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Research Article

ROLE OF PLANT GROWTH PROMOTING MICROBIAL CONSORTIUM AND EFFECTIVE MICROORGANISMS (EM) ON THE GROWTH AND YIELD OF VIGNA UNGUICULATA L

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ABSTRACT

In this present study, Soil samples were collected from Vilakkudi, Thiruthuraiipoondi (TK), Thiruvarur district, Tamil Nadu, India. Bacterial and fungal species were isolated, identified and confirmed using Bergey's Manual of Systematic Bacteriology and the Manual of Soil Fungi. Bacterial species such as *Lactobacillus casei* and fungal species such as *Trichoderma harzianum* were identified. The activated EM was verified by pH (3.2) and by sweet sour smell. The seeds of *Vigna unguiculata* were sown in six pots of equal size and noted as T1, T2, T3, T4, T5 and Control. In the experimental set up, T1 pot was treated with *Lactobacillus casei*, T2 pot was treated with *Trichoderma harzianum*, T3 was treated with *Saccharomyces cerevisiae*, T4 with Combined formulation of *Lactobacillus casei*+ *Trichoderma harzianum*+ *Saccharomyces cerevisiae*, T5 was treated with EM and the un inoculated pot was denoted as Control. T5 showed the highest results in all the growth parameters and yield of the plant.

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INTRODUCTION

Soil is the outer covering of the earth which consists of loosely arranged layers of materials composed of inorganic and organic constituents in different stages of organization. In the developing countries, where organic farming is practiced by small holders due to the high cost and/or non-availability of agrichemicals the scientific aspects of these enterprises are being evaluated for their productivity and sustainability (Gliessman, 1991; NRC, 1993).

Liquid biofertilizer defined as suspension having fix atmospheric nitrogen and solubilize and insoluble phosphate and make available for the plants. It is alternative to chemical and organic fertilizer.

Liquid biofertilizer formulation is the promising and updated technology which inspite of many advantage over the agrochemical, leaf a considerable dispute among the farmer community in terms of several reason major being the viability of the organisms. Traditionally liquid biofertilizer produced from fermentation of effective microorganism (EM) was recommended to be used within three months. Nowadays the production of ready to use liquid biofertilizer from EM is becoming available in market. (Harisan Nagapimol, 2008)

Effective microorganisms are not a substitute for other management practices. It is however, an added dimension for optimizing our best soil and crop management practices such as crop rotation, use of organic amendments, conservation tillage, crop residue recycling and bio control of pests. If used properly, EM can significantly enhance the beneficial effect of these practices (Parr and Hornick, 1992).

Microbial inoculants containing many kinds of naturally occurring beneficial microbes called "Effective microorganism" has been used widely in nature and organic farming (Hui-Lian, 2000). EM Technology uses a laboratory cultured mixture of microorganisms consisting mainly of Lactic acid bacteria, purple bacteria and yeast which co-exist for the benefit of whenever environment they are introduced, as has been claimed by the various EM-like culture purveyors.

Trichoderma harzianum. Have been widely studied, are among the microorganisms most commonly used as biological control agents and are presently marketed as active ingredients of bio-pesticides, biofertilizers, growth enhancers and stimulants of natural resistance. This is due to their ability to protect plants, enhance vegetative growth and contain pathogen populations under numerous agricultural conditions, as well as to act as soil amendments inoculants for improvement of nutrient ability, decomposition and biodegradation (Kubicek, Harman 1998).

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Lactic acid bacteria (LAB) mainly *Lactobacillus* and *Lactococcus* species, are useful microorganism in much biotechnology process in the food and food industries. Bacteriophages contamination of this important group of Lactic acid bacteria (LAB) are a heterogeneous group of non-sporulating Gram-positive bacteria which grow under microaerophilic to strictly anaerobic conditions. Have been reported since the 1930. *Lactobacillus* represent a major genus of the lactic acid bacteria that wide spread use in fermented food production.

"*Saccharomyces*" derives from Latinized Greek and means "sugar-mold" or "sugar-fungus", *Saccharo* being the combining form "sugar" and *myces* being "fungus". *cerevisiae* comes from Latin and means "of beer". *Saccharomyces cerevisiae* is a species of yeast. It has been instrumental to wine making, baking, and brewing since ancient times.

