Isolation, identification and phosphate solubilization of fungi from soil in Jaipur district of Rajasthan

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Abstract
The soil act as a great reservoir of microorganisms, play different roles in the environment. Fungi is one of kind of microorganisms that have a major role in converting the dead and decaying matter into organic substances which are further used as fertilizers and increase the soil nutrients. The aim of the present study is to isolate and identify fungal species present in different soil samples of Jaipur, Rajasthan. The isolated fungal species were also subjected to phosphate solubilization activity which was calculated by solubilization index (SI) value. A total of ten fungal species including *Aspergillus niger, Aspergillus nidulans, Aspergillus flavus, Aspergillus fumigatus, Fusarium oxysporum, Rhodotorula mucilaginosa, Rhizopus stolonifer, Sepedonium chrysospermum, Penicillum expansum,* and *Cladiosporium sphaerosperimum* were isolated from different locations on the basis of morphological and microscopic characterization. The results obtained for phosphate solubilization; it was observed that all the isolated fungal species showed halo zone around the fungal growth with SI value varying from 1.0 to 1.4. Phosphate-solubilizing saprophytic fungi have a potential application in plant nutrition as phosphate is a macronutrient and play a vital role in every aspect of plant growth and development. With the help of phosphate solubilizing fungi present in soil, it can hydrolyze organic and inorganic insoluble phosphorous compound to soluble ones that can easily be assimilated by plant therefore can be used in bio fertilizer industries.

Keywords: Soil, Fungi, Microscopy, Phosphate solubilization, Solubilization index

INTRODUCTION

Several microorganisms of plants and herbs that can produce the CO_2 and nitrogen cycle found in the soil as a reservoir. The soil ecology is greatly influenced by the microorganisms. By nutrient recycling, biological control, and the breakdown of organic matter, microbial diversity and function alter the quality of the soil (Stefanis *et al.*, 2013). The soil is an oligotrophic substrate for the fungi to develop because the fungi's growths are usually quite confined, easily accessible, and present for only brief durations in a small zone (Ratnakumar *et al.*, 2015).

Fungi spend the most of their time either dormant or slowly growing while metabolizing a variety of chemical compounds. Organic debris is dispersed by fungi far from the roots. The majority of microorganisms are typically found near to the surface of roots (rhizosphere) and hyphae of arbuscular mycorrhizal fungus (myco-rhizosphere), where exudates are a crucial source of organic energy coming from soils (Dick, 2009 and Kirk, 2004). Over 32,000 different species of Ascomycetes, the biggest class of fungi, are known to exist. In natural circumstances, carbon, nitrogen, vitamins, and other necessary trace components influence spores develop. It has been shown that microorganisms play a crucial part in the fertility of the soil; their abundance and survival exclusively rely on the state of the eco-system (Lowenfels and Lewis, 2006). In addition to being attractive, fungi are also useful to humans in a variety of ways, including industry, agriculture, medicine, food industry, textiles, bioremediation,

natural cycling, and as bio-fertilizers. The advancement of fungus biotechnology has significantly impacted human welfare (Karthikeyan *et al.,* 2014).

The majority of plants benefit from fungus because they prevent plant root illnesses and make plants healthier by using fungal enzymes to combat plant infections. In order to lessen rivalry, fungi also produce antibodies that prevent the growth of competing bacteria. Many vitamins that they make help plants flourish. In order to safeguard the host plants, beneficial fungus also construct protective webs and nets around the roots and leaves (Sylvia *et al.*, 2005). By providing a protective health to give water and phosphorus to the plant roots during droughts, fungi also defend plants (Magdoff and VanEs, 2009).

It is challenging to preserve the interaction between biodiversity and soil fungi due to the change in global temperature and human interference of ecological processes. The largest species, saprophytes, play a significant role in the breakdown of plant polymers like cellulose, lignin, and hemicelluloses.

There are some fungi that are widely spread in the soil, but there are also some that are scarce. The primary factor in the biased distribution of organisms is soil fertility. In the present study different soil samples were collected from parts of Jaipur, Rajasthan and subjected for fungal isolation and were characterized through morphological identification (Macroscopic and Microscopic identification) and screened using phosphate solubilizing screening test.

