used as a VAM inoculant.

Results

The microorganisms in the biofertilizer were tested for Phosphate Utilization Test, Siderophore Production Test, NH_3 Production Test and Indoleacetic Acid (IAA) Production test.

Phosphate solubilization test of the inoculum

B. subtilis, *B. cereus* and *Pseudomonas fluorescence* were found positive for Phosphate solubilization test. The inoculum was tested using Pikovskaya's (PVK) agar containing TCP as the phosphate source. Plates were incubated at 30°C for 72 h and colonies with clear halo were marked for phosphate solubilization.

Siderophore production test of the inoculum

Bacterial isolates were assayed for Siderophores on the Chrome azural S agar medium. The plates containing this medium was inoculated with the organisms and incubated at 28°C for 48 h development of yellow–orange halo around the growth was considered as positive for Siderophore production. The bacteria were found positive for the test. *Azotobacter*, *Pseudomonas fluorescence* and *B. cereus* were found positive for the test.

NH_3 production test of the inoculum

Bacterial isolates were tested for the production of ammonia in peptone water. Freshly grown cultures were inoculated in 10 mL peptone water in each tube and inoculated for 48h at 28°C. 0.5 mL of Nessler's reagent was added to each tube. The development of brown to yellow colour was a positive test for ammonia production. *B. subtilis*, *B. cereus* and *B. megaterium* were found positive for the test.

Indoleacetic Acid (IAA) Production Test of the inoculum

Bacterial cultures were grown for 72 h (*Azotobacter*) and 48 h (*Pseudomonas* and *Bacillus*) on their respective media at 28°C. Fully grown cultures were centrifuged at 3000 rpm for 30 min. The supernatant (2 mL) was mixed with two drops of Orthophosphoric acid and 4 mL of the Salkowski reagent (50 mL, 35% of Perchloric acid, 1 mL 0.5 M $feCl_3$ solution). Development of pink colour indicates IAA production. The bacteria were found positive for the test.

Physical parameters of the plants

The plants treated biofertilizer developed the green and fresh leaves with no visible marks on the surface and were significantly larger in dimension compared to the other two sets. In the case of plants treated with chemical fertilizer, the leaves were curled with marks on the surface, particularly where the fertilizer came in contact and smaller in dimension compared to the bio-fertilizer set. The soil texture and colour changed from the initial one week, with visible differences from the other two sets. The soil colour got darker with decreased water holding capacity. In addition, the application of a higher dose of the fertilizer than prescribed resulted in the death of the plants. The leaves were fresh and green in the control with small marks on the surface which are very distinct from the marks that were seen on the chemical fertilizer set. The marks on the control set leaves were probably due to insect injury and the leaves were smaller in dimension compared to the other two sets. Approximately, application of bio-fertilizer significantly increased leaf length by 16-50%, the total number of leaves by 50-80%, plant size by 19.15-63.15%, and gel volume by 124-147% when compared to the control and the set treated with chemical fertilizer. Details of the results are given in (Table 1).

Internal parameter: chlorophyll content of the plants

Chlorophyll was extracted from the leaves of *Brassica juncea*, *Basella alba* and *Amaranthus dubius.* The concentration of the chlorophyll was detected using "Arnon's method for Chlorophyll estimation".

The chlorophyll content was higher in the case of the sets treated with bio-organic fertilizer, than the other two sets for all the three plants- *Brassica juncea*, *Basella alba* and *Amaranthus Dubius* (Figs 1-6). The final calculated data of the plants treated with biofertilizer showed a significantly higher value in the studied parameters (Table 1).

Discussions

For *Brassica juncea*, the plants treated with biofertilizer showed an increase of 41.67% in plant size/length compared to the control and 36% to that of the set treated with chemical fertilizer. The percentage of increase of gel volume was 147 compared to control and 141 compared to the set with chemical fertilizer. The chlorophyll content of the plants was 221.9% higher than the control and 25.9% higher than the set treated with chemical fertilizer. Though the diameter of the leaves had no significant difference, the leaves were longer compared to the control and the set treated with chemical fertilizer. The block set of *Basella alba* showed a 63.55% increase in plant size/length in