MATERIALS AND METHODS

Collection of soil sample

The soil samples were collected from paddy field at Vilakkudi, Thiruthuraiipoondi (TK), Thiruvarur District, Tamil Nadu, India. A V- shaped cut was made with a spade to remove 1 to 2cm slice of soil .The samples were collected on the blade of the spade and put in a bucket. In this way sample was collected all the spots marked for one sampling unit. The soil was poured from the bucket on a piece of clean paper or cloth and mixed thoroughly. The soil was spread evenly and divided it into 4 quarters. Two opposite quarters were rejected and the rest of the soil was mixed again. The process was repeated till left with about half Kg of the soil. Collect put in a clean cloth bag. Each bag should properly marked to identify the sample. The collected sample was brought to the laboratory in sterilized polythene bags were air dried and stored in containers for future use.

Analysis of nutrient status of soil

Sample was collected first, air dried at room temperature, then crushed using a porcelain mortar and pestle and then sieved for further analysis. The physicochemical parameters such as the available nitrogen (Subbaiah and Asija, 1956), available phosphate (Jackson and Bray, 1973), available potassium (Toth and Prince, 1752) were tested before and after the pot cultivation of *Vigna unguiculata* treated with EM.

Isolation of bacteria from soil

Serial dilution technique

Microbiological analysis of the soil was also carried out to analyze the soil quality. Serial dilution was performed by using the collected soil sample to isolate the bacteria, fungi. 1gm of soil sample was diluted in the tube containing 9ml of sterile distilled water and mixed thoroughly to make a 1:10 dilution (10^{-1}). 1ml of diluted sample was transferred to the next test tube and serially diluted in to the series of test tubes having 9ml of sterile pipettes up to 10^{-7} th dilution (Ronald Atlas, 1998).

Biochemical test

The following biochemical tests were carried out to find out the enzymatic activities of bacterial culture. Indole Test, Methyl

Red and Voges- Proskauer Test, Citrate utilization Test, Catalase Test, Triple sugar iron test, Carbohydrate Fermentation Test (Cappuccino and Shermann, 1988).

Isolation of fungi from soil

Plating technique

0.05g of soil was scattered on the bottom of a sterile petridish and molten cooled (40-45°C) agar medium potato dextrose agar (PDA) was added, which was then rotated gently to disperse the soil particles in the medium. The petridishes were then incubated at $28 \pm 2^\circ\text{C}$ for three days (warcup, 1950).

Isolation of yeast

Dry yeast purchased from Lakshmi Department store at Mannargudi, Thiruvarur District, Tamil Nadu, India. YPD medium was used for pour plate method. Medium was sterilized at 121°C for 15 minutes. Petri plates were sterilized and labeled properly. 1ml of sample from 10^{-3} and 10^{-5} , dilution was transferred into the respective plates. Finally, the cooled medium was poured into the sample containing plates and incubated at 30°C for 72 hours and the colonies were counted. Different colonies were observed and transferred to other specific media for identification.

Activation (EMA) of EM stock solution

For most applications, EM stock solution is to be "activated" prior to use. One liter of EM stock solution and 1 kg of jaggery were mixed with 20 liter of water. The water has to be clean and free from chlorine. The container should be of good-grade plastics.

For the period of activation, the container was placed in shade at ambient temperature (20-40°C) without exposure to strong temperature fluctuation. Activated EM (abbreviated as EMA) will be ready after 5-10 days. It can be verified by a pH of 3.5 or lower and a pleasant sweet sour smell (APNAN, 1995).

Compatibility of the selected antagonistic isolates under invitro conditions

Cell suspensions of 24-hours old bacteria was prepared by culturing them on Nutrient broth. These were mixed with their respective selective agar media, poured into sterile petriplates and 5mm Diam disc of *Trichoderma harzianum* cultures were inoculated separately on the media (medium previously mixed with 24-hours old suspension of bacterial antagonists) and observed for the inhibition of growth of *Trichoderma harzianum*

Compatibility test was carried out to show that *Trichoderma harzianum*, and *Lacto bacillus* were compatible to each other in the cultures. There was no inhibition of either of the antagonistic forms after 7 days of incubation (Rini and Sulochana, 2006).

