Appl Biochem Biotechnol (2015) 175:813–824
DOI 10.1007/s12010-014-1309-0

Effect of an Organophosphate Pesticide, Monocrotophos, on Phosphate-Solubilizing Efficiency of Soil Fungal Isolates

Rachna Jain · Veena Garg · Jyoti Saxena

Received: 22 April 2014 / Accepted: 15 October 2014 /
Published online: 25 October 2014

Abstract Soil is a sink of pesticide residues as well as microorganisms. Fungi are well known for solubilization of inorganic phosphates, and this activity of fungal isolates may be affected by the presence of pesticide residues in the soil. In the present study, five generically different fungal isolates, viz. *Aspergillus niger* JQ660373, *Aspergillus flavus*, *Penicillium aculeatum* JQ660374, *Fusarium pallidoroseum* and *Macrophomina* sp., were tested and compared for their phosphate-solubilizing ability in the absence and presence of monocrotophos (500 mg L^{-1}). After 168 h of incubation, four times high amount of tricalcium phosphate was solubilized by isolates in the growth medium containing monocrotophos in comparison to control (without monocrotophos). Concurrently, 78 % of the applied monocrotophos was degraded by these fungal isolates. Kinetics of phosphate solubilization shifted from logarithmic to power model in the presence of monocrotophos. Similarly, the phosphatase activity was also found significantly high in the presence of monocrotophos. The combined order of phosphate solubilization as well as monocrotophos degradation was found to be *A. niger* JQ660373 > *P. aculeatum* JQ660374 > *A. flavus* > *F. pallidoroseum* > *Macrophomina* sp. On the contrary, phosphate solubilization negatively correlated with the pH of the growth medium. Hence, it could be concluded that these fungal species efficiently solubilize inorganic phosphates and monocrotophos poses a positive effect on their ability and in turn degraded by them. To the best of our knowledge, this is the first report on P solubilization by *Macrophomina* sp. and *F. pallidoroseum*.

Keywords Pesticide · Degradation · Inorganic phosphate · Phosphatase · Tricalcium phosphate

Introduction

In modern agriculture, pesticides are frequently used in field to increase crop yield. Besides combating insect pests, insecticides also affect the population and activity of beneficial

R. Jain (✉) · V. Garg
Department of Bioscience and Biotechnology, Banasthali Vidhyapeeth, Banasthali University, Rajasthan 304022, India
e-mail: rachna11587@gmail.com

J. Saxena
Biochemical Engineering Department, BT Kumaon Institute of Technology, Dwarahat 263653, India

microbial communities in soil [1]. The pesticide residues may influence the microbial populations present in rhizosphere which carry out different processes, such as nitrogen fixation, nitrification, ammonification, organic matter decomposition, sulfur oxidation and phosphorus solubilization. However, these effects vary with different types of insecticides, their doses as well as field conditions. Low concentration of lannate stimulated the growth of microorganisms, whereas high doses caused short-term toxicity [2, 3]. In contrast, a population of microorganisms in the soil treated with *cis*- and *trans*-isomers of cypermethrin at different time durations had no adverse effect on soil microbes [4].

Monocrotophos (MCP) is a highly toxic, broad-spectrum, fast-acting organophosphate insecticide with both systemic and contact actions. While mainly applied against cotton pests, it is also used for citrus, olives, rice, maize, sorghum, sugar cane, sugar beet, peanuts, potatoes, green peas, soya beans, vegetables, ornamentals, strawberries, bananas, melons and tobacco. Its 40 and 25 % solutions come under the 'highly hazardous' class; however, stimulatory effect of low doses (≥250 ppm) of MCP under laboratory conditions has been reported on microorganisms [5, 6]. Its half-life depends upon the soil and aqueous environments, i.e. pH, temperature, etc. Half-life is reported to be 131 days at pH 3, 26 days at pH 9 and 30 days at neutral pH at 25 °C in the dark [7].

Phosphorus is an essential nutrient for biological growth and development of plants [8]. For over a century, agricultural microbiologists and microbial ecologists have been interested in testing the ability of microorganisms to dissolve poorly soluble mineral phosphates such as tricalcium phosphate and hydroxyapatite present in soil [9, 10]. Pure cultures of many phosphate-solubilizing microorganisms (PSMs) belonging to bacterial, fungal, yeast and actinomycetous groups have been isolated [11–13]. These are used to develop biofertilizer to increase the crop yield through P supply [14]. PSMs render insoluble phosphate into soluble form through the process of acidification, chelation and exchange reaction in soils [15–19].

