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Antagonistic and Phosphate Solubilization Potential of Trichoderma SP From Rhizosphere of Red Gram Cultivated in Marathwada Region

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ABSTRACT

The region where soil and roots makes contact is called rhizosphere. The microbial growth is enhanced by nutritional substances such as amino acids and vitamins released from plant root tissues. It represents a tremendously complex biological system. The growth of plant is also influenced by the products of microbial metabolism. The inorganic phosphorus which is unavailable to plant is solubilized by fungi & bacteria. Biocontrol of plant disease is an alternative to chemical pesticides which causes environmental pollution and development of resistant strain. In the present work screening of phosphate solubilizing Trichoderma sp. from rhizosphere of red gram cultivated in Marathwada region was done by using PVK medium. Soil samples were collected from different rhizosphere region of red gram cultivated in Marathwada region. From this isolates of Trichoderma sp. were isolated & discussed in this paper. Isolates of Trichoderma sp. were identified depending upon their morphological and cultural characters. Efficient isolates of PSTS were further tested for antimicrobial activity against Bacillus subtilis NCIM2010; E. coli NCIM2064; Proteus vulgaris NCIM2027; Xanthomonas compestris NCIM2956 & Fusarium oxysporum NCIM1281.It was observed that one isolate showed high PSE & antimicrobial activity; hence it could be exploited as biofertilizer and biocontrol agent in agriculture.

Keywords: Antagonism, Biocontrol, Phosphate Solubilization, Rhizosphere, Trichoderma SP

I. INTRODUCTION

Cajanus cajan L. (Red gram) is also known as pigeon pea (Arhar or tur in local language).It is one of the most extensively used pulses in India. Pigeon pea probably evolved in South Asia and appeared about 2000 BC in West Africa, which is considered a second major center of origin. It is a leguminous shrub that can attain height of 5 M. It is a major pulse crop of India. Among total pulses, the red gram accounts for 14.5% in area and 15.5% in productivity. Maharashtra is the largest producer with approximately 10.51 lakh hector area with average productivity of 6.03 Q/ha. Being important nitrogen fixing crop, it is widely grown for enriching the soil. Its deep penetrating roots helps in bringing nutrients from deeper layers of soil.

The term rhizosphere was introduced by German scientist Hiltner. It is the region where soil and roots makes contact. It is a unique subterranean habitat for microorganisms [1]. The microbial growth is enhanced by nutritional substances like amino acids, vitamins and other nutrients released from plant tissues. The growth of plant is also influenced by the products of microbial metabolism that are released into the soil [2]. It is a hotspot of microbial interaction. There are several beneficial microorganisms in the rhizosphere, which can improve soil quality, enhance crop production, protection, conserve natural resources and ultimately create more sustainable agricultural production and safe environment[1], [2] . Phosphorus is a major growth limiting nutrient for plants. Like the case for nitrogen there is no large atmospheric source for it [3]. The inorganic phosphorus which is unavailable to plant is solubilized by fungi and bacteria. Trichoderma is free-living fungi that are highly interactive in root, soil and foliar environments. Trichoderma was found to be the most effective organic phosphorus mobilizers as compared to other fungi [4].

Biocontrol of plant pathogen is an alternative to chemical pesticides, which cause environmental pollution and development of resistant strain. The use of beneficial microorganisms (biopesticides) is considered as one of the most promising method for disease control [5]. The nature and practice strategies for detection and characterization systems for biological control of plant and soil born pathogen have been elucidated earlier [6]. Trichoderma sp have been shown to act, and are commercially applied as biological control agents against fungal pathogens [7, 8]. Biological control agents colonize the rhizosphere, the site requiring protection & leave no toxic residues, as opposed to chemicals. In the present work screening of Trichoderma species from rhizosphere soil of red gram cultivated in Marathwada region was done. Then its phosphate solubilizing efficiency & antagonistic activity was studied.

II. METHODS AND MATERIAL

Collection of rhizosphere soil sample

Soil samples were collected from rhizosphere of red gram cultivated in different locations of Aurangabad, Jalna, Latur, Osmanabad, Nanded, Parbhani, Hingoli and Beed district area in sterile plastic bags & stored at low temperature.

Isolation & identification of Trichoderma sp

The soil sample was serially diluted by serial dilution method. The last dilutions were spread on Czapek Dox agar and PDA medium .Plate was incubated at 25 ºC for 3-5 days. Morphologically distinct colonies were picked on the basis of their morphology [9]. The isolates Trichoderma sp. were identified by morphological character, cultural characters and reproductive structure [8], [10], [11], [12].

Phosphate solubilization

The isolates of Trichoderma sp were spot inoculated on PVK agar (M520 Hi-media) plates containing: Glucose 10.0 gram, Tri calcium phosphate – 5.0 gm, $(NH_4)_2SO_4$-0.50 gm, KCl-0.20 gm, $MgSO_4.7H_2O$-0.10 gm, $MnSO_4$- Trace, $FeSO_4$- Trace, Yeast extract-0.50 gm, Agar-15gm in 1000 ml of distilled water. The plates were incubated at 28 ºC for 4-6 days. After incubation phosphate solubilizing fungi were detected by the appearance of transferent halo zone around its growth [11]. The zone diameter around the colony is measured and phosphate solubilizing efficiency was calculated by using following formula [13].

$$PSE = \text{Solubilization diameter} \div \text{Growth diameter} \times 100 \quad (1)$$

Detection of antimicrobial activity

The antimicrobial activity of isolates of Trichoderma sp. was determined by using agar diffusion (well) method. The water extract of isolates of PSTS was loaded into the well

bored and test organism seeded agar plates. For bacteria nutrient agar & for fungi Czapek Dox agar was used. The test organisms used were Bacillus subtilis NCIM2010; E. coli NCIM2064; Proteus vulgaris NCIM2027; Xanthomonas compestris NCIM2956 & Fusarium oxysporum NCIM1281.The plates were kept in freeze for 20-30 minutes and incubated at 30 ºC for 24 hours for bacteria & 25 ºC for 2-3 days for fungi. The diameter of zone of inhibition was measured & noted in the table.