Pot culture Technique

A pot culture experiment was conducted using garden soil. Treatments (T1, T2,T3,T4 and T5) and control (C) pots were maintained. The pots were provided with water facilities. The plant chosen for this study was *Vigna unguiculata*. Six treatments were done. All the pots were arranged in a randomized design. The pots were maintained in the open

shade at the temperature of 27-30°C. The seeds were soaked with EM stock culture for overnight and pot culture were carried out (Parvathi *et al.*, 1985).

There were 6 treatments resulting from the combination of

T₁ - *Lactobacillus casei*

T₂ - *Trichoderma harzianum*

T₃ - *Saccharomyces cerevisiae*

T₄ - Liquid formulation of *Lactobacillus casei*+ *Trichoderma harzianum*+ *Saccharomyces cerevisiae*

T₅ - EM Control

Effective microorganism application Schedule

Effective microorganism culture under the commercial name of EM. The stock culture was diluted to prepare 0.2% solution by adding tap water. The fresh solution was used immediately after preparation. The respective pots of the treatments with soil effective microorganism application were irrigated with a 0.2% dilute solution 15 days prior to sowing. Each pot received 1 L of dilute solution. These pots were further supplemented with 1L 0.2% effective microorganism solution fortnightly throughout the experimental period. Plants of the treatment with foliar application of effective microorganism were sprayed fortnightly with 0.2% solution just to moisten the plant surface, throughout the experimental period (Chowdhary *et al.*, 2002)

Analysis of plant growth parameters of cultivated plants

plants were uprooted along with the rhizospheric soil and the following parameters were studied. Height of the plant (in cm), Shoot length (in cm), Root Length (in cm), Number of leaves (per plant), Leaf fresh weight (in mg), Leaf dry weight(in mg), Number of nodules(per plant), carbohydrate, and chlorophyll contents were recorded at both the flowering and harvesting stage. Number of flowers was recorded in flowering stage only. Number of pods (per plant), Length of the pods (in cm) and Yield (in gms) were recorded. All the data were analyzed statistically (Gupta, 2004).

RESULTS

Analysis of nutrient status

The availability of nitrogen was estimated by using Subbaiah and Asija method. The total nitrogen value was recorded as 0.87ppm. The availability of phosphorus was estimated by using Jackson and Bray method. The total phosphorus contents was recorded as 87.5 ppm. The availability of potassium contents was estimated by using Toth and prince method. The total potassium value was recorded as 56 ppm

Isolation and identification of bacteria

Serial dilution technique was used to isolate the bacteria by streak plate method. In Gram staining technique Gram positive and motile bacteria was observed (Table 1), motility test and biochemical test, Indole, Methyl Red, Voges - Proskauer, Citrate utilization test, Triple Sugar Iron test, and Carbohydrate Fermentation test, positive result Catalase test was negative result was used to identify bacterial species. confirmed using Bergey's Manual of Determinative Bacteriology. Thus the bacteria was identified and confirmed as *Lactobacillus casei*.

Table – 1 Morphological and Biochemical Characteristics of *Lactobacillus casei*

S.No	Characteristics	Organisms <i>Lactobacillus casei</i>
Cultural characteristics		
1	Appearance	Green coloured colonies
Morphological Characteristics		
2	Motility	Motile
3	Gram staining	Gram Positive
4	Shape	Rod Shape
Biochemical Characteristics		
5	Indole test	+
6	Methyl Red test	+
7	Voges- Proskauer test	+
8	Citrate Utilization test	+
9	Catalase test	-
10	Triple Sugar Iron test	A/A

(+) – Positive

(-) – Negative

(A/A) – Acid slant and Acid butt

Isolation of fungi

Lacto phenol cotton blue technique was used to identify the fungal species. The colonies showed a characteristic colour of green confirmed and identified by the morphological characters and confirmed using the fungal manual, Dematiaceous Hypomycetes (Ellis,1971) The fungal species *Trichoderma harzianum* was isolated and identified.

Compatibility of the selected antagonistic isolates under in vitro conditions

Compatibility test carried out showed that *Trichoderma harzianum*, and *lactobacillus casei* were compatible to each other in the cultures. There was no inhibition of either of the antagonistic forms after 7 days of incubation also. The average radii of *T. harzianum* on the 4th day in the dual plate cultures were 2.6 cm whereas 3.9 cm in the control plates. By the 9th day, the colonies of *T. harzianum* and *Lactobacillus* met and neither organism grew any further. No clear inhibition zone was observed between the bacterial and the fungal colonies.

