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Isolation of Phosphate Solubilizing Fungi from The Rhizospheric Soil of Wheat plant in Raipur

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ABSTRACT

Phosphate solubilizing fungi were isolated from the rhizosphere soil of wheat plant of Raipur region, Chhattisgarh state of India.. In this study total of 19 fungi were isolated from rhizospheric soil of wheat plants. Out of 19, twelve fungi were showed as phosphate solubilizers on the Pikovskaya's Agar medium. Aspergillus species, a soil isolate had excellent potential to solubilize phosphate in vitro. In present study fungal strains isolated from soil having potential to solubilize phosphate were characterized and fungal strain used as bioinoculent.

Keywords: Aspergillus, Penicillium , Phosphate solubilizing fungi & Solubilization

I.INTRODUCTION

Improving soil fertility is one of the most common practices in agricultural production. Phosphorus (P) is one of the most essential plant nutrients for maximizing crop productivity. This nutrient is limited in soils, which remain as a major challenge to agriculturists and land managers. (1). Phosphorus is one of the major nutrients, second only to nitrogen in requirement for plants. A greater part of soil phosphorus, approximately 95–99% is present in the form of insoluble phosphates and cannot be utilized by the plants (2). Compared with the other major nutrients, phosphorus is by far the least mobile and available to plants in most soil conditions. Although phosphorus is abundant in soils in both organic and inorganic forms, it is frequently a major or even the prime limiting factor for plant growth (3).

Phosphate solubilizing fungi and bacteria are known as effective organisms in this process (4,5). Fungi are the important components of soil microbes typically constituting more of the soil biomass than bacteria, depending on soil depth and nutrient conditions. Fungi have been reported to have greater ability to solubilize insoluble phosphate than bacteria (6). A wide range of soil fungi are reported to solubilize insoluble phosphorous such as *Aspergillus niger* and *Penicillium sp*. which are the most common fungi capable of phosphate solubilization (7).

Exploration of phosphate solubilizing microorganisms has been conducted by many researchers from soils, mangrove and rhizosphere (8). Since large population of Chhattisgarh state is dependent on agriculture the present investigation is aimed to isolate some fungal strains that may have high efficiency for phosphate solubilization.

II . MATERIAL AND METHOD

The present investigation was carried out in the SoS Life Sciences, Pt. Ravishankar Shukla University, Raipur, Chhattisgarh. (INDIA)

II.1-Study Site

Raipur is situated in East Central part of Chhattisgarh at latitude of $21^0 16^{'}$ N, longitude $81^0 36^{'}$ E and altitude 289.5 m above mean sea level. The climate of Raipur is falling under sub-humid with mean annual rainfall of about 1489 mm out of which 90 per cent (1348 mm) is received during monsoon (June to September). During *rabi*, (December to February) only 8 mm of rainfall is being received on an average. The maximum temperature ranged from 24.4 to 42.6 ^{0}C and minimum temperature ranged between 10.0 to 27.5^{0}C respectively.

II.2-Collection of soil samples

Soil samples were collected from rhizosphere of Wheat plantation from 3 different villages of Raipur city of Chhattisgarh state. Samples were collected in polythene bags, transported to laboratory and stored in refrigerator for further processing. Soil samples were separated from roots, air dried at room temperature, crushed, sieved and collected in separate polythene bags. pH of the samples was recorded using pH meter (Elico made).

II.3-Culture media for isolation

Pikovskaya's (9) agar medium (HIMEDIA) was used for the isolation and maintenance of phosphate solubilizing fungi. It contained (g litre^{-1}) Dextrose 10; Calcium phosphate 5; Ammonium sulphate 0.5; Potasium chloride 0.2; Magnesium sulphate 0.1; Manganease suplhate 0.0001; Yeast extract 0.5; Ferrous sulphate 0.0001, Agar 15. The pH of medium was 7.0 (± 0.2).

Potato dextrose agar (PDA, HIMEDIA) was used for the isolation maintenance of fungal cultures. It contained (g.litre^{-1}) potato infusion 200; Dextrose 20; Agar 15 and the pH of medium was 5.6 (± 0.2) The pH of culture media was adjusted using 1N NaOH or 1N HCl. Media were sterilized by autoclaving at 121°C for 15 min.

II.4- Isolation

All the soil samples collected were used for the isolation of phosphate solubilizing fungi on Potato Dextrose agar medium by dilution and plating method. Ten gram of soil from each sample was aseptically weighed and transferred to 250 ml Erlenmeyer flask containing 90 ml of sterile double distilled water. Aliquots of 1 ml of the

supernatant from the sample were transferred to 9 ml of 0.85 per cent NaCl dispensed in test tubes and serially diluted to 10^{-1}, $10^{-2,}$ 10^{-3} 10^{-4}, 10^{-5} and 10^{-6}. One ml sample from each of these dilutions was transferred to sterilized 20 ml Potato Dextrose agar medium before solidification (450C temperature) and poured in sterile Petri dish after mixing. After solidification plates were kept in incubator at 28±2ºC for 5-7days. Fungal colonies were subcultured several times on PDA plates till the appearance of pure cultures. Pre cultures thus isolated were kept in refrigerator after growing on slants of PDA and used for further studies.