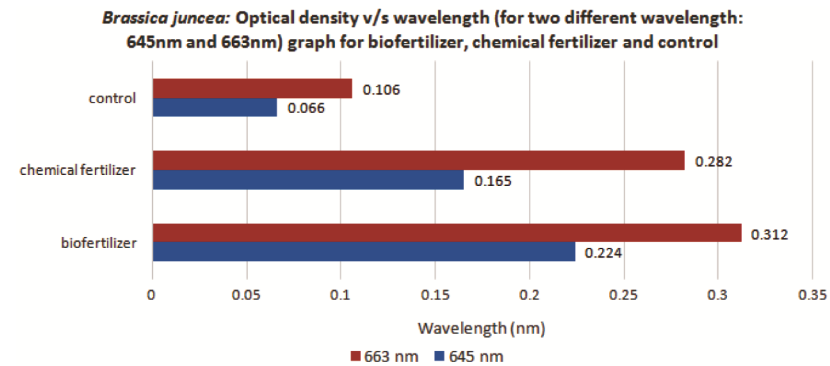

Fig.1 — Optical density *vs* wavelength (for two different wavelengths: 645 and 663 nm) graph for biofertilizer, chemical fertilizer and control as measured spectrophotometrically for *Brassica juncea*

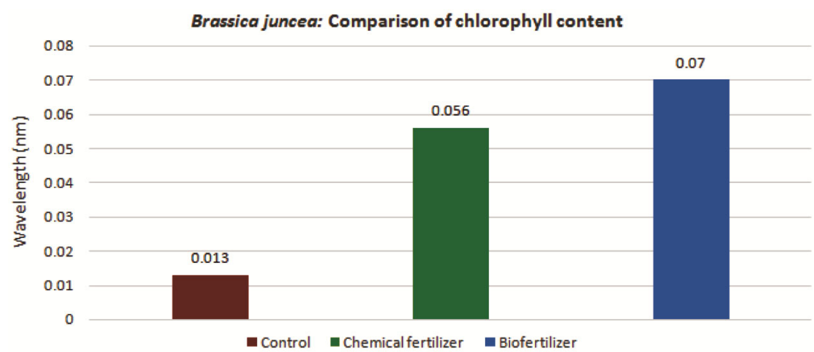

Fig. 2 — Comparison of chlorophyll content for biofertilizer, chemical fertilizer and control as measured spectrophotometrically for *Brassica juncea*

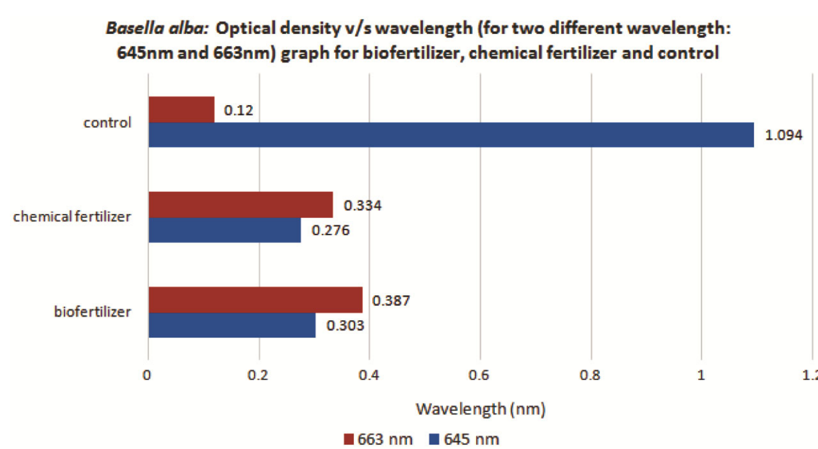

Fig. 3 — Optical density *vs* wavelength (for two different wavelengths: 645 and 663 nm) graph for biofertilizer, chemical fertilizer and control as measured spectrophotometrically for *Basella alba*

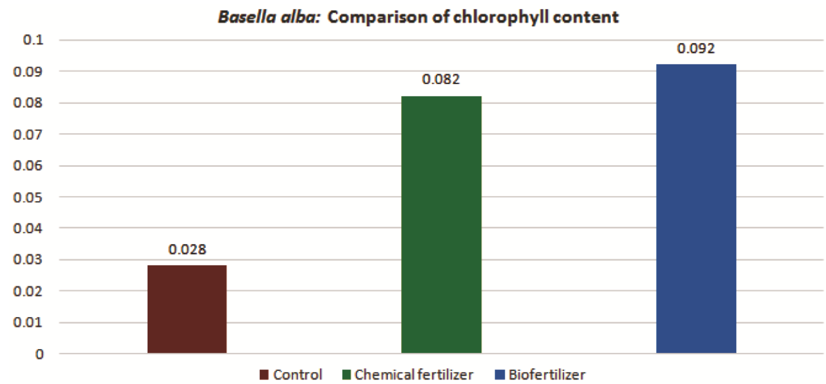

Fig. 4 — Comparison of chlorophyll content for biofertilizer, chemical fertilizer and control as measured spectrophotometrically for *Basella alba*

