

Research article

Isolation and screening of phosphate solubilising fungi from Okra rhizosphere soil and their effect on the growth of Okra plant (*Abelmoschous esculentus* L.)

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Abstract: Soil microbiota plays an important role in enhances the available nutrients in the soil for plants. In this study twenty-two filamentous fungi were isolated from the Okra rhizosphere soil. Based on primary screening for phosphate solubilizing potential on solid Pikovskaya's medium ten isolates were selected for quantitative estimation in liquid medium amended with Tricalcium phosphate (TCP) and Rock Phosphate (RP). *Aspergillus niger* strain (AMB) solubilized maximum TCP & RP and release 295.1 & 221.8 $\mu\text{g ml}^{-1}$ inorganic-P, while *Aspergillus japonicas* released maximum P in liquid media amended with TCP (298.5 $\mu\text{g ml}^{-1}$). The other tested isolates *Penicillium* sp., two strains of *Trichoderma viride* and *Chaetomium globosum* released a significant amount of P in liquid media from 0.5% TCP&RP after ten days. Furthermore, the application of selected phosphate solubilizers in the form of consortium and in a combination of the recommended dose of fertilizers resulted good growth of Okra plants in compare to recommended dose of chemical fertilizers. This fungal consortium may serve as very good phosphate solubilizers and can be used as a biofertilizer.

Keywords: Phosphate solubilizers - Rhizosphere fungi - Biofertilizers - Fungal consortium.

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INTRODUCTION

Okra (*Abelmoschus esculentus* L. Moench) commonly known as Bhindi or Lady's finger belongs to the family Malvaceae is one of the main vegetables grown extensively throughout India. For good yield plants need balance and a sufficient dose of fertilizers (Rathava *et al.* 2018). The cultivation of Okra in and around the suburban part of Mumbai is mostly limited near the railway track lands. The soil is highly polluted and less fertile, but rich in phosphorus content due to several chemical industries located near the track. The rhizosphere soil of Okra plant has shown a varied group of microorganisms such as *Aspergillus* sp., *Penicillium* sp., *Fusarium* sp., etc. some of which have been reported potent phosphate-solubilizers and growth promoters (Prajapati *et al.* 2013).

Phosphorus is one of the important plant macronutrients that plays a major role in several physiological processes & metabolism and contribute in the growth and yield of crop plants. The majority of soils rich in insoluble inorganic phosphates, which are of no use to plants unless they are solubilized. Plants can absorb Phosphorus from soil solution in the form of phosphate anions. However, phosphate anions are very reactive and may be immobilized through precipitation with several cations and very quickly become crystalline. The crystalline P is insoluble and unavailable to plants. Phosphate solubilizing micro-organisms convert these insoluble phosphates into soluble form through the biochemical processes of acidification, chelation and exchange reaction (Starkanova *et al.* 1999, Narsian & Patel 2000, Reyes *et al.* 2002).

Many phosphate solubilizing bacteria, fungi and actinomycetes have been isolated from tropical as well as temperate soil (Gaur 1990, Chabot *et al.* 1993, Khalil 1995, Vassileva *et al.* 1998, Subba Rao 1999, Trivedi &

Pandey 2007, Pandey *et al.* 2008, Singh *et al.* 2011, Kasana *et al.* 2017). Fungi are important components of soil microbiota in all types of soil and colonize the rhizosphere region of plant. Rhizosphere microflora helps to plants in many way, they increase the fertility level of soil resulted in high yield of crops (Kundu *et al.* 2009, Prajapati *et al.* 2013). Several rhizosphere soil fungi like genus *Aspergillus* and *Penicillium* have been reported as good phosphate solubilizers (Asea & Kucey 1988, Gaur 1990, Vassilev *et al.* 1996, Narsian & Patel 2000, Pandey *et al.* 2008, Padmavathi & Usha 2012). These fungi have the potential to be used as biofertilizers. The objective of this study is to isolate potent phosphate solubilizers and make fungal consortium which can be served as biofertilizer to increase agricultural yields.