MATERIAL AND METHOD

Sample collection

Soil samples were collected as of the ten diverse zones of Jaipur district of Rajasthan including Agra Road, Brahmpuri, Dravyavatti river, Gopalpura, Kalyanpuri Colony, Muhana Mandi, Mahapura, Riico Industrial Area, Sanganer Dye Textile Area and Transport Nagar. The collection time of soil samples were in the months of January 2017 to May 2017. The sample were collected from 8-10cm depth using a sterile spatula and transferred to sterilized polybags. Each bag of sample was attached a tag suitably by marked of the collection sites, collection time, collection date or collection place of collected area. The ready soil samples were then allowed to taken in the laboratory for future experiments.

Figure 1: Collection of soil samples from different areas of Jaipur district.

Preparation of media to isolated fungi

The potato dextrose agar (PDA) media was used to grow fungi successfully. In this regard 250 gram of potato, 20gram dextrose in 1000ml distilled water (Aina *et.al*. 2011) was arranged. pH adjusted with 7.0. The media was exaggerated with 15% agar and allowed to keep for 15 min in autoclave at 15 psi. Sterilized glass-plates were used to poured autoclaved containing media under aseptic condition and allowed to solidify.

Isolation and Enumeration of isolated fungi

Potato dextrose agar media was used for isolation and enumerated the fungi from different soil sample by soil dilution

method (Waksman, 1922). In this method soil dilutions were made by suspends 1 gram soil of every sample in 10ml of sterilized in distilled water. Dilution 10^{-3}, 10^{-6} was carried to isolate of fungi from soil sample. The selected dilution factor was 10^{-3} for future experiments. Equally 1ml of microbial suspension of each dilution factor concentrations were add to hygienic plates in triplicates of every dilution that contain autoclaved PDA media and spread by sterilized spreader. 1mg/ml concentration of ciprofloxacin solution was added before pouring to prevented bacterial growth. The plates were allowed to incubated at 28˚C for 7 seven days.

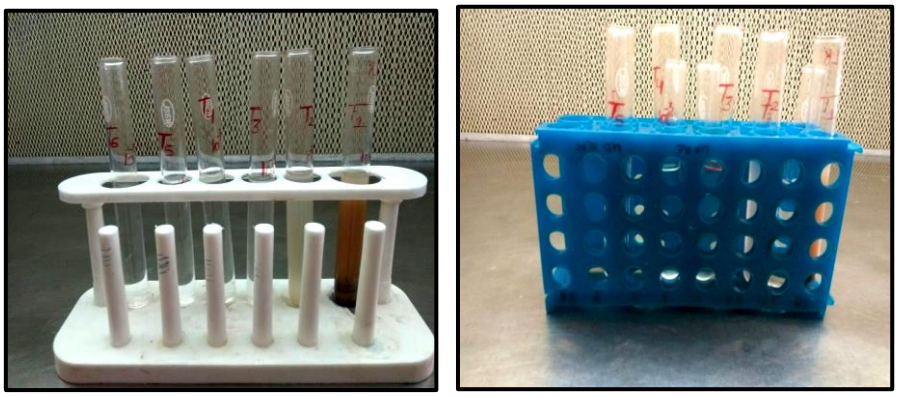


Figure 2: Isolation of fungi from the soil sample

Inoculums preparation

PDA broth was made to transfer the fungal colony into the potato dextrose broth, by aseptically punching out 5mm of the agar plate culture with a cutter. In addition of this added 24-gram potato dextrose broth in 1000-liter distilled water and mixed well. Heated and boiled 1 minute to dissolved powder. Autoclaved at 121˚C for 15 minutes (Mac Faddin, 1985). The pH was adjusted at 3.5, then allowed to cool the base to 45-50˚C and add suitable sterile amount 10% of tartaric acid and mixed well. The inoculums were from time to time tested at every 24 hours for various growth parameters.

Macroscopic and Microscopic identification of isolated fungi

In macroscopic the colony features color, shape, size, mycelia, and hyphae of fungal morphology were identified and in microscopic lactophenol cotton blue and compound microscope were used to observe. Staining procedure was used to isolated and identified of fungi. Potato dextrose agar media used to grow of fungi and morphology of fungi was examined by lacto phenol staining procedure. In this method lacto phenol cotton blue and standard cover-slip were used. Forceps was used to taken out the cover slip and allowed to set in inverted position. A pinch fall of lacto phenol cotton blue stain spread on the slide and visualized under the compound microscope. The characterization or identification of isolated fungi was ended by staining procedure. PDA media used to grow fungal colony and the morphologically analysis was done by used average cover slip method and lacto phenol cotton blue. The morphologically identification were done by the study of shape, hyphae, size and color. A small portion of the mycelium was also help to study the identification of fungi under compound microscope (Gaddeyya *et al*., 2012).

