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RESEARCH ARTICLE

RHIZOBACTERIAL BIOINOCULANTS EFFECT ON TURMERIC (*Curcuma longa* L.) GROWTH IMPROVEMENT UNDER *IN VITRO* CONDITIONS

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ABSTRACT

Among a total of 18 cultivable rhizobacteria isolated from *Curcuma longa* grown tropical region of Tamil Nadu, India. Two isolates were evaluated screened for different PGP traits. Based on the above studied parameters, rhizobacterial isolates AUPF25 and AUBM29 were selected for investigation and identified as *Pseudomonas fluorescens* and *Bacillus megaterium* by 16S r RNA gene sequencing. The ability of this isolates to assess the impact of bioinoculants application on turmeric (*Curcuma Longa* L.) plant productivity under pot culture conditions. Understanding the growth performances of turmeric plants inoculated with bacterial biofertilizers under pot culture conditions is a key to improve crop production further in field (15-20%). Turmeric plants with 5 different combinations of bacterial bioinoculants in the liquid formulation were applied as single and dual combinations. The experimental plants were maintained based on complete randomized block design (CRBD). Both biotic and a biotic factors that influence the plant growth including soil nutrient status, plant hypotrophy, phyotochemical composition, bacterial population density were taken into consideration and those were analyzed. Among the bioinoculants combinations applied worked out better than the single and chemical inoculation. The treatment such as T_4 concluded as best combinations of bioinoculants and those were also found as compatible.

INTRODUCTION

The high usage of agrochemicals has made soil infertile, accumulation of toxic chemicals in the soil and food products and imbalanced nutrient cycling and ecosystem also occur. In order to maximize the agricultural productivity with minimum soil loss, a cheap, better and safest way is necessary. All these criteria can be achieved through application of microbial bioinoculants. Because, these microorganisms are known to possess vast range of capabilities by producing growth promoting substances, enhancing the plant nutrients, biological N2 fixation and crop protection against stress and diseased conditions. These PGPR have been shown to cause very real and positive effects when matched correctly to the right plant and the right environmental situation.

Turmeric (*Curcuma longa* L.) is a perennial crop belongs to Zingiberaceae family. It is widely cultivated in India and other parts of the world. Curcumin, a bioactive component of turmeric is responsible for the wide spectrum of medicinal properties and have commercial applications. The bacterial bioinoculants such as *Pseudomonas fluorescens* AUPF27 and *Bacillus megaterium* AUBM29 were chosen for the study was based on the metabolic activities they carry out. An important goal of nursery experiments is to produce consistence results of target morphometric, biochemical and microbiological characteristics with an input of microbial bioinoculants on turmeric growth and identification of nursery cultural treatments that promote these characteristics should help to improve field cultivation success (Jacobs *et al.*, 2003). If a positive effect of microbial biofertilizers is seen on a specific crop in nursery studies, there is a strong likelihood that those benefits will carry through to field conditions (Lusy *et al.*, 2004). The present study focused to find out the suitable combination of rhizobacterial bioinoculants that successfully increase the yield and quality of turmeric (15-20%).

MATERIALS AND METHODS

Culture used

The plant growth promoting rhizobacteria such as *Pseudomonas fluorecens* (AUPF25), *Bacillus megaterium* (AUBM29) were isolated from Curcuma longa (Boominathan et al.2012; unpublished manuscript) and the Nucleotide Sequence (16S rRNA) of this isolates were deposited to NCBI Genebank under the accession numbers. The isolates have been maintained in respected agar slants at 30±2ºC with monthly transfer.

Strain Name	Accession No	Genus and species
AUPF 25	JQ 638587	*Pseudomonas fluorecens*
AUBM 29	JN 990602	*Bacillus megaterium*

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Experimental setup

The experiments were carried out at the Annamalainagar, Annamalai University, Chidambaram, Tamil Nadu, India. The polybag media ($1x10^9$ CFU ml^{-1}) were prepared by mixing vermiculite carrier. The pot culture plants were maintained in randomized block design with three replication (RBD). Seeds were inoculated with biofertilizers (*Pseudomonas fluorescens*, *Bacillus megaterium*) individually or in combination and the control plants were raised in polythene bags. Plants were watered as and whenever necessary throughout the experimental duration. Various combinations of biofertilizers introduced were follows:

T_1-Control
T_2-*P.fluorescens** (AUPF25)
T_3-*B.megaterium** (AUBM29)
T_4- *B.megaterium* + *P.fluorescens*** (AUPF25+AUBM29)
T_6- 75% N (30 kg N/ha)*

*-Nitrogen was applied as urea in two split doses (30DAS, 90DAS)

**- The vermiculite based inoculum (100 g containing minimum inoculation

Load of 1×10^9 CFU/ g dry wt of carrier)

Sample collection

Root, rhizome and rhizosphere soil samples were collected from turmeric plantations at the harvesting period. The rhizosphere soils (1kg) from a composite soil sample; air dried in room temperature, packed in polythene bags and kept at 4ºC for further analysis.