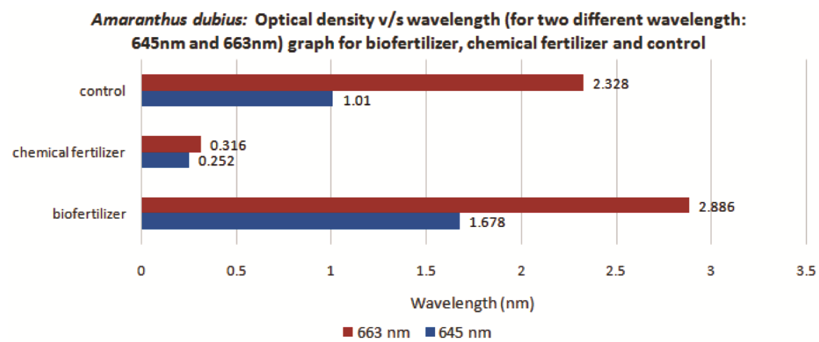

Fig. 5 — Optical density *vs* wavelength (for two different wavelengths: 645 and 663 nm) graph for biofertilizer, chemical fertilizer and control as measured spectrophotometrically for *Amaranthus dubius*

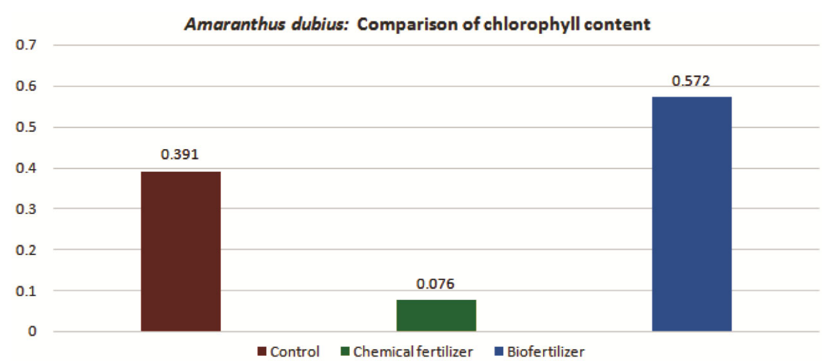

Fig. 6 — Comparison of chlorophyll content for biofertilizer, chemical fertilizer and control as measured spectrophotometrically for *Amaranthus dubius*

the replica treated with biofertilizer in comparison with the control and a 42.92% increase to that of the set treated with chemical fertilizer. The percentage increase in gel volume was 147 and 143 compared to the control and the replica treated with chemical fertilizer. The size and number of the leaves were significantly higher than the control and chemical fertilizer set. The chlorophyll content was 222.3% higher than the control and 11.71% higher than the chemical fertilizer treated set. The study showed an increase of 37.5% of the number of leaves in *Amaranthus dubius* compared to the control and 10% to the set with chemical fertilizer. There was an increase of 61.58% of plant size/length compared to the control set and 19.15 compared to the chemical fertilizer set. The gel volume increased by 143% and 124% compared to the control and the chemical fertilizer set respectively. The chlorophyll content of the plants was 46.03% higher than the control and surprisingly, 648.7% higher than the set treated with chemical fertilizer. The average leaf size was higher in the case of the plants treated with