The studies on interrelationship between microorganisms and pesticides have increased considerably in recent years. The stimulatory effect of certain pesticides such as monocrotophos on rhizosphere microorganisms showed beneficial effects on crops; thus, they may help in sustainable agriculture. This paper reports the ability of fungal strains to solubilize tricalcium phosphate (TCP) and the effect of monocrotophos on their phosphate-solubilizing efficiency as well as alkaline phosphatase activity of tested fungal strains.

Material and Methods

Chemicals

Analytical grade monocrotophos (Sigma, 99.5 %) was used for the study. A stock solution of 1 mg ml^{-1} was prepared in 100 % ethanol. Working solutions were prepared by appropriate dilution of stock. All the other chemicals used in the study were of analytical grade.

Soil Sampling

Soil samples were collected from agricultural soil containing chickpea (*Cicer arietinum*), pearl millet (*Pennisetum glaucum*) and mung beans (*Vigna radiata*) within a 10-km range of Banasthali campus, Rajasthan, India. The collected samples were air-dried, sieved and mixed through a 2-mm mesh prior to use. Soil sample has been treated with OP pesticide 1 year prior to the experiment.

Medium

Pikovskaya agar medium (PVK) agar medium with the following composition was used: glucose 10, yeast extract 0.5, $(NH_4)_2SO_4$ 0.5, $MgSO_4 \cdot 7H_2O$ 0.1, $Ca_3(PO_4)_2$ 5.0, NaCl 0.2, KCl 0.2, $MnSO_4 \cdot 2H_2O$ 0.002, $FeSO_4 \cdot 7H_2O$ 0.002 and agar 12 g per litre.

Physico-chemical Analysis of Soil

Physico-chemical properties of soil were analyzed using standard methods. Particle size, organic carbon percentage, exchangeable cations and pH were as follows: particle size—clay 8.9 %, silt 5.3 %, sand 85.8 % and texture class loamy sand; organic carbon 0.33 %; exchangeable cations—Ca 7.5 meq/100 g soil, Mg 2.00 meq/100 g soil, Na 0.65 meq/100 g soil and K 0.039 meq/100 g soil; and soil reaction (pH) 7.88.

Isolation and Screening of Phosphate-Solubilizing Fungal Strains

Fungal strains were isolated from local agricultural soil on potato dextrose agar by soil enrichment culture technique [20]. Screening of phosphate-solubilizing strains was done by inoculating these isolates on PVK agar as well as on broth medium. Released inorganic phosphate (micrograms per millilitre) was measured calorimetrically by the method of Murphy and Riley [21] in liquid culture medium. In solid medium, solubilization index (halo zone around the fungal colony) was used as a measure of phosphate solubilization. Solubilization index was calculated according to the ratio of the total diameter (colony + halo zone) and the colony diameter [22].

Pure cultures of phosphate-solubilizing fungi were identified by staining with lactophenol cotton blue under a phase-contrast microscope (×100) with the help of literature and expertise available in the department and thereafter were sent to Indian Type Culture Collection, Indian Agricultural Research Institute (IARI), New Delhi for confirmation.

Effect of Monocrotophos on Solubilization Index of Fungal Strains

To see the effect of MCP on the phosphate-solubilizing ability of fungal strains, all the selected fungal strains were point-inoculated on PVK agar medium supplemented with 0.5 % TCP and 0.5 % TCP +0.5 % MCP (stock solution), respectively. Solubilization index of both medium was calculated and compared to estimate the effect of MCP on the phosphate solubilization ability of fungal isolates.

Effect of Monocrotophos on Solubilization Efficiency in Liquid Cultures

Similarly, quantitative estimation and comparison of phosphate solubilization ability of fungal isolates was done in PVK broth medium. Experiment was carried out in medium amended with 0.5 % TCP and 0.5 % TCP +0.5 % MCP (stock solutions). In triplicate, a 100-μl suspension of each fungal culture (1×10^8 spores ml^{-1}) was added to the broth. A control without any inoculation was maintained with each of the medium used. The cultures were incubated on a rotary shaker at 28±2 °C for 168 h. Available phosphorus in broth was estimated at a regular time interval by the modified molybdenum-blue method of Murphy and Riley [21]. It was expressed in terms of micrograms per millilitre phosphorus released in culture medium. The decline in pH of the growth medium was also recorded after each withdrawal. Simultaneously, the growth of fungal isolates was estimated and compared by

calculating the dry mass (milligrams per millilitre) of fungi after 168 h in the absence and presence of MCP.