Effect of liquid biofertilizer and EM on *Vigna unguiculata*

All the treatments were observed for the increase in the height of the plants. In 15th, 30th,45th,60th day showed the highest result in 60th day.

Height of the plant

Among the six treatments, treatment with EM (T₅) in 60th day, showed maximum response on height of the plant (66.9±0.89cm), followed by T₄- (58.5±0.6),T₂-(54.3±0.2cm) T₁-(51.7±0.2cm), T₃-(42.7±0.7cm) and C-(22.6±0.2cm) respectively (Table- IV). In 15th day T₅ (27.7 ± 0.42cm) when compared to other treatments showed the minimum response on height of the plant.

Number of leaves

Among the six treatments, treatment with EM (T₅) in 60th day, showed maximum response on number of leaves (46±0.42cm) followed by T₄- (43±0.82cm),T₂-(42±0.67cm) T₁-(38±0.79cm) ,T₃-(34±0.83cm) and C-(28±0.83cm) respectively (Table- IV). In 30th day T₅ (24 ± 0.7 cm) when compared to other treatments showed the minimum response on number of leaves.

Table – 2 Counting of Microbial population (Cfu/ml) by Total cell count

S.No	Sample	Microorganisms	Total number of colonies (Cfu/ml)
1	Soil	Bacteria	41.2×10^7
2	Soil	Fungi	54.1×10^4
3	Dry Yeast	Yeast	27.1×10^5

Table – 3 Analysis of physico-chemical parameters of soil sample before and after treatment

S.No	Nitrogen (ppm)	Physico-Chemical parameters		pH
		Available potassium(ppm)	Available phosphorus(ppm)	
1	0.60	Before treatment	84.25	6.4
		39		
2	0.87	After treatment	87.5	6.8
		56		

Shoot Length

Among the six treatments, treatments with EM (T5) in 60th day, showed maximum response on shoot length of the plant (47.2 ± 0.6 cm) followed by T4- (44.1 ± 0.4), T2- (42.5 ± 0.7 cm) when compared to other treatments and control *Lactobacillus* and *Saccharomyces cerevisiae* was showed the minimum response on shoot length of the plant T1- (41.7 ± 0.9 cm) , T3- (40.4 ± 0.6 cm) and C- (31.9 ± 0.2 cm) respectively (Table - IV).

Root Length

Among the six treatments, treatment with EM (T5) in 60th day, showed maximum response on root length of the plant (25.7 ± 0.4 cm) followed by T4- (22 ± 0.51), T2- (21 ± 0.76 cm) when compared to other treatments and control *Lactobacillus* and *Saccharomyces cerevisiae* was showed the minimum response on root length of the plant T1- (20.5 ± 0.8 cm) , T3- (17.3 ± 0.74 cm) and C- (16 ± 0.76 cm) respectively (Table- IV).

Fresh weight of plant

Among the six treatments, treatments with EM (T5) in 60th day, showed maximum response on Fresh weight of the plant (15.6 ± 0.9 cm) followed by T4- (15.2 ± 0.8), T2- (14 ± 0.8 cm) T1- (11.6 ± 0.7 cm), T3- (13.3 ± 0.1 cm) and C- (10.1 ± 0.9 cm) respectively (Table- IV).

Dry weight of plant

Among the six treatments, treatment with EM (T5) in 60th day, showed maximum response on dry weight of the plant (3.83 ± 0.7 cm) followed by T4- (3.5 ± 0.5), T2- (3.3 ± 0.9 cm) when compared to other treatments and control *Lactobacillus* and *Saccharomyces cerevisiae* was showed the minimum response on dry weight of the plant T1- (2.92 ± 0.5 cm) , T3- (2.85 ± 0.1 cm) and C- (2.15 ± 0.1 cm) respectively (Table- IV).