MATERIALS AND METHODS

Study sites and sampling

In the present study, soil samples were collected from the surface to 10 cm depth from Okra field near the railway track lands in the area around Kalyan, Thane- Mumbai during the 2018. The samples were placed in sterile plastic containers and stored at room temperature for further study. Four soil samples were collected from four different places and designated as AMB, UNR, KYN and DOV (Table 1). 1 g of soil sample was taken for the isolation of fungi by serial dilution method (Waksman 1916). Samples were serially diluted up to 10^{-7} and 0.2 ml suspension was used as inoculums, pour plate technique applied for the isolation of fungi on three different culture media: malt extract agar, corn meal agar and potato dextrose agar. To avoid bacterial growth 1ppm solution of streptomycin added in media. The plates were incubated at room temperature. The growing fungal colonies on different media having different morphological features were transferred on fresh PDA plates, purified and transferred onto potato dextrose agar slants for detailed study. Pure isolates were identified based on morphotaxonomy with the help of standard literature (Barnett 1960, Rapper & Fennell 1965, Ellis 1971, 1976, Von Arx 1974, Barron 1977, Pitt 1979, Carmichael *et al.* 1980, Gams *et al.* 1980, Kirk *et al.* 2008).

Table 1. Isolates obtained from different samples of rhizosphere soil of Okra plants.

S.N.	Name of Isolates	Sample AMB	Sample UNR	Sample KYN	Sample DOV
1	<i>Aspergillus niger</i> Tieghem	✓	✓	✓	✓
2	<i>Aspergillus terious</i> Thom & Raper	✓	✓	✓	✓
3	<i>Aspergillus aculiatu</i> s Iizuka	-	✓	✓	-
4	<i>Aspergillus japonicus</i> var. <i>japonicus</i> Saito	✓	-	-	-
5	<i>Aspergillus flavus</i> Link	-	✓	✓	✓
6	<i>Aspergillus wentii</i> Wehmer,	-	-	-	✓
7	<i>Emmericella nidulans</i> (Eidam) Vuill	-	-	-	✓
8	<i>Penicillium citrinum</i> Thom	✓	-	✓	-
9	<i>Penicillium chrysogenum</i>	-	-	-	✓
10	<i>Penicillium restrictum</i> J.C. Gilman & E.V. Abbott	✓	-	-	-
11	<i>Penicillium</i> sp.	✓	✓	✓	✓
12	<i>Pacelomyces variotii</i> Bainier	-	-	-	✓
13	<i>Trichoderma viride</i> Pers	✓	✓	✓	✓
14	<i>Trichoderma harzianum</i> Rifai	-	-	-	✓
15	<i>Trichoderma</i> sp.	✓	-	-	-
16	<i>Chaetomium globosum</i> Kunze	✓	-	-	✓
17	<i>Syncephalastrum</i> sp.	-	✓	-	-
18	<i>Rhizopus microsporus</i> (Cohn) Schipper & Stalpers.	✓	-	✓	✓
19	<i>Rhizoctonia</i> sp.	✓	-	-	-
20	<i>Chladosporium chladosporoides</i> (Fresen.) G.A. de Vries.	-	✓	✓	-
21	<i>Fusarium oxisporum</i> E.F. Sm. & Swingle	✓	-	-	-
22	<i>Fusarium solani</i> (Mart.) Saccardo	-	-	✓	-

Screening for Phosphate solubilizing ability

All pure and identified isolates of four soil samples were screened for their phosphate solubilizing activity on solid Pikovskaya's medium (Hi-media) by spot inoculation on the culture plate. Plates were incubated at room temperature for seven days (Pikovskaya 1948). A clear transparent zone around the colony indicated phosphate solubilization. After the primary screening, the positive isolates were selected for detailed study. The positive isolates were inoculated separately in the centre onto the solid Pikovskaya's medium and incubated at room temperature. Photograph of culture plates were taken and the Phosphate solubilization index was calculated using the following formula (Edi-Premono *et al.* 1996):

$$\text{Solubilizing index} = \frac{\text{Colony diameter clearing zone}}{\text{Colony diameter}}$$

Quantitative estimation of phosphate solubilisation in broth culture

Pikovskaya's broth medium (pH 7.2) was prepared; 100 ml media dispensed in 250 ml conical flasks for quantitative estimation of phosphate. Insoluble phosphate in the form of TCP & Rock Phosphate (500 mg) were added separately to each flask and was then sterilized at 15 lb pressure for 20 min. Ten-day-old culture grown on PDA medium was used as an inoculum source and 1.0 ml of spore suspension of the isolates was inoculated in triplicate. An un-inoculated flask containing 100 ml media was maintained as a control. Flasks were kept on a rotary shaker at 140rpm and room temperature for seven days. After incubation, the contents of each flask were filtered through Whatman filter paper grade 42. Water-soluble phosphorus was measured using the chlorostanous-reduced molybdophosphoric acid blue method (Jackson 1973).