Phosphatase Activity

Alkaline phosphatase activity of each fungal strain in the presence and absence of MCP was estimated by the method of Tham et al. [23]. Cultures grown in PVK broth were sampled at regular intervals from 72 to 168 h and centrifuged at 5,000 rpm for 10 min. Cell-free supernatant was used as a crude enzyme sample, and *p*-nitrophenyl phosphate was used as a substrate. Enzyme activity was expressed as units per millilitre (micromolars of *p*-nitrophenol formed per minute per millilitre).

Kinetics of P Solubilization

The P solubilization rate constant (k) was determined using the following equation [24]:

$$k = \frac{2.303(\log C_t\text{-}\log C_0)}{t(\text{time})}$$

where k = solubilization rate, log C_0 = initial P solubilization, log C_t = P solubilization at different time interval and t = time.

Degradation Study of Monocrotophos by Fungal Isolates

In order to find out the basic mechanism of inducibility of MCP towards phosphate solubilization, the percent degradation of MCP during phosphate solubilization was also determined. All the experiments were set up in triplicates. Pure culture of each of the isolated strain was suspended in 1.0 ml of 0.85 % saline, and 100 μl of this suspension was inoculated in 125 ml of PVK broth medium containing 0.5 % MCP and incubated at 28±2 °C for 168 h in an orbital shaking incubator at 90 rpm under aerated culture conditions. Thereafter, at a regular time interval, residual MCP was extracted twice with equal amount of ethyl acetate (1:1). The solvent was evaporated, and the residue was re-dissolved in 1 ml of ethyl acetate. Further, recovery of biodegradable products of MCP was determined by the spectrophotometric method of Janghel et al. [25], modified by using sulphanilamide instead of *p*-aminoacetophenone. This method is based on alkaline hydrolysis of monocrotophos to *N*-methyl acetoacetamide followed by coupling with diazotized sulphanilamide in alkaline medium. Of each sample, 100 μl was taken in a 12-ml graduated tube, and to it, 1.0 ml of 20 % sodium hydroxide was added and kept for 10 min at room temperature for complete hydrolysis. Then, 1 ml of diazotized sulphanilamide was added and shaken thoroughly and kept at 0–5 °C for 15 min for full colour development. The initial yellow colour turns to reddish violet. The solution was then diluted to the mark with water, and absorbance was measured at 560 nm against a reagent blank. Percentage of degradation was calculated with respect to control using the following formula:

$$\%\,\text{of degradation} = \frac{\text{Amt of } N\text{-methylacetoacetamide in sample} - \text{Amt of } N\text{-methylacetoacetamide in control}}{\text{Amount of } N\text{-methylacetoacetamide in sample}} \times 100$$

Molecular Characterization of Efficient Phosphate-Solubilizing Fungal Isolate

For the molecular characterization, genomic DNA of efficient phosphate-solubilizing fungi was isolated by the cetyltrimethylammonium bromide (CTAB) method [26], and the interspecific transcribed spacer (ITS) was amplified by polymerase chain reaction (Biometra, Germany, model personnel) using the forward primer: ITS1: 5′-TCC GTA GGT GAA CCT GCG G-3′ and reverse primer ITS4: 5′-TCC TCC GCT TAT TGA TAT GC-3′. The amplified gene fragment was purified by agarose gel electrophoresis and cloned into pGEM-T vector (Promega Scientific, Santa Barbara, CA). Both the strands of cloned 18S rDNA fragment were sequenced using sequencing facility provided by Banglore Genei, India. The sequences were compared to those in GenBank using Blast Alignment Tool and deposited to the GenBank, EMBL and DDBJ library.

Statistical Analysis

The statistical analysis was done by using SPSS 17 program (Statistical Package for the Sciences System 17). The variables were subjected to ANOVA and Student's t test and analysis of correlation (significance was set at $*p<0.05$).