Phosphate solubilizing screening test

The screening test of isolated fungi was based on plate assay. For screening of phosphate solubilizing test Pikovskaya's media was used (Pikovskaya, 1948). It allowed to add extract of yeast 0.50 (g/l), dextrose 10.00 (g/l), calcium phosphate 5.00 (g/l), ammonium sulphate 0.50 (g/l), potassium chloride 0.20 (g/l), magnesium sulphate 0.10 (g/l), manganese sulphate 0.0001 (g/l), ferrous sulphate 0.0001(g/l).

Prepared medium was adjusted to pH 7.0. The prepared media was exaggerated by agar 1.5% and keep in autoclave at 15 psi. for 15 min. Poured form was allowed to solidify. Inoculation of fungal colonies on plates done properly and incubated in inverted position at 28°C at 72 hours. Positive culture was examined by detect halo zone in Pikovskaya's medium.

Pure cultures of phosphate solubilizing fungal species

Sub cultured was done of positive fungal colonies on sterilized plates for plate assay. To isolate fungal cultures the plates were allowed to incubate in inverted position at 28°C for 72 hours. The test was examined by observe halo zone of positive culture in the medium of Pikovskaya's because of insoluble tricalcium phosphate solubilized in the soluble form.

Solubilization index (SI)

Each PSF preserved culture of 200 mg-500 mg ml in sterilized water were positioned on prepared medium (Pikovskaya, 1948). The glass petri-dishes were allowed to incubate for as a minimum 5 to 7 days. The Index of solubilization was considered by given principle (Edi-Premono *et al*., 1996).