Preparation of fungal consortium of Phosphate solubilizers

Based on phosphate solubilizing potential of isolates, some of isolates were shortlisted for their further use as biofertilizers. The shortlisted isolates were grown on agricultural waste (Ground nut shell) separately. Mass multiplication of isolates on agricultural waste was done in plastic bags. Sterilized agricultural waste 1kg inoculated with 5ml of spore suspension of respective isolates and kept for incubation at RT, once mycelium of fungus colonized the substrate at this stage appropriate amount of colonized organic waste used as biofertilizer. Okra was selected for trial for fungal phosphate solubilizers consortium to assess their effect on growth in pots. Surface sterilized seeds were shown in pots filled with soil and amended consortium @5%. The other treatments were also planned for a comparative study like one set kept as control; next treatment were recommended dose of chemical fertilizers and soil amended with compost and fungal phosphate solubilizers consortium @5%. All treatments were set in triplicates. After germination and thirty days of growth the physical parameters of plants were recorded.

RESULTS

Isolation and Identification of Fungi

In total 42 isolates were obtained during isolation of fungi from Okra rhizosphere soil samples collected from four different localities. Based on morphological identification, they belong to 21 species under 11 genera (Table 1). The taxa isolated from the soil samples are listed in table 1. The most common genus was *Aspergillus* with six species, which were found in soil samples of all four localities (AMB, UNR, KYN and DOV). *Aspergillus niger* was more dominant species. *Penicillium* sp. and *Trichoderma viride* were also commonly found in all samples. The highest number of fungal isolates obtained from AMB, while the lowest number was from UNR. Two known pathogenic genus viz., *Rhizoctonia* sp. and *Fusarium* also obtained from rhizosphere soil sample of AMB.

Screening

Screening of all isolates for their P solubilising ability on solid Pikovskaya's media revealed that among 42 isolates 24 isolates (Table 2) were found to be P solubilizers and they were marked (+, ++, +++) their potential. The phosphate solubilizing isolates obtained from different samples are given in table 2. The maximum number of P solubilizers were obtained from sample AMB and DOV (8 isolates from each) while four isolates were showed P solubilization potential from sample UNR & KYN.

Table 2. Primary screening of isolates for their Phosphate solubilising activity on Pikovskaya's media.

S.N.	Name of Isolates	Sample AMB	Sample UNR	Sample KYN	Sample DOV
1	<i>Aspergillus niger</i> Tieghem	+++	+	++	+++
2	<i>Aspergillus terious</i> Thom & Raper	-	-	-	-
3	<i>Aspergillus aculiatus</i> Iizuka	-	++	++	-
4	<i>Aspergillus japonicas</i> var. <i>japonicus</i> Saito	+++	-	-	-
5	<i>Aspergillus flavus</i> Link	-	-	-	++
6	<i>Aspergillus wentii</i> Wehmer,	-	-	-	-
7	<i>Emericella nidulans</i> (Eidam) Vuill	-	-	-	-
8	<i>Penicillium citrinum</i> Thom	++	-	+++	-
9	<i>Penicillium chrysogenum</i>	-	-	-	++
10	<i>Penicillium restrictum</i> J.C. Gilman & E.V. Abbott	-	-	-	-
11	<i>Penicillium</i> sp.	-	-	-	++
12	<i>Pacelomyces variotii</i> Bainier	-	-	-	+

13	<i>Trichoderma viride</i> Pers	+++	+++	+	-
14	<i>Trichoderma harzianum</i> Rifai	-	-	-	++
15	<i>Trichoderma</i> sp.	+++	-	-	-
16	<i>Chaetomium globosum</i> Kunze	++	-	-	+++
17	<i>Syncephalastrum</i> sp.	-	+++	-	-
18	<i>Rhizopus microsporus</i> (Cohn) Schipper & Stalpers.	-	-	--	++
19	<i>Rhizoctonia</i> sp.	+++	-	-	-
20	<i>Chladosporium chladosporoides</i> (Fresen.) G.A. de Vries.	-	-	-	-
21	<i>Fusarium oxisporum</i> E.F. Sm. & Swingle	++	-	-	-
22	<i>Fusarium solani</i> (Mart.) Saccardo	-	-	-	-

Note: +poor, ++ moderate, +++ high.