Experimental 3; Estimating curcumin(%) in turmeric plants

The quality of turmeric products was assessed by estimating curcumin (cur) based on spectrophotometric method (Wright and Upadhyaya, 1996).

Experimental 4; Study turmeric Rhizosphere Bacterial population

The bacteria, such as Pseudomonas, Bacillus and total bacterial populations were isolated and enumerated to assess the population density by using standard microbiological techniques.

Statistical analysis

All data were subjected to analysis of variance (ANOVA) and the means separated using Duncan's Multiple Range Test (DMRT).

RESULTS

Influence of bioinocluants on the soil factors

The rhizosphere soil pH of various biofertilizers treatments existed from 8.4 to 9.2 the combination of *B. megaterium*, and *P. fluorescens* (T_4) were involved in the increment of rhizosphere soil pH to the maximum level when compared to all other treatments. However, trails of T_1, T_2 and T_3 has maintained pH level equivalent to control. The Electerical conductivity of rhizosphere soils varied from 0.10 to 0.16 among all the treatments assessed. The combination of *B. megaterium*, and *P. fluorescens (*T_4) inoculated had raised the EC value to its highest extent of 0.16. The maximum amount of OC% was accumulated when inoculated with *P. fluorescens* and *B. megaterium* (T_4) combined (Table -1).

Table 1 Influence of rhizobacterial bioinoculants on rhizosphere soil edaphic factor

Treatment	pH	EC	OC	N	P	K	Fe	Mn	Zn
T_1	8.6 ± 0.3^c	0.10 ± 0.004^c	0.16 ± 0.006^e	50±3e	4.0 ± 0.2^e	45 ± 2^e	4.0 ± 0.2^e	0.65 ± 0.13^e	0.66±0.02
T_2	8.9 ± 0.4^b	0.11 ± 0.003^b	0.84 ± 0.04^b	55 ± 2^d	4.4 ± 0.1^c	54 ± 1^c	4.2 ± 0.1^c	1.20 ± 0.21^b	0.86±0.03
T_3	8.6 ± 0.3^c	0.10 ± 0.002^c	0.75 ± 0.06^c	60 ± 2^c	5.2 ± 0.3^b	60 ± 3^b	5.4 ± 0.3^b	1.10 ± 0.3^c	0.76±0.02
T_4	9.2 ± 0.3^a	0.16 ± 0.003^a	1.42 ± 0.05^a	76 ± 4^a	7.1 ± 0.2^a	73 ± 3^a	5.7 ± 0.4^a	1.80 ± 0.11^a	0.96±0.04
T_5	8.4 ± 0.3^d	0.10 ± 0.004^c	0.56 ± 0.006^d	61 ± 2^b	4.1 ± 0.2^d	49 ± 2^d	4.1 ± 0.2^d	0.80 ± 0.13^d	0.72±0.02

Note: T_1- Control; T_2- *P. fluorescens*; T_3- *B. megaterium*; T_4- *P. fluorescens* + *B. megaterium* T_5- 75% N

The values bearing the same letters are not significantly different at 5% level according to DMRT.

Experimental 1; Study of Soil physic - chemical properties

The soil physic-chemical properties such as pH, EC and nitrogen (N), phosphorus (P) and potassium (K) were analyzed (Jackson, 1973). Organic carbon and organic matter was estimated according to walkey and black method (Walky and Black, 1934). The micronutrients such as cu, zn, Fe, and Mn were estimated as described by Lindsay and norvell (Lindsay and Norvel, 1978).

Experimental 2; Study of plants morphological parameters

The shoot height, root length, shoot and root biomass and number of leaves present in each plant were recorded. The shoot and root dry weights were obtained from each sample by oven drying at 80ºC to get a constant weight. The total chlorophyll, carbohydrate and protein concentration was determined by the methods of Hedge and Hofreiter (Hedge and Hofreiter, 1962) and Lowry's (Lowry *et al.*, 1951) respectively. The concentration of phenol content present in the leaves was analyzed according to the method of Malick and Singh (Malick and Singh, 1980).

The influence of microorganism in increasing the N content at the first level was observed in treatment (T_4).The available P content of all the treatments occurred between the range of 4.0 to 7.1 Kg/acre. The combined efforts of T_4 Treatment had elevated avilable P and K level to the maximum when compared to control.

On considering the Fe content, input of biofertilizers had towards increase of Fe based on their capability and the values from 4.0 to 5.7 ppm .The treatments of T_4 leads all other treatments with maximum Mn content of 0.65 and 1.80 ppm. The maximum and minimum range of Zn between 0.66 and 0.96 ppm. The treatment of T_4 were well known by producing lengthier roots. The dual combination of bioinoculants was perceptible in performance compared to single inoculation.

