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STUDIES ON POPULATION DENSITY OF DIFFERENT BIOINOCULANTS IN TURMERIC PLANTATION SOILS FOR BIOCONTROL ACTIVITY

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ABSTRACT

Turmeric (*Curcuma longa* L*)* is an herbaceous annual plant belonging to the family Zingiberaceae in which curcumin has been identified as the active principle compound. The quality of turmeric rhizomes is being affected by various pests and diseases which in turn reduce the quality of rhizomes. Turmeric is an important exportable spice for India. In most of the foreign countries, turmeric price is fixed based on the appearance of rhizomes and curcumin content. In order to get the maximum yield in turmeric, planters used to apply a variety of inorganic chemical fertilizers and agrochemicals for controlling pests and diseases. Furthermore, constant and indiscriminate applications of agrochemicals and mineral fertilizers pollute the environment and soil and also hazardous to animal and human health. Organic farming is a crop production method which encourages sustainable agriculture by enhancing the biological cycles in nature. The present study envisages studying the population density of different known bioinoculants strains in turmeric rhizosphere soil samples. The antagonistic activity of Bioinoculants was studied by performing test against rhizome rot pathogen, *Pythium aphanidermatum*. Plant growth promoting rhizobacteria (Bioinoculants) have been proved as efficient biocontrol agents in controlling various plant diseases besides, enhancing the plant growth significantly. The results revealed that Bioinoculants such as *Bacillus*, *Pseudomonas*, *Trichoderma* and *Streptomyces* spp. were identified and present predominantly in the soils of turmeric plantations. The population diversity was further correlated with the soil nutrient status. The microbial population of Bioinoculants and soil nutrient contents were carried out both in the rhizosphere and non-rhizosphere soils. The results indicated that the microbial population was found to be more in rhizosphere than in non-rhizosphere soils and there was a positive correlation between microbial population and soil nutrient contents.

Keywords: turmeric, *Curcuma longa,* PGPR, Bioinoculants, biocontrol activity, population density

INTRODUCTION

Turmeric (*Curcuma longa* L*)* is an herbaceous annual plant belonging to the family Zingiberaceae. It has a large oval rhizome with sessile cylindrical tubers containing curcumin content and commonly used as a flavouring, colouring agent and preservatives including biomedical applications. In India, turmeric is grown in its 18 states in which Tamil Nadu is the largest producer in India. The states like Andhra Pradesh, Tamil Nadu, Karnataka, Orissa and West Bengal are the major turmeric-producing states in India. It has been well studied in Asian, African and European countries due to its economic importance. It is native to tropical South Asia but is now widely cultivated in the tropical and subtropical regions of the world (Reddy, 2009). India occupies the first position in area, *viz*. 1,75,300 ha and also in production, *viz*.7,94,400 tonnes during 2009-10. The major countries that export turmeric are: India, China, Myanmar and Bangladesh. Indian turmeric fetches a premium price due to its superior quality in terms of high curcumin content, size and organoleptic characters in the international market. Curcumin has been identified as the active principle of turmeric. India has occupied around 60% of the world trade in turmeric. Curcumin is having anticancer and anti inflammatory properties apart from its potent antioxidant capacity, its mechanisms of action include inhibition of several cell signaling pathways, effects on cellular enzymes, immuno-modulation and effects on angiogenesis and cell–cell adhesion are well documented (Aggarwal *et al*., 2003).

The important turmeric varieties grown in India are, 'Alleppey Finger' (Kerala) and 'Erode and Salem turmeric' (Tamil Nadu), 'Raja pore' and 'Sangli turmeric' (Maharashtra) and 'Nizamabad Bulb' (Andhra Pradesh). In Tamil Nadu, the important varieties cultivated are Erode local, BSR-1 and 2, PTS-10, Roma, Suguna,

Sudarsana and Salem local. Among these varieties, 70-75% is occupied by the traditional varieties (Kandiannan *et al*., 2008). 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 safe way is necessary. All these criteria can be achieved through the application of microbial inoculants like Bioinoculants (Dinesh *et al*., 2010). Because these microorganisms are known to possess vast range of capabilities by producing growth promoting substances, enzymes, vitamins, organic aids, bioactive compounds, enhancing the plant nutrients, biological N_2 fixation, phosphorous/potassium-solubilization and crop protection against stress and diseased conditions. Plant growth promoting rhizobacteria (Bioinoculants) have been proved as efficient biocontrol agents in controlling various plant diseases besides, enhancing the plant growth significantly.