The isolates showed high ability were shortlisted (Table 3; Fig. 1) and P solubilizing index was calculated at three different temperatures. Table 3 revealed that most of isolates showed maximum solubilisation index at $35\pm 2^\circ\text{C}$, while *Aspergillus niger* strain (DOV-2.11), *Penicillium citrinum* (2.0) and *Chaetomium globosum* (2.30) were exhibited maximum P solubilising index at $40\pm 2^\circ\text{C}$. Both *Trichoderma viride* strain showed minimum P solubilising index (1.2 & 1.0) at $40\pm 2^\circ\text{C}$. Phosphate solubilization in Pikovskaya's broth medium using two types of inorganic phosphates, tricalcium and rock for selected isolates were analysed quantitatively. *Aspergillus japonicas* showed maximum solubilization of phosphorous ($298.5 \mu\text{g ml}^{-1}$) followed by *Aspergillus niger* isolate AMB & DOV (285.1 and 286), *Penicillium* sp. strain AMB ($284.5 \mu\text{g ml}^{-1}$), *Trichoderma viride* strain UNR ($282.6 \mu\text{g ml}^{-1}$) and *Chaetomium globosum* ($278.54 \mu\text{g ml}^{-1}$) whereas *Syncephalastrum* sp. showed minimum of ($180 \mu\text{g ml}^{-1}$) of phosphorous solubilization when the media were supplemented with tricalcium phosphate. When the medium was supplemented with rock phosphate, *Aspergillus niger* strain DOV & AMB were exhibited maximum of 225 & $221.80 \mu\text{g ml}^{-1}$ phosphorous solubilisation, followed by *Aspergillus japonicas* ($220 \mu\text{g ml}^{-1}$) and *Trichoderma viride* strain AMB ($212.5 \mu\text{g ml}^{-1}$). *Syncephalastrum* sp. showed a minimum of $156.4 \mu\text{g ml}^{-1}$ phosphorous solubilization (Table 4).

Table 3. Phosphate solubilizing index of selected isolates at different temperature on Pikovskaya's media.

S.N.	Name of Isolates and their code	Phosphate SI Index ($28\pm 2^\circ\text{C}$)	Phosphate SI Index ($35\pm 2^\circ\text{C}$)	Phosphate SI Index ($40\pm 2^\circ\text{C}$)
1	<i>Aspergillus niger</i> Tieghem (AMB)	1.730	2.088	1.75
2	<i>Aspergillus niger</i> Tieghem (DOV)	2.00	2.10	2.11
3	<i>Aspergillus japonicas</i>	1.85	2.07	1.80
4	<i>Penicillium citrinum</i> Thom	1.56	1.81	2.0
5	<i>Penicillium</i> sp. (AMB)	1.45	1.73	1.7
6	<i>Trichoderma viride</i> Pers (AMB)	2.05	2.07	1.2
7	<i>Trichoderma viride</i> Pers (UNR)	1.80	1.85	1.00
8	<i>Trichoderma</i> sp.	1.43	1.72	1.52
9	<i>Chaetomium globosum</i>	1.92	2.25	2.30
10	<i>Syncephalastrum</i> sp.	1.40	1.85	1.32

Table 4. Quantitative estimation of phosphorus solubilization by fungal isolates in Pikovskaya's liquid medium amended with Tricalcium phosphate and Rock Phosphate.

S.N.	Name of Isolates and their code	Tri calcium Phosphate solubilised ($\mu\text{g ml}^{-1}$)	Rock Phosphate solubilised ($\mu\text{g ml}^{-1}$)
1	<i>Aspergillus niger</i> (AMB)	295.1	221.80
2	<i>Aspergillus niger</i> (DOV)	286	225.0
3	<i>Aspergillus japonicas</i>	298.5	220.0
4	<i>Penicillium citrinum</i>	235.2	194.0
5	<i>Penicillium</i> sp. (AMB)	284.5	190.5
6	<i>Trichoderma viride</i> (AMB)	268.0	212.5
7	<i>Trichoderma viride</i> (UNR)	282.6	197.2
8	<i>Trichoderma</i> sp.	230.8	200.0
9	<i>Chaetomium globosum</i>	278.54	210.3
10	<i>Syncephalastrum</i> sp.	180.0	156.4