Table -4 Growth of morphological parameters of *Vigna unguiculata L* (60th days)

	T1	T2	T3	T4	T5	Control
Yield (seeds in gm)	20±0.76	25.5±0.1	21.5±0.4	30.6±0.3	50.5±0.7	16±0.6
No.of pods / plant	8.1±0.7	11.8±0.8	11.3±0.8	12.5±0.9	12.9±0.4	7.6±0.4
Dry weight of plant	2.92±0.5	3.3±0.9	2.85±0.1	3.5±0.5	3.83±0.7	2.15±0.1
Fresh weight of plant	11.6±0.7	14±0.8	13.5±0.1	15.2±0.8	15.6±0.9	10.1±0.9
No.of leaves/Plant	38±0.79	42±0.67	34±0.83	43±0.82	46±0.42	28±0.83
Root length(cm)	20.5±0.8	21±0.76	17.3±0.4	22±0.51	25.7±0.4	16±0.76
No.of nodules/plant	6±0.43	8±0.81	7±0.26	10±0.23	12±0.84	5±0.9
Shoot length(cm)	41.7±0.9	42.5±0.7	40.4±0.6	44.1±0.4	47.2±0.6	31.9±0.2
Height of plant(cm)	51.7±0.8	54.3±0.2	42.7±0.7	58.5±0.6	66.9±0.8	22.6±0.2
Treatments	T1	T2	T3	T4	T5	Control

Values are triplicates, mean± standard deviation

Number of nodules

Among the six treatments, treatment with EM (T5) in 60th day, showed maximum response on number of nodules (12 ± 0.8 cm), followed by T4- (10 ± 0.4), T2- (8 ± 0.1 cm), T1- (6 ± 0.3 cm), T3- (7 ± 0.8 cm) and C- (5 ± 0.1 cm) respectively (Table- IV). In 30th day T5 (8 ± 0.1 cm) showed better response on number of nodules.

Number of flowers

In 45th day, T5 showed maximum response in number of flowers (10 ± 0.82 cm) combined inoculation of *Lactobacillus* + *Trichoderma harzianum* + *Saccharomyces cerevisiae* was showed better response on T4- (8.1 ± 0.9) T2- (8 ± 0.4) and when compared to other treatments *Lactobacillus* and *Saccharomyces cerevisiae* showed the minimum response on number of nodules T1- (5.58 ± 0.5) , T3- (7.66 ± 0.4) and C- (4.58 ± 0.3) respectively (Table- IV).

Internodal length

In 45th day, T5 showed maximum response in Internodal length (7.5 ± 0.1 cm), T4- (6.7 ± 0.57) T2- (4.5 ± 0.20), T1- (4.2 ± 0.26), T3- (3.8 ± 0.20) and C- (3 ± 0.31) respectively (Table- IV),

Length of pods

Length of the pods for analyzing the improvement of seed yield per plant .increased pods length was observed in the treatments (T5) (12.9 ± 0.4 cm) followed by T4 (12.5 ± 0.9 cm) T2- (11.8 ± 0.8) was showed the better response in result. When compared to other treatments and control the minimum response T1- (11.3 ± 0.8), T3- (8.1 ± 0.7), C - (7.6 ± 0.5) respectively (Table-IV)

Number of seeds

Overall treatment combined inoculation of EM (T5) showed in 30th day showed the maximum response on number of seeds (128 ± 6.01) followed by other treatments and control. Similar observation were made, in 45th day *Trichoderma* (T2) was showed the better response in number of seeds (146 ± 7.3) and the other treatments and control.

Yields (seeds in grams)

Yield of plants result due to the increase in all the parameters studied above. In 60th day EM T5- (50.5 ± 0.2) showed the highest yield. In T4 was showed the better response in T4- (30.63 ± 0.5) T2- (25.5 ± 0.1) when compared to other treatments and control T1- (20.0 ± 0.7) T3- (21.5 ± 0.2) and C - (18.1 ± 0.5) respectively thus the result the yield of the plant increased due to the EM from the soil . (Table-IV)

Phytochemical Analysis of *Vigna Unguiculata*

The chlorophyll content of the plants was increased in the treatments inoculated with EM. If the chlorophyll content was increased then the synthesis of carotenoids and protein content were also increased which in turn increases the yield of the plants. The total chlorophyll was calculated by analyzing the chlorophyll- a and chlorophyll- b contents of the plants. T5 showed the highest chlorophyll content (0.290 ± 0.1 mg/g) followed by T1, T2, T3, T4, and control (Table-5). Thus, EM showed an increased level in all the parameters followed by other treatments and the control showed similar results in most of the parameters.