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

RESULT AND DISCUSSION

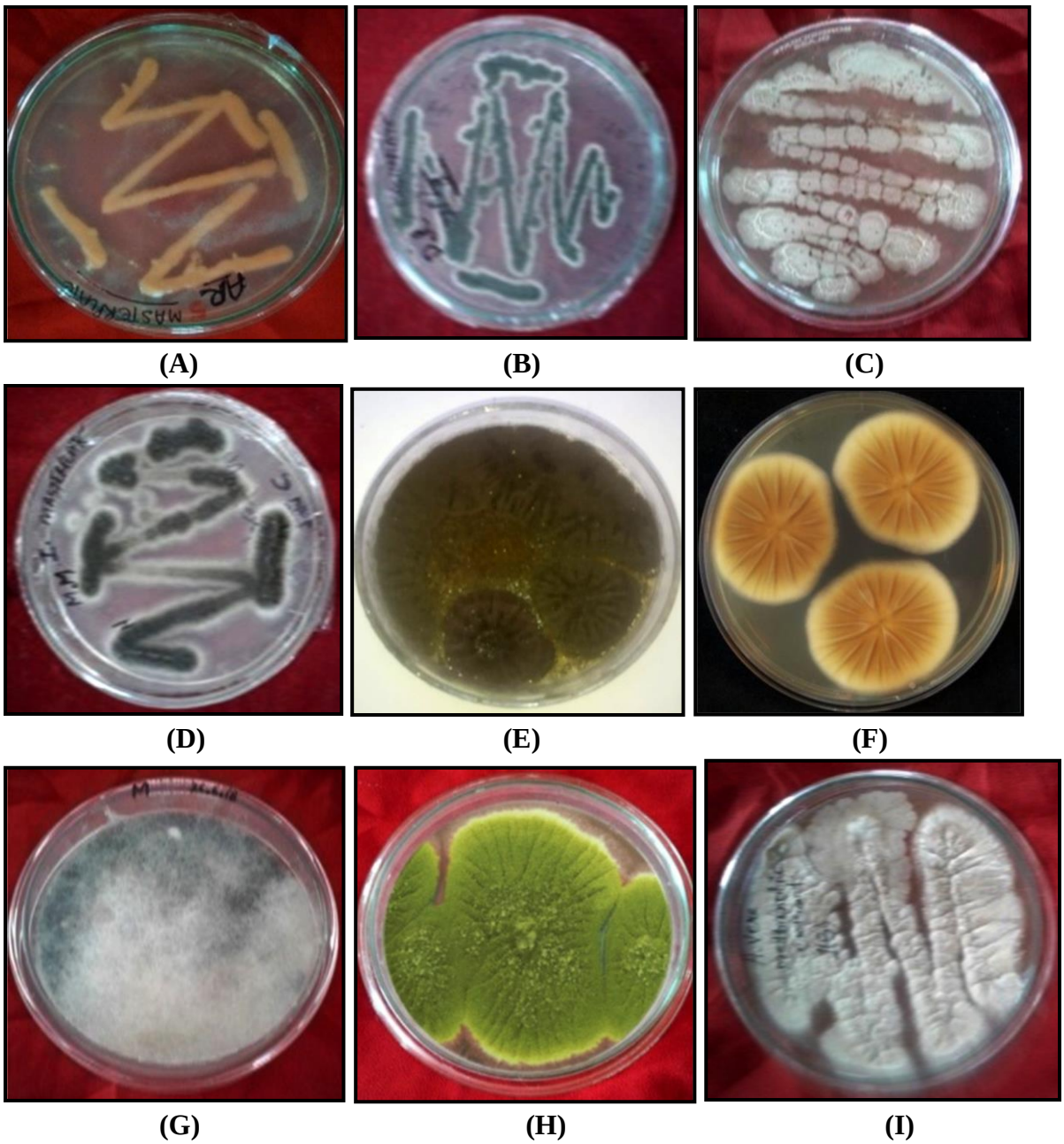
Culture of isolated pure fungal culture

The ten diverse groups of fungi were enumerated and allowed to culture on potato dextrose media (PDA) to obtained pure culture of *Aspergillus niger, Aspergillus nidulans, Aspergillus flavus, Aspergillus fumigatus, Fusarium oxysporum, Rhodotorula mucilaginosa, Rhizopus stolonifer, Sepedonium chrysospermum, Penicillum expansum,* and *Cladiosporium sphaerosperimum*. In a study of Islam *et al.,* 2019 isolated fungal species form subtropical soils for environment friendly biofertilizer development. Sixteen fungal strains were isolated and identified as *Aspergillus* spp., *Penicillium* spp. and *Talaromyces* spp. Also in 2020, Wang *et al.,* isolated fungal species from the soil samples collected from the corn farm in Jiagedaqi region in China. The strains isolated were identified as P*enicillium oxalicum.* Benguenab and Chibani, 2021 isolated two filamentous fungi namely *Aspergillus ustus* and *Purpureocillium lilacinum* from the engine oil contaminated soil of Kherrouba, Mostaganem State, Algeria.

Table 1: Enumeration of total soil borne fungal species

Name of the place	**Enumeration of total soil borne fungus present in different zones**
Agra road	*Aspergillus fumigates, Aspergillus niger, Rhodotorula mucilaginosa*
Brahmpuri Road	*Aspergillus nidulans, Aspergillus niger, Fusarium oxysporum*

Dravyavatti River	*Aspergillus fumigatus, Penicillium expansum*
Gopalpura	*Aspergillu niger, Aspergillus fumigatus*
Kalyanpuri Colony	*Aspergillus fumigatus, Aspergillus niger*
Muhana Mandi	*Cladiosporium sphaerosperimum, Aspergillus niger*
Mahapura	*Rhizopus stolonifer*
Riico Industrial Area	*Aspergillus flavus*
Sanganer Dye Textile Area	*Fusarium oxysporium*
Transport Nagar	*Sepedonium chrysospermum*



(A) **(B)** **(C)**

(D) **(E)** **(F)**

(G) **(H)** **(I)**

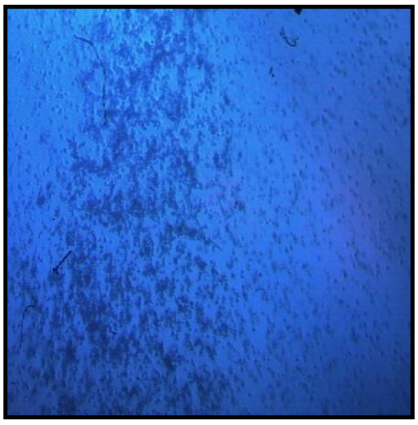
(J)

Figure 3: Pure culture of isolated fungi: A) *Rhodotorula mucilaginosa*, **B)** *Aspergillus nidulans*, **C)** *Aspergillus fumigatus*, **D)** *Aspergillus niger*, **E)** *Cladiosporium spharosperimum*, **F)** *Penicillium expansum*, **G)** *Rhizopus stolonifer*, **H)** *Aspergillus flavus*, **I)** *Fusarium oxysporium*, **J)** *Sepedonium chrysospermum*