Turmeric growth and yield improvement through bacterial application

The impact of combined bioinoculants application on growth and yield of turmeric was assessed by measuring morphological parameters they exhibit. The maximum number

Table 2 Impact of rhizobacterial bioinoculants on growth and yield of turmeric under pot trials

Treatment	Lf. no	Inter Sht	s.ht(cm)	R. Lth(cm)	1°rhi	2 °rhi	Shoot Bio(g)	Root Bio(g)	Rhi Bio(g)
T_1	5±0.4	0±0.0	73±6.3	15.2±1.3	4±0.2	11±0.6	14.3±1.3	8.0±0.3	98.5±8.2
T_2	5±0.2	1±0.1	78±6.6	16.0±1.5	5±0.2	14±0.4	19.2±1.4	9.±0.65	112±10.4
T_3	6±0.3	1±0.1	80±7.6	16.8±1.2	7±0.3	16±0.8	27.5±2.0	11.2±0.3	98±8.3
T_4	8±0.3	2±0.1	88±7.4	17.4±1.6	9±1.0	18±1.3	29.8±2.6	13.±1.3	192±14.5
T_5	6±0.2	1±0.1	76±6.0	16.1±1.0	6±0.5	12±1.0	21.6±2.1	10.5±0.6	103±10

Note: T_1- Control; T_2- *P. fluorescens*; T_3- *B. megaterium*; T_4- *P. fluorescens* + *B. megaterium*; T_5- 75% N
The values bearing the same letters are not significantly different at 5% level according to DMRT.

Table 3 Impact of Rhizobacterial bioinoculants on bacterial density of turmeric Rhizosphere Soil under pot trials

Treatments	*Pseudomonas* (10^{-4} /g soil)	*Bacillus* (10^{-4} /g soil)	Total bacteria (10^{-5} /g soil)
T_1	$0±0^c$	$0±0^c$	$8±0.3^d$
T_2	$3±0.1^a$	$0±0^c$	$9±0.3^c$
T_3	$0±0^c$	$4±0.2^a$	$11±0.6^b$
T_4	$2±0.2^b$	$3±0.2^b$	$13±0.8^a$
T_5	$0±0^c$	$0±0^c$	$9±1.4^c$

Note: T_1- Control; T_2- *P. fluorescens*; T_3- *B. megaterium*; T_4- *P. fluorescens* + *B. megaterium* T_5- 75% N
The values bearing the same letters are not significantly different at 5% level according to DMRT.

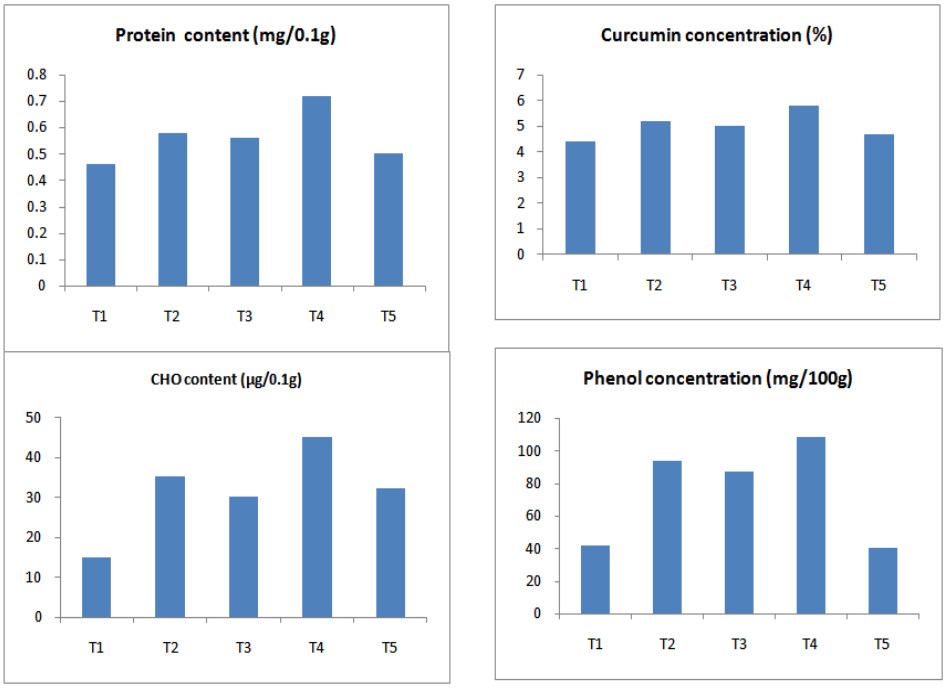

Fig.1
T_1- Control; T2- *P. fluorescens*; T_3- *B. megaterium*; T_4- *P. fluorescens* + *B. megaterium* T_5- 75% N

of leaves was present in treatment of T_4. Minimum number of leaves was shown by treatment T_1. The multiple combinations of biofertilizers had helped in attaining maximum number of intercalary shoots when compared to single inoculation. Both T_4 and T_2 were performing better in increasing shoot height then other treatments and show a least variation among them. Likewise, the treatments T_2 showed second maximum height and statistically they were equal to each other in their performance level.