Effect of Phosphate solubilizers on test plat Okra

Based on quantitative estimation of phosphate solubilized by isolates, five best isolates viz., *Aspergillus niger* strain AMB, *Aspergillus japonicas*, *Penicillium* sp., *Trichoderma viride* strain UNR and *Chaetomium globosum* were selected for making fungal consortium for application as biofertilizer for cultivation of Okra. The fungal consortium (mixed culture of all five isolates) was tested on Okra plant against other treatment like www.tropicalplantresearch.com

recommended dose of chemical fertilizer and Chemical Fertilizer with fungal consortium in pots (Table 4). The effect of consortium and other treatments on Okra plants in terms of height, number of nodes, fresh weight of plant and dry weight of plants were recorded in triplicate after thirty days of showing, test of significance calculated and given in table 5. Pots supplemented with fungal consortium@5% and Chemical fertilizers showed the maximum response in all attributes at the end of 30 days. It was observed that pots supplemented with fungal consortium showed very good response in compare to control and chemical fertilizers.

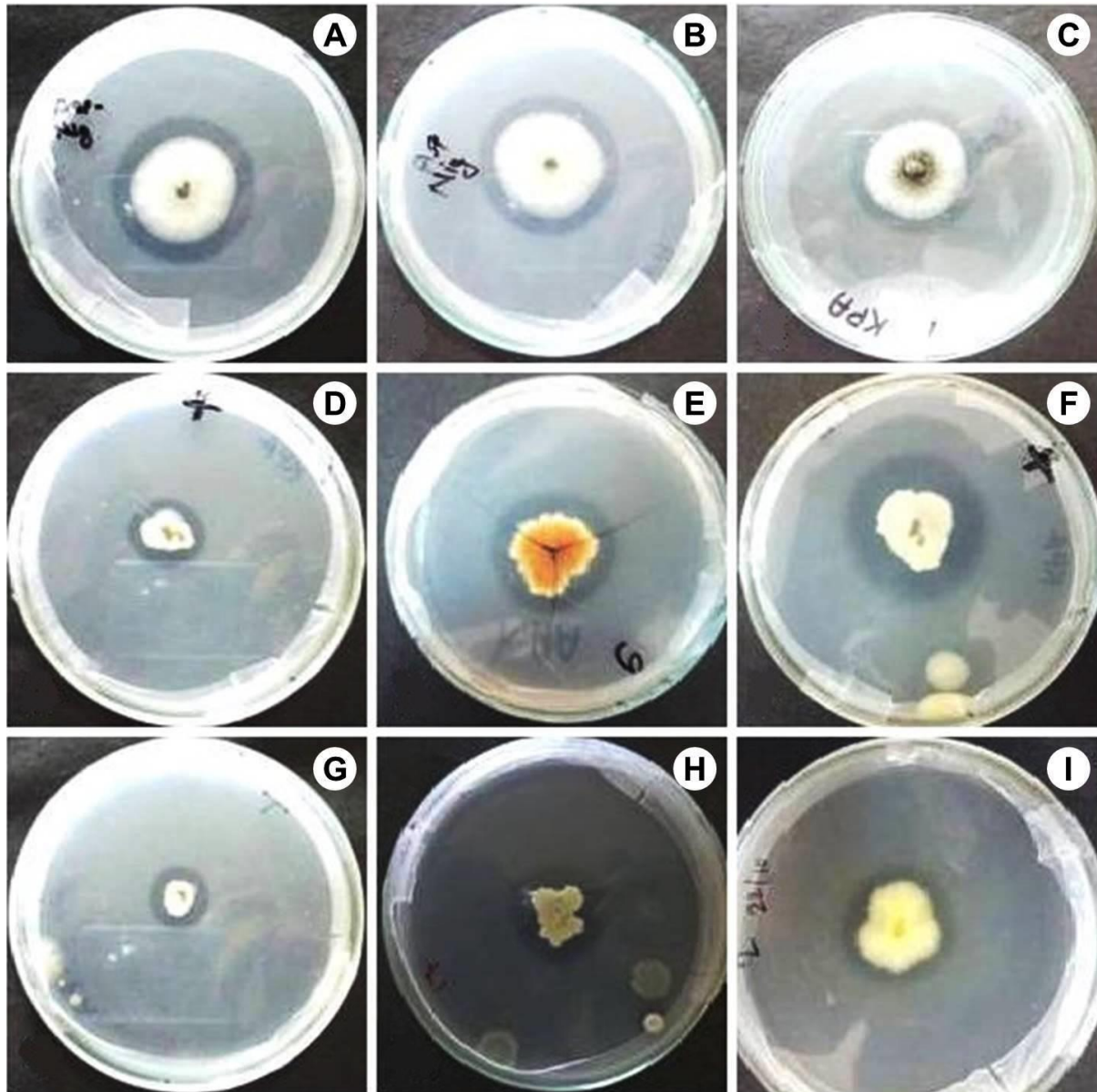


Figure 1. Phosphate solubilizing activity of isolates at room temperature: **A**, *Aspergillus niger* strain (AMB); **B**, *Aspergillus niger* strain (DOV); **C**, *Aspergillus japonicas*; **D**, *Penicillium citrinum*; **E**, *Penicillium* sp. Strain (AMB); **F**, *Trichoderma viride* strain (AMB); **G**, *Trichoderma viride* strain (UNR); **H**, *Chaetomium globosum*; **I**, *Syncephalustrum* sp.

Table 5. Effect of phosphate solubilising fungal consortium on growth of Okara Plant after 30 days of showing.

Treatments	Height of plant (cm)	No. of node per plant	Fresh weight per plant (gm)	Dry weight per plant (gm)
Control	14.6	3.6	8.3	3.3
Chemical Fertilizers	19.0	5.2	30.27	10.9
Soil amended with fungal consortium@5%	17.8	5.6	30.2	10.0
Chemical fertilizers+ fungal consortium @ 5%	22.01	6.1	36.78	12.0
F-test	Sig.	Sig.	Sig.	Non-sign
SE	0.60	0.16	2.2	-
CD@5%	3.72	3.5	3.0	-

DISCUSSIONS AND CONCLUSION

Present study showed that almost fifty percent of isolates from Okra rhizosphere soil collected from different location were phosphate solubilizers. The soil sample designated as AMB collected from Ambernath area from railway track land where Okra cultivation taken place was little acidic, many chemical industries are located near to railway track they realised their effluents in nearby water bodies, such polluted water is used to irrigate the Okra crop might be one of the reason to get more phosphate solubilizers. Zhang *et al.