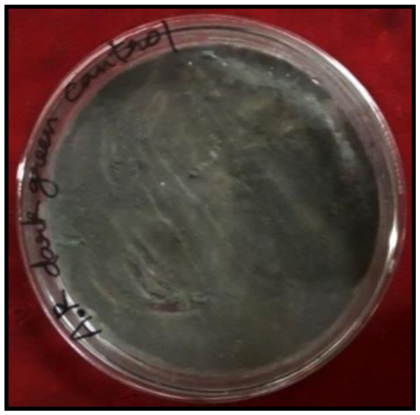
Morphological identification of isolated fungi

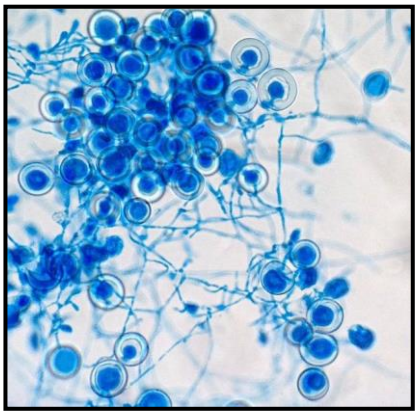
Isolated fungi were identified by their microscopic and macroscopic characterization according to their color, shape, types of the fungal species, mycelium and by spores in Table 2.

Table 2: Morphological characterizations of isolated fungi by microscopic examination

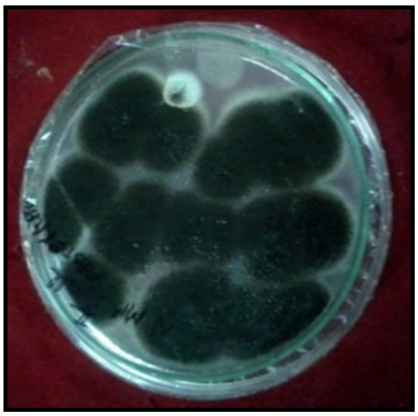
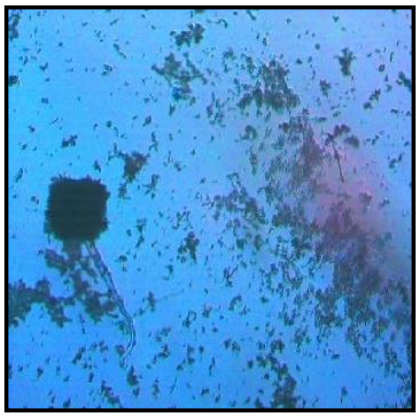


Rhodotorula mucilaginosa

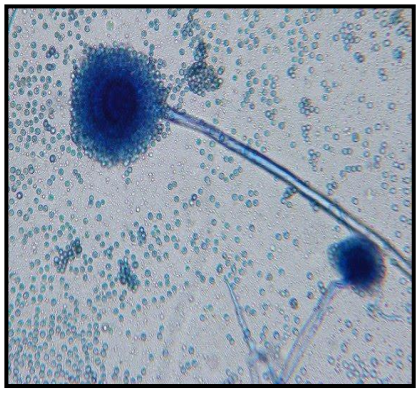




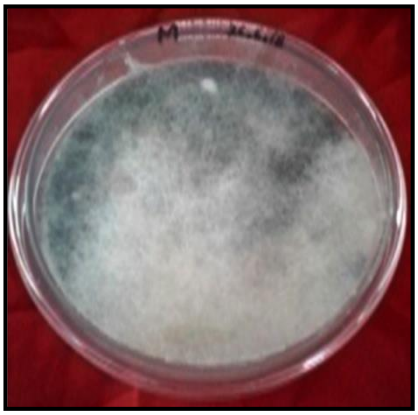
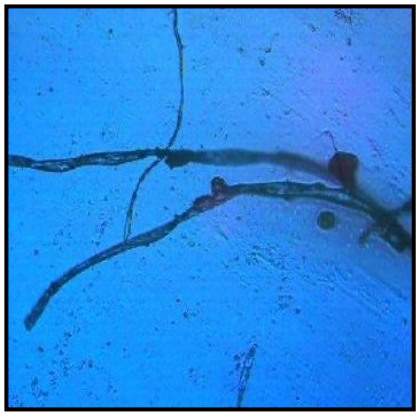
Aspergillus nidulans