Results and Discussion

Screening and Isolation of Phosphate-Solubilizing Fungal Isolates

Initially 10 different fungal strains were isolated from soil by enrichment culture method. All the ten fungal isolates showed halo zone formation. These ten isolates were further tested for their phosphate-solubilizing ability in liquid culture medium. The amount of released inorganic phosphates varied from 95.3 to 342.3 μg ml^{-1} with the maximum for the strain MCP3 and minimum for MCP10. The released inorganic phosphate content of other fungal isolates was observed to be 320.45, 267.6, 197.8, 199.4, 123.2, 110.2, 104 and 98.7 μg ml^{-1} for MCP1, MCP2, MCP4, MCP5, MCP6, MCP7, MCP8 and MCP9, respectively. Hence, the first five strains were considered as efficient phosphate solubilizers which was also evident from the halo formed around the colonies on PVK agar medium. These five strains were primarily identified as *Aspergillus niger* (MCP 1), *A. flavus* (MCP 2), *Penicillium aculeatum* (MCP 3), *Fusarium pallidoroseum* (MCP 4) and *Macrophomina* sp. (MCP 5), respectively, and were used for further studies. *A. niger* and *A. flavus* had been previously reported to be as efficient phosphate solubilizer [27]. Similar types of findings were also shown by Pradhan and Sukla [28]. These workers isolated *Aspergillus* species and *Penicillium* species from soil which proved themselves to be efficient phosphate solubilizer. These five fungal isolates were further confirmed by IARI as *P. aculeatum*: 7,980.10, *A. flavus*: 7,981.10, *A. niger*: 7,982.10, *Macrophomina* sp: 7,984.10 and *F. pallidoroseum*: 7,985.10 respectively. To the best of our knowledge, this is the first report of phosphate solubilization by *Macrophomina* sp. Even *F. pallidoroseum* has rarely been associated with phosphate solubilization activity [29].

Effect of Monocrotophos on Solubilization Index of Fungal Isolates

Solubilization index of all the five fungal strains was compared by growing these fungi on PVK medium with and without MCP. *A. niger* was found to have the largest solubilization index in the presence as well as i the absence of MCP with the values of 157 and 180,

respectively, as shown in Table 1. *Aspergillus* sp. had also previously been reported as extensive phosphate solubilizers and even better than bacteria [29, 27]. The solubilization index of other fungal isolates in the presence as well as in the absence of MCP followed the order of *P. aculeatum* > *A. flavus* > *F. pallidoroseum* > *Macrophomina* sp.

Effect of Monocrotophos on P Solubilization Ability in Liquid Culture Medium

After confirming the phosphate-solubilizing ability on solid medium, phosphate solubilization by the fungal isolates in liquid medium was also compared. Inorganic soluble phosphate content increased linearly up to 168 h of incubation both in the presence and absence of MCP. Figure 1a, b clearly indicates that MCP significantly induced the released inorganic phosphate content by all the fungal isolates. *A. niger* showed the maximum phosphate solubilization followed by *P. aculeatum* both in the presence as well as in the absence of MCP. *A. niger* solubilized 465.4 μg ml^{-1} of inorganic phosphate in the absence of MCP which increased 3.3 folds in the presence of MCP. In the case of *P. aculeatum*, a 3.5-fold increase was observed within 168 h of incubation. Initially, phosphate solubilization of MCP3 was low but it increased rapidly, and after 168 h, it showed the second highest phosphate solubilization among the five tested strains. The rest order of P solubilization was observed to be *A. flavus* > *F. pallidoroseum* > *Macrophomina* sp.

The mechanism of induction of P solubilization by fungal isolates might be the utilization of MCP as a phosphorus source. Inorganic phosphates are released as a by-product of MCP degradation by various microorganisms [30, 31].

Phosphate solubilization was accompanied by a decrease in pH of the medium. PSMs have previously been reported to dissolve insoluble phosphates by the production of inorganic or organic acids and/or by the decrease of the pH [32–35, 13]. Release of extracellular acid phosphatases and phytases play an important role in phosphate solubilization [36–38]. Some phosphatase- and phytase-producing fungi from arid soils of Rajasthan, India were isolated by Tarafdar et al. [37] and Aseri et al. [38]. Most of the previous reports stated that calcium phosphates are mostly dissolved by acidification. Therefore, any microorganism that acidifies its external medium will show some level of phosphorus-solubilizing activity. In most soils, proton substitution reactions are driven by microbial production of organic acids, represented generically by the following equation:

$$\left(Ca^{2+}\right)m\left(PO_4{}^{3-}\right)(HA) = (H+)\left(PO_4{}^{3-}\right) + \left(Ca^{2+}\right)(A^-)$$

Table 1 Solubilizing index of five fungal strains in the presence and absence of monocrotophos