* (2018) also reported potent fungal phosphate solubilizers from acidic soil. In this study ten isolates showed desired P-solubilizing capability in two recalcitrant phosphates like TCP and RP. The P-solubilizing potential of *A. Japonicas* was maximum to solubilise TCP ($298.5 \mu\text{g ml}^{-1}$) while *Aspergillus niger* isolates AMB& DOV exhibited excellent ability to solubilize TCP and RP (285.1 and $286 \mu\text{g ml}^{-1}$). Similar observations have been made by several researcher working with fungi from tropical habitats in India (Das 1963, Ahmad & Jha 1968, Sethi & Subba-Rao 1968, Narsian & Patel 2000). Verma *et al.* (2015) has also reported that the TCP solubilisation ability is higher than RP by *Aspergillus* and *penicillium* isolated from arid soil. Phosphate solubilising activity of *Aspergillus* and *Penicillium* isolated from rhizosphere soil showed maximum level of phosphate solubilization activity *in vitro* when liquid medium was supplemented with both tricalcium phosphate and rock phosphate separately reported by Pradhan & Shukla (2005). Sapatnekar *et al.* (2003), Zayed & Abdel-Motaal (2005) reported phosphate solubilising *Trichoderma viride* isolated from black soil from Maharashtra. Apart of commonly reported phosphate solubilizers like *Aspergillus* and *Penicillium* some others interesting genus, *Chaetomium* and *Syncephalastrum* have been reported in this study. The isolate *Chaetomium globosum* showed maximum P-solubilizing index at $40\pm 2^\circ\text{C}$, this isolate may be utilized as a biofertilizer to increase crops yield in hot regions. In this study, variation in phosphate solubilising potential of different strains of same species has been noticed, it might be due to the chemical nature of soil and other relative factors. The potential of any strains mostly influenced by conditions in which they survive. Consortium of highly active isolates showed positively enhanced the Okra growth in pots study. Similar to Plant Growth Promoting Rhizobacteria, these rhizosphere fungi are also able to promote plant growth and can be used as biofertilizers. Present-day agricultural practices solely rely on high inputs of inorganic fertilizers to get maximum yield, however, the detrimental effect of such practices are now being realized and are coming under increased scrutiny as our awareness of environmental conservation from the impact of chemical overuse. This type of research is now a prime need to improve soil conditions by adding organic manures and beneficial microbes for environmentally friendly agriculture.

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