Impact of Rhizobacterial bioinoculants on turmeric biochemicals

It is indeed necessary to understand the biochemistry of plants in addition to its morphological parameters. The single inoculation of *P. fluorescens* (was involved largely in accumulation of carbohydrates in the turmeric leaves to its higher extent (Table-2). The maximum carbohydrate level was shown by T_4. The total phenol concentration among biofertilizer treatments lies between 40 and 108 µg/ 100g leaf (fig, 1). In the case of protein concentration exists from 0.46 to 0.72 mg/0.1g (fig, 1). The combined effect of all different rhizobactrial inoculation had raised total phenols to its first place with the concentration of T_4 treatment 108 µg leaf. The high quality rhizomes were T_4 treatments. Which involve multiple combinations of biofrtilizers (fig, 1). Then, the treatment T_4 was also capable of providing quality turmeric rhizomes with 5.8 % curcumin.

Status of microbial population in the rhizospher soil

The rhizosphere soil that contained bacterial load to its maximum was present in the sample of soil sample of treatment T_4 were also potentially colonized by bacteria (13 x 10^{-5}/g soil). The *P. fluorescens* colonization was higher in the rhizospher soils of T_2 (3 x 10^{-4}g soil). There was a moderate other rhizobacterial colonization viz.. *Pseudomonas* sp and *Bacillus* sp in dual inoculation treatments when relating to control and singal inoculation (Table-3).

DISCUSSION

The initial assessment of rhizobacterial biofertilizer application in order to increase turmeric yield and quality under pot trails conditions are essential, sience the outcome of pot trails would almost express the same even when tested under field conditions (Lucey *et al.,* 2004). Considering this, the pot trials were made using bacterial inoculants based on their ability to mobilize soil nutrients and conferring biocontrol activity to plants. The influence of biotic and a biotic factors on the crop productivity was also reported (Sumathi *et al.,* 2008). The soil pH is a main factor for colonization of these agriculturally important microorganisms. Some of the treatments had shown that pH found reduced. It indirectly means enhanced microbial activity that happens in the rhizosphere region due to production of organic acids (Rengel and Marschner. 2005). The treatments that effectively raised soil pH were T_4 and T_2. The microorganisms involved in these treatments had played a best phenomenon in raising soil pH . The increase in soil pH is related to the availability of soil nutrients (Luizao et al.,2007). The treatments involving *P. fluorescens* and *B. megaterium* had resulted in accumulation of OC and OM at greater extent.

Like the combination involving *B.* megaterium and *P. fluorescens* can strongly influence the soil phosphorus content. *Pseudomonas* sp. was known to be an efficient solubilizer of complex phosphates releasing inorganic phosphates. In turn, the only slightless variation was observed between T_4 and T_3. In the case of T_4 which involves the work of *B. magaterium* had raised available phosphorus content. The strategies followed by microorganisms have been previously explained (Rodriguez *et al.,* 1999).

he treatment of T_4 showing increased macro and micronutrients contents such as k, fe, mn, crop yield, quality and number of intercalary shoots to its maximum level. The concentrations of these nutrients in improving the crop yield (Renal *et al.,* 1999).

The single inoculation of *P. fluorescens* (T_2) had increased carbohydrate content of the plant than any other combinations. Under greenhouse conditions, vegetative growth parameters like height, fresh and dry weight, leaf area and yield were significantly enhanced over the control when inoculated with *Azospirillum* sp (Raj *et al.,* 2005). *B.* megaterium jointly with *P. fluorescens* increased total phenol contents. Rich in phenolic composition of plant provides defense against various diseases (Van Loon *et al.,* 1998). The colonization of *B. megaterium* in rhizosphere was significantly correlated to intercalary shoots, root and rhizome biomass, curcumiun and than native soil bacteria. *B. megaterium* was the one which positive and significantly inhficantly influenced the turmeric quality (curicumin) than any other microorganisms involved.

CONCULATION

Among the bioinoculant combinations applied, multiple combinations worked out better than single inoculation. Of all the Biotic and A biotic factors analyzed, the treatments such as T_4 was concluded as best combinations of biofertilizers and those were also found as compatible, so, these combinations of biofertilizers can be recommended for application in turmeric fields.

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