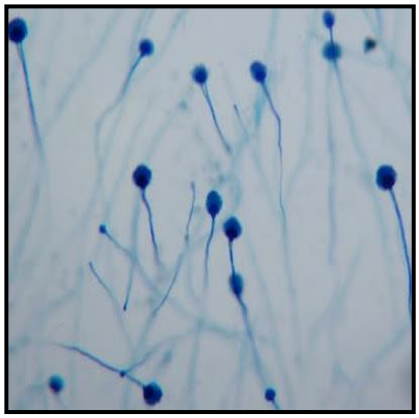
Aspergillus niger

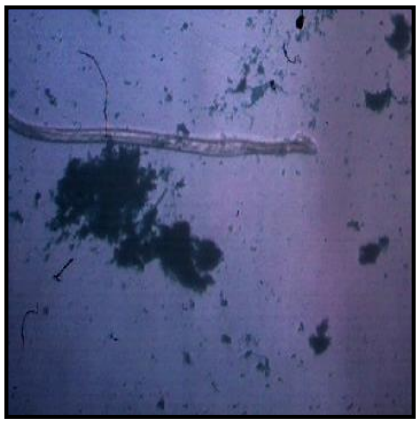
Aspergillus fumigates

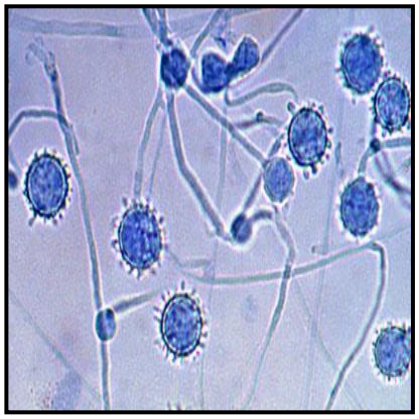
Rhizopus stolonifer



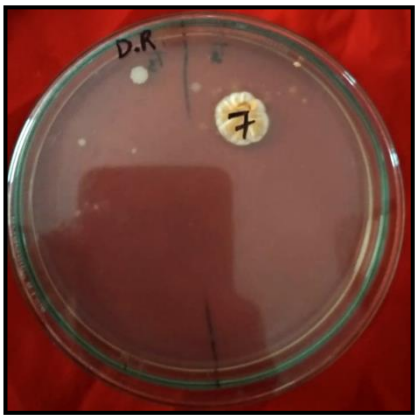
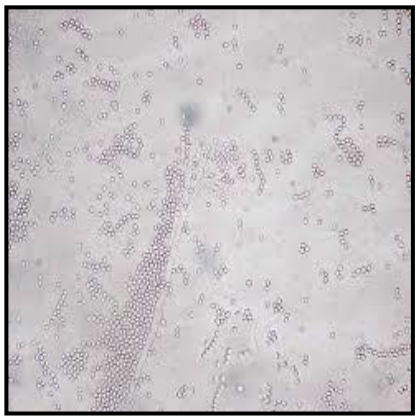
Aspergillus flavus