Sample no.	Strains	Solubilization index (%)		Dry mass (mg ml^{-1})	
		Without MCP	With MCP	Without MCP	With MCP
1	*A. niger*	*157.68±1.32*	*180.01±0.88**	*0.89±0.0013*	*1.94±0.01**
2	*A. flavus*	113.95±1.02	163.01±0.88*	0.67±0.001	1.35±0.1*
3	*P.aculeatum*	117.2±0.52	169.01±1.22*	0.8±0.01	1.78±0.01*
4	*F. pallidoroseum*	108.01±0.37	135.01±3.61*	0.57±0.01	1.22±0.12*
5	*Macrophomina* sp	92.32±2.90	137.7±2.98*	0.54±0.01	1.16±0.12*

Highest values are shown in italics

*$p<0.05$ (significant)

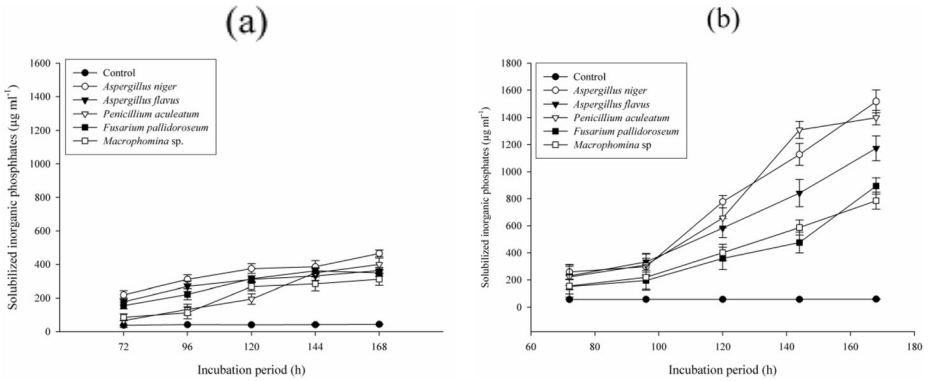

Fig. 1 Inorganic phosphate content solubilized by different fungal strains, *A. niger*, *A. flavus*, *P. aculeatum*, *Fusarium pallidoroseum* and *Macrophomina* sp., at different incubation periods (72, 96, 120, 144 and 168 h) in the (**a**) absence of monocrotophos and (**b**) presence of 0.5 % monocrotophos. *Error bars* indicate standard deviation

There is no stoichiometry in the equation because of the complexity of CaP chemistry and the multiplicity of microbially produced organic acids (HAs) with differing numbers of dissociable protons [39].

A. niger showed the utmost decrease in pH, from initial 8 to 2.4 and 1.5 after 168 h of incubation in the absence and presence of MCP, respectively (Fig. 2a, b). The order of decrease in pH was also found similar to the rate of P solubilization. It was also experiential that the decrease was high in the medium supplemented with 0.5 % MCP. This might be due to the degradation of MCP which resulted in the release of organic acids [30].

The results of dry mass analysis showed that fungal growth positively correlated with phosphate-solubilizing ability, i.e. the higher the growth, the higher is the rate of phosphate solubilization. *A. niger* showed maximum value of dry microbial mass, i.e. 0.89 and 1.90 mg ml^{-1} after 168 h of incubation without and with MCP (Table 1). The growth of other fungal isolates also increased in the presence of MCP in the order of *P. aculeatum* > *A. flavus* > *F. pallidoroseum* > *Macrophomina* sp. Therefore, it could be concluded that monocrotophos posed a significant effect on the phosphate-solubilizing ability as well as on the growth of fungal isolates.

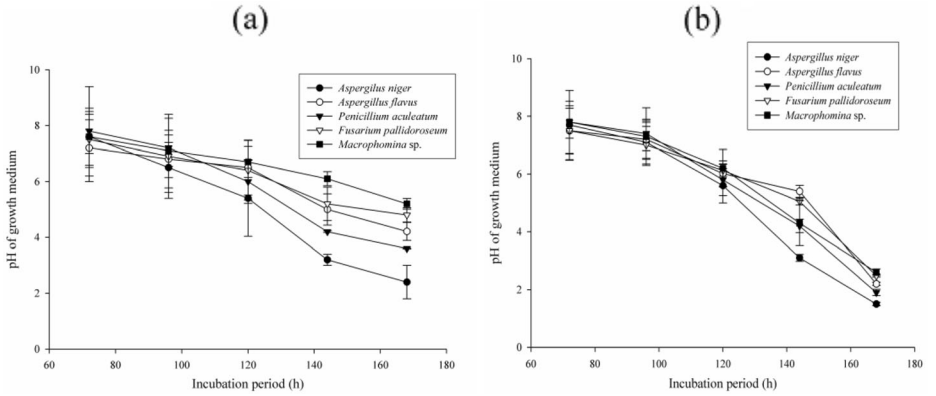

Fig. 2 pH drop during phosphate solubilization in the (**a**) absence of monocrotophos (**b**) presence of 0.5 % monocrotophos. *Error bars* indicate standard deviation