II.5- Screening

The isolates were screened by inoculating on plates containing Pikovskaya's Agar (PKA) medium (9) amended with 0.5% tricalcium phosphate (TCP) as insoluble phosphate source and were incubated at 28±2ºC for 5 days. Fungal colonies with clear halozone around them were screened as phosphate solubilizers.

II.6- Identification

The fungal cultures were identified on the basis of colony characteristics and microscopic examination (10, 11, and 12). Some of the fungal isolates have been sent and deposited to NFCCI for identification

II.7-Solubilization Index (SI):

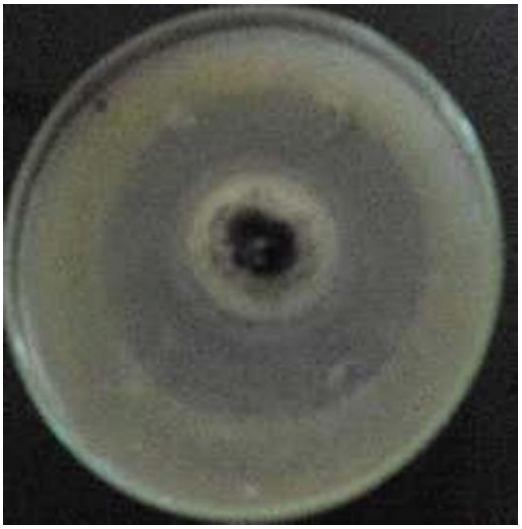
Suspensions were prepared in sterile saline (0.85 per cent NaCl) from isolate fungal cultures. Optical density of each culture was adjusted at 0.3 using colorimeter (Elico CL 157) at wavelength 520 nm. The 10 µl suspension of each isolate was placed on Pikovskaya's agar plate and incubated at 28±2ºC for 5days in incubator. Solubilization index (SI) was measured using the following formula [13].

$$SI = \frac{\text{Colony diameter + Halo zone diameter}}{\text{Colony diameter}}$$

III.RESULTS AND DISCUSSIONS

Total 19 fungi were isolated from rhizospheric soils of chickpea plantation and 12 were screened as phosphate solubilizers based on appearance of clear halozone on Pikovskaya's agar medium (**Table**). Fungi that showed halo zones around the colony are good phosphate solubilizers and belong mainly to the genera of *Aspergillus*, and *Penicillium. Aspergillus niger* was found to be the dominant group followed by Penicillium sp. and other species of Aspergillus. The similar results were highlighted by Mahamuni *et al.* (14) and Deepa *et al.* (15).

IV.PHOTOGRAPH OF HALOZONE FORMATION & *ASPERGILLUS NIGER*



V.TABLE-1

S. No	Village Name (Raipur)	Soil p^H	Isolated Fungi name	Formation of Halozone
01	Jora Village	7.70	*Aspergillus* sp.JW1	Yes
			Aspergillus niger	Yes
			Aspergillus fumigatus	Yes
			Penicillium sp.JW1	No
			Aspcergillus sp.JW2	Yes
			Rhizopus sp.JW	No
			Fusarium sp.JW	No
			Penicillium sp. JW2	Yes
02	Tekari	6.8	*Curvularia sp.TW*	No
			Aspergillus sp. TW	Yes
			Fusarium spTW	No
			Aspergillus niger	Yes
			Penicillium sp.TW	Yes
03	Nakti	7.2	*Aspergillus niger*	Yes
			Aspergillus sp.NW	Yes
			Penicillium sp.NW	Yes
			Curvularia sp.NW	No
			Mucor sp.NW	No
			Alternaria spNW	Yes

Table: Screening of the phosphate solublizing properties by the isolated fungi on the basis of halo zone formation.

Qualitative assay: The solubilization indices of different isolates ranged from 1.10 to 1.49 (**Table 2**). Fungal strains isolated from sugarcane and sugar beet rhizosphere showed SI in range of 1.13 to 1.59 (14). Alam et al. (16) reported SI of the fungal strains isolated from maize rhizosphere that ranged from 1.53 to 1.80.

TABLE-II

S. No	Name of fungi	Solubilization Index (SI)		
		A(mm)	B(mm)	SI
1	*Aspergillus* sp.JW1	49	33	1.48
2	*Aspergillus niger*	50	35	1.42
3	*Aspergillus fumigatus*	52	37	1.40
4	*Aspcergillus sp.JW2*	50.5	33.8	1.49
5	*Penicillium* sp. JW2	42	31	1.35
6	*Aspergillus* sp. TW	78	69	1.13
7	*Aspergillus niger*	44	39.9	1.10
8	*Penicillium* sp.TW	43	29.9	1.43
9	*Aspergillus niger*	51	36	1.41
10	*Aspergillus* sp.NW	58	45	1.28
11	*Penicillium* sp.NW	44	31.3	1.40
12	*Alternaria sp.NW*	40	32	1.25

A= (Halozone + Colony) diameter, B= Colony Diameter.

VI.CONCLUSION

It is concluded from this study that wheat rhizosphere contains various types of phosphate solubilizing fungi; *Aspergillus* a nd *Penicillium* are found as dominant strains. *Aspergillus* species showed maximum phosphate solubilization and it can be used as potential phosphate biofertilizer for the cultivation of wheat and other crop plants. Further nursery and field trials are required to confirm its inoculation effects on different crop plants.

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