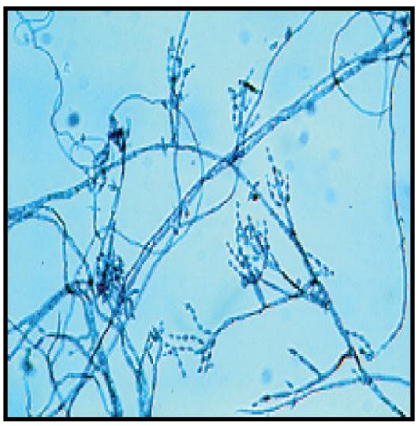
Fusarium oxysporium

Sepedonium chrysospermum

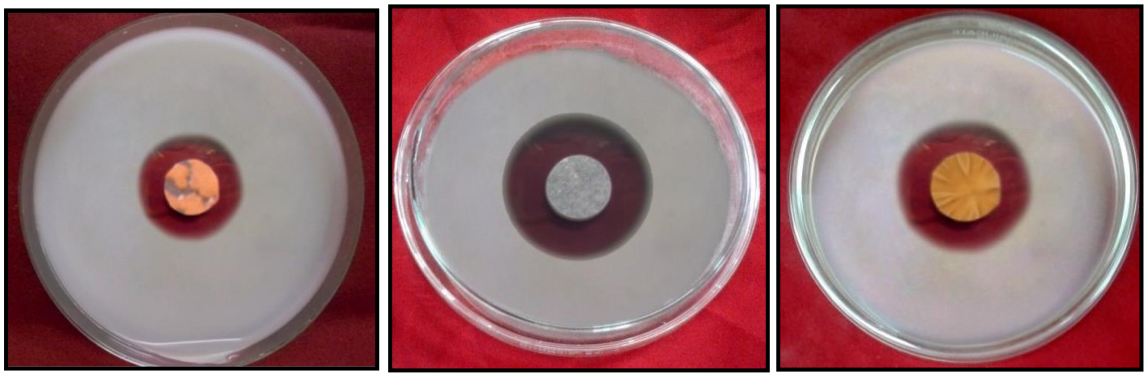
Penicillium expansum

Cladiosporium sphaerosperimum

Screening test of phosphate solubilization of isolated fungi

Phosphate solubilization screening test for fungi were identified. The results showed in Figure 4 that all different fungi included *Aspergillus niger, Aspergillus nidulans, Aspergillus flavus, Aspergillus fumigatus, Fusarium oxysporum, Rhodotorula mucilaginosa, Rhizopus stolonifer, Sepedonium chrysospermum, Penicillum expansum,* and *Cladiosporium sphaerosperimum* showed halo zone around the fungal growth. The solubilized indexes of different fungi were recorded between 1.0-1.4 and mentioned in Table 3. Also, Islam *et al.,* 2019 study focused to isolate and identify potential phosphate solubilizing fungi from subtropical soils for environment friendly biofertilizer development and isolated sixteen fungal strains identified as *Aspergillus* spp., *Penicillium* spp. and *Talaromyces* spp. from subtropical dark red soil, red soil and grey soil based on phosphate solubilization index and morphological studies. Remarkably, *Aspergillus niger* isolates (strain SI-10URAgr, SI-11URAgr and SI-12URAgr) had marked phosphate solubilization ability regardless of the substrates followed by *Penicillium oxalicum* and *Talaromyces pinophilus*. Likewise, in the study of Elfiati *et al.,* 2021 collected peat soil samples from the peat ecosystem in Nagasaribu Village, North Sumatra, Indonesia. They isolated soil samples to obtain phosphate-solubilizing fungi using the Pikovskaya selective medium and were tested for their ability to dissolve phosphate qualitatively by calculating the dissolution index values and quantitatively by calculating the available phosphorus on Pikovskaya medium. Total of 12 isolates of phosphate-solubilizing fungi was obtained during the study and the isolates were able to dissolve phosphate in the value range from 17.77 ppm to 69.86 ppm and the qualitative test obtains dissolution index values that vary from 2.55 to 4.25. The top five fungal isolates with highest phosphate-solubilizing potential were identified as *Aspergillus niger* based on molecular identification. In 2020, Wang *et al.,* isolated *Penicillium oxalicum* fungal strain from the soil samples of a corn farm in Jaigedaqi region, China and found high phosphate solubilization activity of it. A high soluble phosphorus content was achieved with 529.0 µg/ml, 514.0 µg/ml, and 330.7 µg/ml for *Penicillium oxalicum*.