The quality of turmeric rhizomes is being affected by various pests and diseases. Furthermore, constant and indiscriminate applications of agrochemicals and mineral fertilizers pollute the environment and soil and also hazardous to animal and human health (Raveendra *et al*., 2007). Organic farming is a crop production method which encourages sustainable agriculture by enhancing the biological cycles in nature. The present study envisages to study the population density of different known PGPR strains in turmeric rhizosphere soil samples. The antagonistic activity of Bioinoculants was studied by performing test against rhizome rot pathogen, *Pythium aphanidermatum*.

Materials and Methods

Soil samples were collected from different turmeric planting districts of Tamil Nadu, India such as Coimbatore, Dharmapuri, Erode, Namakkal and Villupuram for the estimation of nutrient contents, enumeration of different Bioinoculants. These samples were allowed to air dry at room temperature and various parameters like soil pH, total organic carbon (Walkley and Black, 1934), total nitrogen (AOAC, 1990) and available phosphorous (Jackson, 1973) were determined. The population density of different Bioinoculants such as *Bacillus*, *Pseudomonas*, *Trichoderma* and *Streptomyces* species were enumerated in the above soil samples using nutrient agar (NA), King's B, *Trichoderma* selective medium (TSM) and casein nitrate agar (CNA) media; respectively.

The pathogen, *P. aphanidermatum* was isolated from infected rhizomes of turmeric plants collected from different agroclimatic zones covering different turmeric planting districts of Tamil Nadu state. The culture was identified and deposited at Centre for Advanced Studies in Botany, University of Madras, Chennai, Tamil Nadu followed by Microbial Type Culture Collection Centre (MTCC), Chandigarh, India. Enumeration and isolation of different Bioinoculants in the soil samples were performed by serial dilution plate technique. Morphological characterizations such as Gram's staining, endospores and motility test and biochemical characterizations such as pigment production, starch hydrolysis, casein hydrolysis, catalase test, nitrate reduction, indole production, gelatin hydrolysis, and hydrogen sulphide production were carried out (Williams and Wilkins, 1994).

Influence of abiotic factors such as pH (4.0 to 6.5) and temperature (5 to 40°C) and various nutrient factors such as carbon, nitrogen, amino acid and vitamin sources on growth of various Bioinoculants were carried out. Six different carbon compounds such as glucose, fructose, maltose, sucrose, starch and cellulose and four nitrogen compounds such as ammonium nitrate, sodium nitrate, potassium nitrate and casein hydrolysate were added by replacing starch and potassium nitrate; respectively in the basal medium. On the other hand, sources of amino acid like alanine, methionine and arginine and vitamins such as thiamine, riboflavin and biotin were selected and supplemented extra in the basal medium to enhance the growth potential of Bioinoculants. The inoculated plates were incubated for 10-15 days depending upon the nature of experiment to record the growth rate of Bioinoculants (Ponmurugan *et al*., 2007).

It was studied by following the method of dual culture (Huang and Hoes, 1976) and Antibiosis (Dennis and Webster, 1971) techniques. In the case of dual culture study, a mixture (50:50 %) of PDA and NA / King's B/ TSM / CNA media containing plates were inoculated with *P. aphanidermatum* as well as BIOINOCULANTS strains on diametrically opposite points. Radial growth of the pathogen and antagonist were measured at 24 hrs interval. For antibiosis study, Petri dishes containing mixture malt extract medium amended with secondary metabolites of Bioinoculants were subsequently inoculated with *P. aphanidermatum*. Radial growth was measured periodically and calculated the per cent inhibition.

Results and Discussion

Fertility of the soil is influenced by its physical, chemical and biological properties including cultivation of crops. In general, beneficial microorganisms in soil decompose various organic substances and thereby improve soil tilth and fertility, which in turn enhance the plant growth. Many beneficial microorganisms promote plant growth by mutualistic or symbiotic relationship by fixing atmospheric nitrogen or mobilizing phosphorous or solubilizing potassium to a greater extend (Jayanthi *et al* 2001). It has been observed that the rhizosphere is a region of dynamic process initiated by root exudations, release of organic nutrients and is influenced by a host of

factors like soil features, environmental conditions, cultural practices and soil microbial interactions (Baby *et al.,* 2002). Among the beneficial mutualistic with non-symbiotic microorganisms, *Bacillus subtilis, Pseudomonas fluorescence, Trichoderma atroviride* and *Streptomyces* species are very important in turmeric plant growth in terms of enhancing the yield potential and maintain the soil health. The results revealed that the total number of various Bioinoculants population in turmeric soil samples was found to be higher in Erode followed by Coimbatore than in Dharmapuri and Vizhupuram districts. Moreover, among the Bioinoculants, bacterial Bioinoculants such as *B.subtilis* and *P.fluorescence* were found to be higher than actinomycetes (*Streptomyces* spp.) and fungal Bioinoculants like *T. atroviride*. Similarly, of the bacterial Bioinoculants, *P.fluorescence* was the highest (14.5 x10^5) population in the soils of Erode while *B.subtilis* was the least (9.72 x10^5 cfu/gm soil dry wt) population.