Table 1 — Physical parameters

Parameters	Bio-fertilizer	Chemical fertilizer	Control
Brassica juncea			
Plant Radical (avg) (17th Feb, 2017)	2 cm	3 cm	2 cm
No. of Leaves (avg) (30th June, 2017)	5	5	5
Leaf size (avg)	Length- 2 cm Diameter- 0.5 cm	Length- 1.7 cm Diameter- 0.5 cm	Length- 1.9 cm Diameter- 0.5 cm
Yield	25/28 survived	13/30 survived	20/27 survived
Plant length (avg) (22nd March, 2017)	16 cm	8 cm	9 cm
Plant Length (avg) (19th April, 2017)	23 cm	15 cm	15 cm
Plant Length (avg) (1st June, 2017)	26 cm	20 cm	18 cm
Final Plant Length (avg) (30th June, 2017)	34 cm	25 cm	24 cm
Basella alba			
Plant Radical (avg) (15th Feb, 2017)	0.5 cm	0.5 cm	0.5 cm
No. of Leaves (avg) (29thMarch, 2017)	9	5	7
Leaf size (avg)	Length-4.5 cm Diameter-3.5 cm	Length- 3 cm Diameter- 2 cm	Length- 3.5 cm Diameter- 2.5 cm
Yield	3/3 survived	2/3 survived	1/3 survived
Plant Length (avg) (13th March, 2017)	9.5 cm	7.8 cm	7.9 cm
Plant Length (avg) (21st March, 2017)	27.8 cm	18.7 cm	16 cm
Final Plant Length (avg) (29th March, 2017)	32.8 cm 31 cm 31.4 cm	22.9 cm 21.5 cm	19.1 cm 19.7 cm
Amaranthus dubius			
Plant Radical (avg) (15th Feb, 2017)	0.6 cm	0.5 cm	0.6 cm
No. of Leaves (avg)	11	10	8
Leaf size (avg)	Length-3.5 cm Diameter-2.9 cm	Length-3 cm Diameter-2 cm	Length-3 cm Diameter-2.5 cm
Yield	3/3 survived	1/3 survived	1/3 survived
Plant Length (avg) (13th March, 2017)	12.5 cm	11.8 cm	9.9 cm
Plant Size (avg) (21st March, 2017)	34.6 cm	30.7 cm	21 cm
Final Plant Length (avg) (29th March, 2017)	38.9 cm 37 cm 38.5 cm	33.1 cm 30.9 cm	23.6 cm

Percentage increase in data

Name of the plant	% increase of leaf size compared to				% increase of no. of leaves compared to		% increase of plant size compared to		% increase of gel volume compared to	
	Control		Chemical fertilizer		Control	Chemical fertilizer	Control	Chemical fertilizer	Control	Chemical fertilizer
	Dia.	length	Dia.	length						
Brassica juncea	0	5.26	0	17.64	0	0	41.67	36	147	141
Basella alba	40	28.57	75	50	28.57	80	63.55	42.92	147	143
Amaranthus dubius	16	16.67	45	16.67	37.5	10	61.58	19.15	143	124

biofertilizer than the other two sets. The results suggested an increase in the yield of the plants with better plant features in comparison with the untreated plants and plants treated with chemical fertilizer. Furthermore, the contact of chemical fertilizer with plant tissues resulted in lesions and changed the soil texture and colour, and decreased the water holding capacity. Applying high dose of chemical fertilizer than prescribed resulted in the plants to die, which was not the case for the set treated with biofertilizer. Biofertilizers didn't adversely affect the soil or the plants, unlike chemical fertilizer.

Conclusion

In the current study, the result showed that the set treated with biofertilizer was significantly higher in-chlorophyll content by 11.71-648.7%, leaf length by 16-50%, the total number of leaves by 50-80%, plant size by 19.15-63.15%, and gel volume by 124-147% in comparison to the control and the set treated with chemical fertilizer.

Continuous use of chemical fertilizers and manures for intensifying soil fertility and crop productivity often results in unexpected harmful effects on the environment. Using microbial inoculants, including plant growth-promoting microorganisms like Rhizobacteria, arbuscular mycorrhizal fungi, PSB, KSB, and bacteria producing siderophore, ammonia and IAA, increases the fertility of the soil, providing primary nutrients to the plants[27-32]. This plant-microbe interaction results in enhanced crop productivity and soil fertility without producing any adverse effects on the environment[33-37]. However, the currently available bio-organic fertilizers in markets are either costly or deficient in nutrients. To address this issue, the present study undertook an approach to composite a mixture of organic and biofertilizer which is not only cost-effective but provides the necessary nutrients for the growth of a plant and has a higher yield of healthy crops compared to the plants treated with chemical fertilizer. Though biofertilizers need special care for their long-term storage, the advantageous effects of biofertilizers overshadow the difficulty. It is hoped that this study would help in solving the issues, the current agricultural world is facing from the disadvantageous effects of chemical fertilizers.

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Conflict of interest

All authors declare no conflict of interest.

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