(A) **(B)** **(C)**

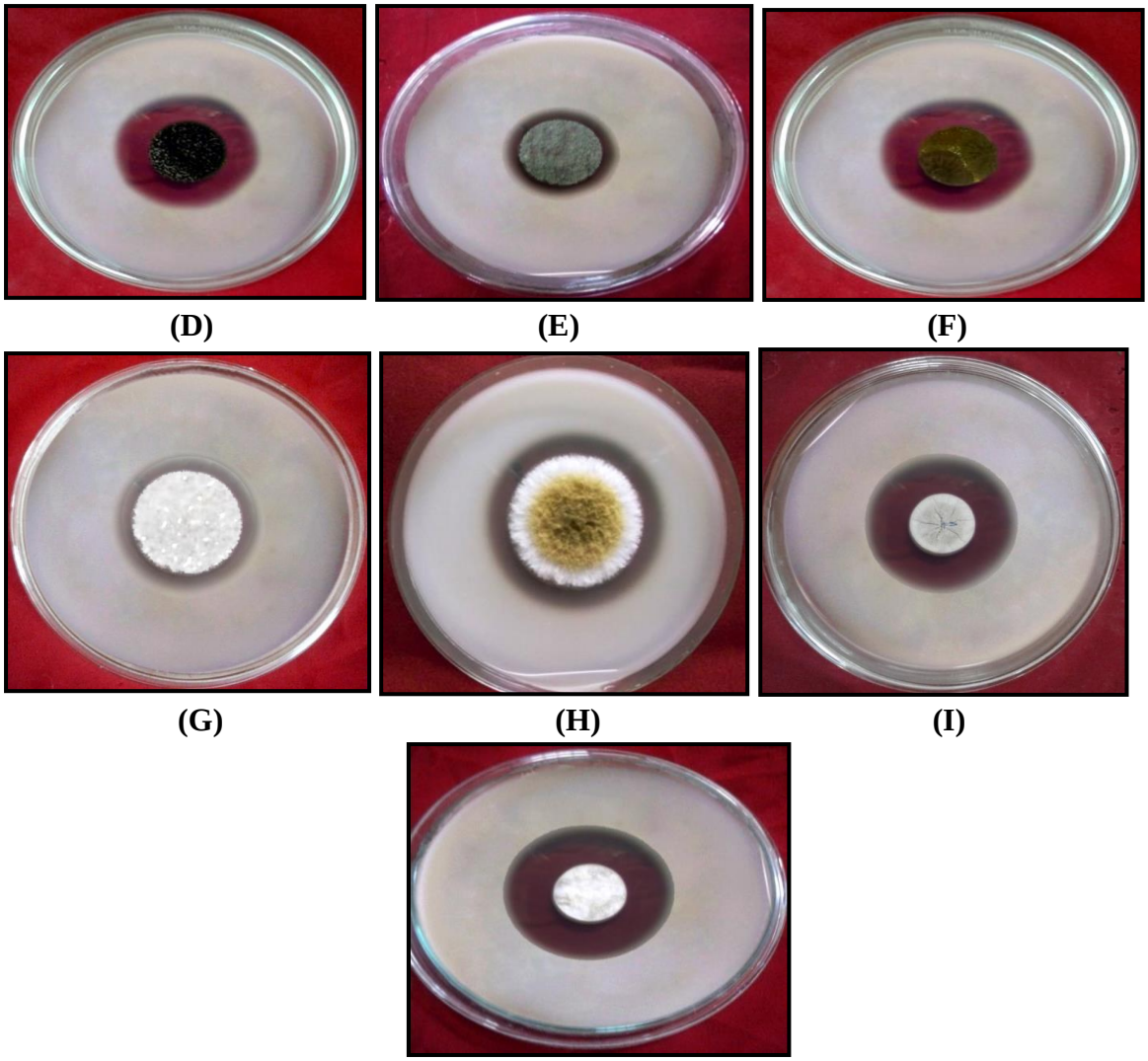


Figure 4: Screening of phosphate solubilizing fungus: A) *Rhodotorula mucilaginosa,* **B)** *Aspergillus nidulans,* **C)** *Penicillium expansum,* **D)** *Aspergillus niger,* **E)** *Aspergillus fumigatus,* **F)** *Cladiosporium sphaerosperimum,* **G)** *Rhizopus stolonifer,* **H)** *Aspergillus flavus,* **I)** *Fusarium oxysporum,* **J)** *Sepedonium chrysosperimum*

Table 3: Investigation of solubilized indices of fungal isolated

Isolated Fungus	**SI**
Rhodotorula mucilaginosa	1.0 ± 0.25
Aspergillus nidulans	1.0 ± 0.32
Penicillium expansum	1.2 ± 0.15
Aspergillus niger	1.4 ± 0.18
Aspergillus fumigates	1.1 ± 0.35
Cladosporium sphaerosperimum	1.1 ± 0.28

Rhizopus stolonifera	1.1 ± 0.37
Aspergillus flavus	1.2 ± 0.13
Fusarium oxysporum	1.4 ± 0.22
Sepedonium chrysospermum	1.4 ± 0.34

SI= Soulibilization index

CONCLUSION

In the present study, fungal species were isolated from the soil samples collected from different locations in Jaipur, Rajasthan. They were then subjected to morphological and microscopic identification and later screened through phosphate solubilization test. Total ten fungal species were isolated from the soil samples collected in which *Aspergillus* species was found maximum and the fungal species gave a solubilization index between 1.0 to 1.4. From the present study it can be concluded that the fungus plays a vital role in soil nutrient balance and can be used as bio-fertilizers as they hydrolyze insoluble phosphorus into soluble one and make available for the plants to uptake for their growth and development.

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