III. RESULT & DISCUSSION

Soil samples were collected from different rhizosphere region of red gram cultivated in Marathwada region & from this isolates of Trichoderma sp. were isolated and identified. Trichoderma sp. was confirmed on the basis of morphological and cultural characters. On the PDA and Czapek Dox agar plate after 3-5 days at 25 ºC, the colonies were initially white in color which turns yellowish green in color after further incubation. Colony characters are: size- large, shape- irregular, elevation- flat, margin- lobate and surface texture-concentric. After staining with lactophenol cotton blue, microscopic observation showed septate hyphae which form firm tufts, conidiophores erect developing from side branching, branching usually opposite, conidiophores bear terminal conidial heads and conidia were oval to elliptical and smooth. Isolates of Trichoderma sp. were further subjected for detection of phosphate solubilization activity. Phosphate solubilizing efficiency was determined by using formula (Eq.1) & noted (Table 1).

Table 1: Phosphate solubilizzation efficiency of isolates of Trichoderma sp. at temp 28 ºC on PVK medium.

Sr. No.	**Isolates of *Trichoderma* sp.**	**PSE**
1.	PSTS01	113
2.	PSTS02	181
3.	PSTS03	175
4.	PSTS 04	134
5.	PSTS 05	147
6.	PSTS 06	180
7.	PSTS 07	207
8.	PSTS 08	132
9.	PSTS 09	187
10.	PSTS 10	158

Identification of Trichoderma sp was done according to morphological character, cultural characters and reproductive structure. Phosphate solubilization was confirmed by observation of clear zone around the colony on PVK agar plate. The zone is formed due to microorganism which cleaves phosphate molecules present in the medium [14]. The isolates of PSTS were subjected for study of antimicrobial activity against Bacillus subtilis NCIM2010; E. coli NCIM2064; Proteus vulgaris NCIM2027; Xanthomonas compestris NCIM2956 & Fusarium oxysporum NCIM1281. Zone diameter was measured & noted (Table 2)

Table 2: Antimicrobial activity of isolates of phosphate solubilizing Trichoderma p.

Sr. No.	Isolates of *Trichoderma* sp	*B. subtilis* NCIM 2010	*E. coli* NCIM2064	*Pr. vulgaris* NCIM2027	*X. compestris* NCIM2956	*F. oxysporum* NCIM 1281
1.	PSTS01	+	+	-	-	+
2.	PSTS02	++	++	+	+	-
3.	PSTS03	+++	-	++	-	++
4.	PSTS 04	-	+	+	+	-
5.	PSTS 05	+	+	-	+	++
6.	PSTS 06	++	-	++	+	+
7.	PSTS 07	+++	++	++	+	++
8.	PSTS 08	+	+	-	-	-
9.	PSTS 09	+++	-	+	+	+
10.	PSTS 10	++	+	-	+	+

(--: no antimicrobial activity, +: antimicrobial activity) It was observed that isolate PSTS07 showed high phosphate solubilization and antimicrobial activity against Bacillus subtilis NCIM2010; E. coli NCIM2064; Proteus vulgaris NCIM2027; Xanthomonas compestris NCIM2956 & Fusarium oxysporum NCIM1281, hence it could be exploited as biofertilizer and biocontrol agent. Phosphate solubilization takes place through different microbial processes. Phosphate solubilizing microorganism is being used as biofertilizer since 1950 [15, 16]. Microorganisms release organic acids which through their hydroxyl and carboxyl groups chelate the cation bound to phosphate, the latter being converted to soluble forms [14, 17]. The Trichoderma sp. are known to produce a number of secondary metabolites like trichodermin, trichodermol, harzianum A, harzianolide etc., apart from lytic enzymes that play significant role in the antagonistic activity [18]. Trichoderma sp. produces different antibiotics against fungal phytopathogens. Among the antibiotics, the production of gliovirin, gliotoxin, viridin, pyrones, peptaibols and others have been described [19].Continuous and indiscriminate use of chemicals leads to development of resistant strain towards pesticides. Chemical fertilizers also adversely affect the useful microorganisms in the soil. Biofertilizer is an attractive alternative to chemical fertilizers which causes environmental pollution.

IV. CONCLUSION

On the basis of plant growth in the present investigation, it was conclude that the organic fertilizer is more effective than the chemical fertilizer. We used more amount of bio fertilizer, organic fertilizer for plant growth. The Banana plants receiving the recommended dose of fertilizers along with bio-fertilizes recorded plants were superior in growth, fruit was more than other plants. The treatments with bio-fertilizers recorded higher values compare to without bio-fertilizers and the organic fertilizer gives more amount of product yield. And also increase the soil fertility for long time the chemical fertilizer casus various disease.

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