The population density of PGPR strains was positively coincided with soil nutrients like total organic matter, available nitrogen, phosphorous and potassium contents (Table 1). Moreover, the population density of Bioinoculants was found to be more in rhizosphere than in non-rhizosphere soil samples collected from turmeric fields. The same trend was registered in population density which was found to be maximum in soil samples obtained from Erode followed by Coimbatore and least with Dharmapuri and Vizhupuram districts. The The soil edaphic parameters like soil reaction (pH), electrical conductivity (Ec), clay, silt, sand content and water holding capacity were varied in varying levels between turmeric planting districts, however, there was a strong relationship between these parameters and population diversity of Bioinoculants . Similar observations were reported in tea soils by Baby *et al.* (2002) and Ponmurugan *et al*. (2011) who observed a correlation between beneficial microorganisms like nitrogen fixers, phosphate solubilizers, antibiotic producing microorganisms (actinomycetes) and biocontrol agents and nutrients in the soil.

A total of 1500 strains of Bioinoculants were isolated from turmeric soil samples and subjected to screen for their antagonistic activity by following paper discs method. Further, morphological and biochemical characteristics of the PGPR strains were studied and results were presented in the Table 2. On morphological characterization showed the isolates were found to be Gram's positive organism. The cells were coiled rods and the endospores were free to tight in nature. The results on biochemical characterization indicated that most of the strains were efficient in hydrolyzing starch, gelatin and casein. Indole production was strictly negative for some strains but catalase test was positive to the rest of strains. Production of hydrogen sulphide and nitrite reduction showed positive result in majority of the isolates (Table 2). The results coincided with the report of Ravel *et al*. (2000) and Ponmurugan et al (2011).

The growth of PGPR strains in the basal medium adjusted with different pH and nutrient sources revealed that a better growth was recorded in a pH at 5.5. This pH level may be correlated with the soil pH. The optimum temperature for the growth of PGPR strains was 25°C followed by 20°C. Among the different carbon and nitrogen sources tested, maltose and potassium nitrate; respectively the good supplementary sources. Of the different vitamin and amino acid sources evaluated, biotin and alanine; respectively were found to be limiting substrates for utmost growth of PGPR strains (Table 3). Production of an array of antifungal metabolites has been known to be influenced by components of medium and cultural conditions such as pH, temperature, carbon, nitrogen and other sources (Augustine *et al*., 2004).

The antagonistic potential of various PGPR strains was studied against a rhizome rot disease causing pathogen namely *P. aphanidermatum* which revealed a remarkable percentage of inhibition of pathogen growth was recorded. Among the two different methods of antagonistic activity studied, antibiosis was better than hyperparasitism in terms of good growth inhibition of the pathogen in which 83.23-92.19% and 75.56-84.56% was registered with antibiosis and hyperparasitism; respectively. These results were coincided with the report of Zahner *et al*. (1979) and Ponmurugan *et al*. (2007; 2011) who observed the inhibition zone of *Phomopsis theae* against *Streptomyces* spp. The growth inhibition of pathogen is due to the production of secondary metabolites by the antagonists (Thangapandian *et al*., 2007) According to Demain and Fang (1995), the most widely accepted theory is that antibiotics are used to complete with other organisms in nutrient depleting environment (Rovel *et al*., 2000 Augustine *et al*., 2004) and the production of exopolysaccharide compounds, diffusible pigments and enzymes such as lipase, caseinase, gelatinase, cellulase and amylase by the antagonists (Ponmurugan *et al*. (2011). In the present study, most of the strains of Bioinoculants were found to be of potential antagonists against rhizome rot pathogen. Based on these results, it can be further inferred that the isolated PGPR strains can be used as soil inoculants to prevent the growth of soil-borne pathogens like *P. aphanidermatum* in turmeric soils.

Table 1: Population density of various Bioinoculants and nutrient status in turmeric soils of Tamil Nadu state of India

Name of planting districts	Population Density				Soil reaction (pH)	Organic carbon (%)	Total nitrogen (ppm)	Available phosphorous (ppm)
	BS	PF	TA	SS				
Coimbatore	8.84	12.7	4.88	6.72	7.80	2.55	2734	14.72
Dharmapuri	8.54	10.3	2.57	3.53	6.92	1.87	1777	13.22
Erode	9.72	14.5	6.75	8.57	7.81	2.77	2844	16.83
Namakkal	7.73	11.4	1.75	5.35	6.82	0.85	1146	12.80
Villupuram	5.00	10.0	3.44	3.90	7.20	1.68	1305	13.55
SE ±	1.00	2.24	1.03	2.24	0.54	0.82	150.47	02.33
CD at P=0.05	2.57	3.08	3.55	3.07	1.38	1.02	253.37	03.47

BS – *Bacillus subtilis* (cfu x 10^5 cfu/gm soil dry wt)

PF – *Pseudomonas fluorescence* (cfu x 10^5cfu/gm soil dry wt)

TA – *Trichoderma atroviride* (cfu x 10^3 cfu/gm soil dry wt)

SS – *Streptomyces species* (cfu x 10^4 cfu/gm soil dry wt)

Table 2: Morphological, physiological and biochemical characterization of various Bioinoculants.