Estimation of carotenoids

Among the overall treatments T5 was showed the higher activity in carotenoids of plants (0.997 ± 0.98 mg/g) followed by T4- (0.667 ± 0.96 mg/g) T2- (0.515 ± 0.64 mg/g) T1- (0.505 ± 0.9 mg/g) , T3- (0.423 ± 0.68 mg/g) and C - (0.412 ± 0.76 mg/g) respectively (Table -V)

Estimation of protein

The protein content was estimated on the growth of plant leaves showed the highest protein content T5- (EM) - ($6.75 \pm 0.87 \mu\text{g/g}$) followed by T4- ($4.43 \pm 0.72 \mu\text{g/g}$) T2- ($3.12 \pm 0.72 \mu\text{g/g}$). When compared to other treatments and control minimum protein content of T1- ($2.13 \pm 0.83 \mu\text{g/g}$), T3- ($2.543 \pm 0.76 \mu\text{g/g}$) and control ($1.44 \pm 0.4 \mu\text{g/g}$) respectively (Table-V),

Moreover, the *Rhodospseudomonas palustris* in the EM solution plays an important role in increasing the photosynthetic capability of the plants. As the solution is commercial, it contains the best formulations when compared to the consortium. This consortium was prepared to include *Trichoderma harzianum*, as the biocontrol agent, to suppress the soil borne pathogens in order to protect plant diseases. As EM contains the organisms for improving the plant growth and lesser effect as plant disease suppressing agent, the inclusion of *Trichoderma harzianum* may increase that ability and single solution can be used for the above said properties such as plant growth promotion and disease suppression.

DISCUSSION

They indicate that inoculation of pigweed with effective microorganisms increased the growth of shoot height, stem diameter, leaf number, leaf area, leaf fresh and dry weights, and root fresh and dry weights. Increased shoot height stem diameter growth probably reflects allocation of resources into shoots rather than roots. Increase in the number of leaves and leaf area are common occurrences in plants that are provided with proper nutrition and this can increase the photosynthetic activity of the plants (Singh et al, 2003)

Increase in chlorophyll 'a' and 'b' contents increase photosynthetic activity. The synthesis and degradation of the photosynthetic pigments are normally associated with the photosynthetic efficiency of the plants and their growth adaptability to different environment (Beadle, 1993). In this study, increase in chlorophyll a and b contents of the *Vigna unguiculata* contributes increase in leaf chlorophyll content could in turn lead to increased carotenoids and protein synthesis of the plants and this could have a direct consequence on the plant growth and photosynthesis.

In this study similar to the work in which EM has significant role in seed germination, shoot length, root length, of cowpea (Rubini and sashi, 2011). Maintaining crop yields and that EM increase the capacity to improve yield, thus maintaining productivity and sustainability. All of these benefit could be attributed to the development of the soil. Following treatments with the EM, which provides a conductive rhizosphere for plant growth. *Trichoderma harzianum* is an efficient biocontrol agent that is commercially produced to prevent development of several soil pathogenic fungi. Different mechanisms have been suggested as being responsible for their biocontrol activity.

Thus, we conclude that, the usage of EM can improve the soil fertility and the microbial consortium also improves the same with additional property of disease suppression. It is evident from the plants in the treatments with *Trichoderma harzianum* and the consortium were disease free when compared to others. Thus in a cost effective way, we can improve the plant growth parameters, yield and make them disease free.

CONCLUSIONS

The present study, EM inoculation increase the plant growth of *Vigna unguiculata*. EM have the maximum effect on plant growth parameters, to improve the nodulation ability of leguminous plants and to fix high atmospheric nitrogen. We conclude that the treated EM used is more suitable. which could be recommended to farmer to insure the public health and for a sustainable agriculture. The microbes in EM produce hormones, amino acids and alcohol substances. It may be play an important role in increased rate of (T5). In addition, EM increases in plant available nutrients. EM can be used to promote soil fertility and quality, reduces or eliminates the use of inorganic input, enhance crops yields and quality, accelerates the production of quality fertilizer by promoting fermentation decomposition of organic matter used in agriculture and lowers the hazards of continued cropping in open and greenhouse environment. *Trichoderma harzianum* is an efficient biocontrol agent that is commercially produced to prevent development of several soil pathogenic fungi. Different mechanisms have been suggested as being responsible for their biocontrol activity.

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