S.No.	Parameters	Strains of various Bioinoculants			
		Bacillus	*Pseudomonas*	*Streptomyces*	*Trichoderma*
1.	Cell morphology	Coiled rods	Rods	Coiled rods Rods Spiral spore chain	Cluster of spore chain
2.	Gram's staining	++	++	++	
3.	Pigment production	+	-	+	-
5.	Starch hydrolysis	++	-	+	++
6.	Casein hydrolysis	++	-	+	++
7.	Catalase test	+	++	++	+
8.	Nitrate reduction	++	++	++	++
9.	Indole production	+	-	+	-
10.	Gelatin hydrolysis	++	+	++	-
11.	Hydrogen sulphide production	++	++	++	-
12.	Presence of endospores	++	++	++	-
13.	Nature of endospores	Free cells	Tight free cells	Tight free cells	-

++ Prominent growth
+ Moderate growth
- No growth

Table 3: Effect of biotic and nutrient factors on the growth of various Bioinoculants.

Parameters	Strains of Bioinoculants			
	Bacillus	*Pseudomonas*	*Streptomyces*	*Trichoderma*
Optimum pH	5.0	5.0	5.5	5.5
Optimum temperature (°C)	25	25	20	25
Glucose *	++	+	+	++
Fructose *	++	+	+	++
Maltose *	++	++	++	++
Sucrose *	++	+	-	-
Starch *	++	+	++	++
Cellulose *	++	++	-	-
Ammonium nitrate **	++	+	++	+
Sodium nitrate **	++	+	+	+
Potassium nitrate **	++	++	++	++
Casein hydrolysate **	++	+	+	+
Alanine #	++	++	++	++
Methionine #	+	+	+	+
Arginine #	+	+	+	+
Thiamine ##	+	-	+	+
Riboflavin ##	+	+	+	+
Biotin ##	++	++	++	++

* Carbon sources
** Nitrogen sources
# Amino acid sources
Vitamin sources

++ Prominent growth
\+ Moderate growth
\- No growth

Table 4: Antagonistic potential of *B. subtilis, P. fluorescence, Streptomyces* spp. and *T. atroviride* against *P. aphanidermatum*

Microorganisms	Hyperparasitism	Antibiosis
*Pythium x B. subtilis**	75.56	83.23
*Pythium x P. fluorescence**	78.89	84.84
Pythium x Streptomyces spp.**	84.56	92.19
*Pythium x T. atroviride****	80.56	83.74
SE ±	02.56	03.23
CD at P=0.05	04.41	04.89

* on 24 hours
** on 72 hours
*** on 96 hours

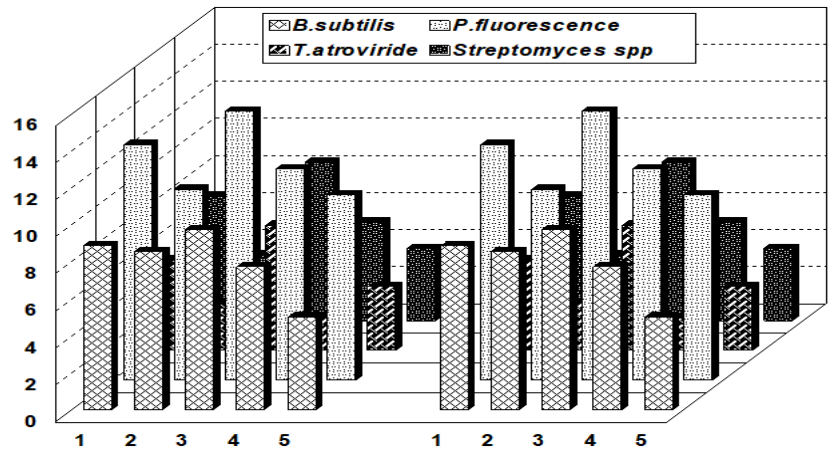

Figure 1: Population density of various Bioinoculants in rhizosphere and non-rhizosphere soils of turmeric plants (1. Coimbatore; 2. Dharmapuri; 3. Erode; 4. Namakkal and 5. Villupuram)

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