Alkaline Phosphatase Activity

Comparative data for the extracellular alkaline phosphatase activities in the absence and presence of MCP by all fungal isolates is represented in Fig. 3a, b. Maximum enzyme activity was observed between 96 and 168 h of incubation. The amount of phosphatase activity varied between 51 and 77 U ml^{-1} in the absence and between 59 and 90 U ml^{-1} in the presence of MCP. This clearly depicts the inductive effect of MCP on enzyme activity. Utilization of MCP as a substrate by different microorganisms had been previously reported by other scientists [30, 31]. *A. niger* showed the highest phosphatase activity followed by *P. aculeatum*, *A. flavus*, *F. pallidoroseum* and *Macrophomina* sp. It is quite clear from the data that though the amount of released inorganic phosphates was high, fungal strains showed a meager amount of alkaline phosphatase activity. This indicates the presence of specific phosphatases which specifically attack on the phosphate group and release inorganic phosphates.

Our results are concurrent with the previous study of Rodriguez and Fraga [40] which also reported substantial acid/alkaline phosphatase activity in bacterial strains with high P-solubilizing ability. Simultaneous exudation of organic acids and phosphatases could increase P solubility by releasing bound organic phosphates and its mineralization by increasing the rate of hydrolytic cleavage [41]. A positive correlation was observed between released inorganic phosphates and phosphatase activity [42]. The P released as a by-product by the action of phosphatases provided the cells with essential nutrients [43].

Kinetics of P Solubilization

Solubilization rate (k) of inorganic phosphates by five fungal isolates at different incubation periods was determined by using first-order kinetics [24]. Further, from regression analysis, it was found that kinetics of P solubilization best fitted in power and logarithmic model for all the isolated strains in both the presence and absence of MCP. In the absence of MCP, all the strains gradually solubilized inorganic phosphate and best fitted in logarithmic model. But in its presence, they showed a steep curve; thus, the values were best fitted in power model (Fig. 4a–e). Therefore, this confirmed that MCP increased the solubilization rate of all the strains studied.

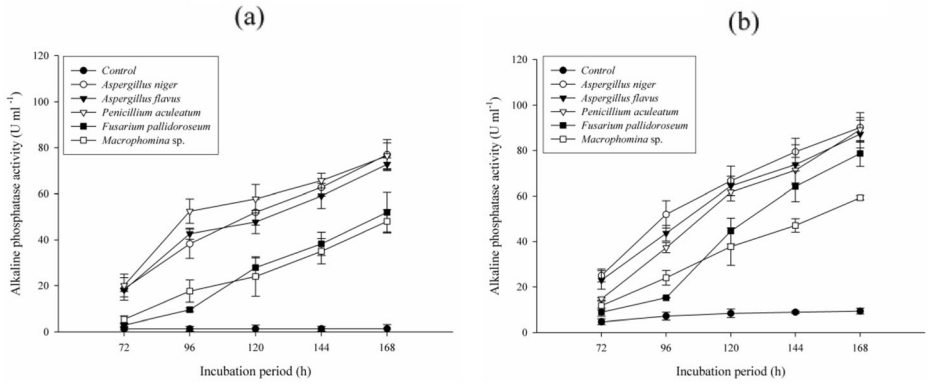

Fig. 3 Alkaline phosphatase activity of fungal strains (units per millilitre) at different incubation periods (72, 96, 120, 144 and 168 h) in the (**a**) absence of monocrotophos and (**b**) presence of 0.5 % monocrotophos. *Error bars* indicate standard deviation

Monocrotophos Degradation

Percentage of degradation of MCP was studied to find out the basic mechanism of inducibility towards phosphate solubilization. It was observed that initially when the amount of MCP was high, the released inorganic phosphate content was large; therefore, it induced phosphate solubilization. Table 2 clearly indicates that only 45–78 % MCP was degraded till 168 h; hence, after that, it also induced the process by its remaining content present in the liquid medium. The maximum degradation of MCP was observed for *A. niger* and the minimum for *Macrophomina* sp. Other researchers had also reported that degradation of MCP was accompanied by the formation of *N*-methyl acetoacetamide with the release of inorganic phosphates

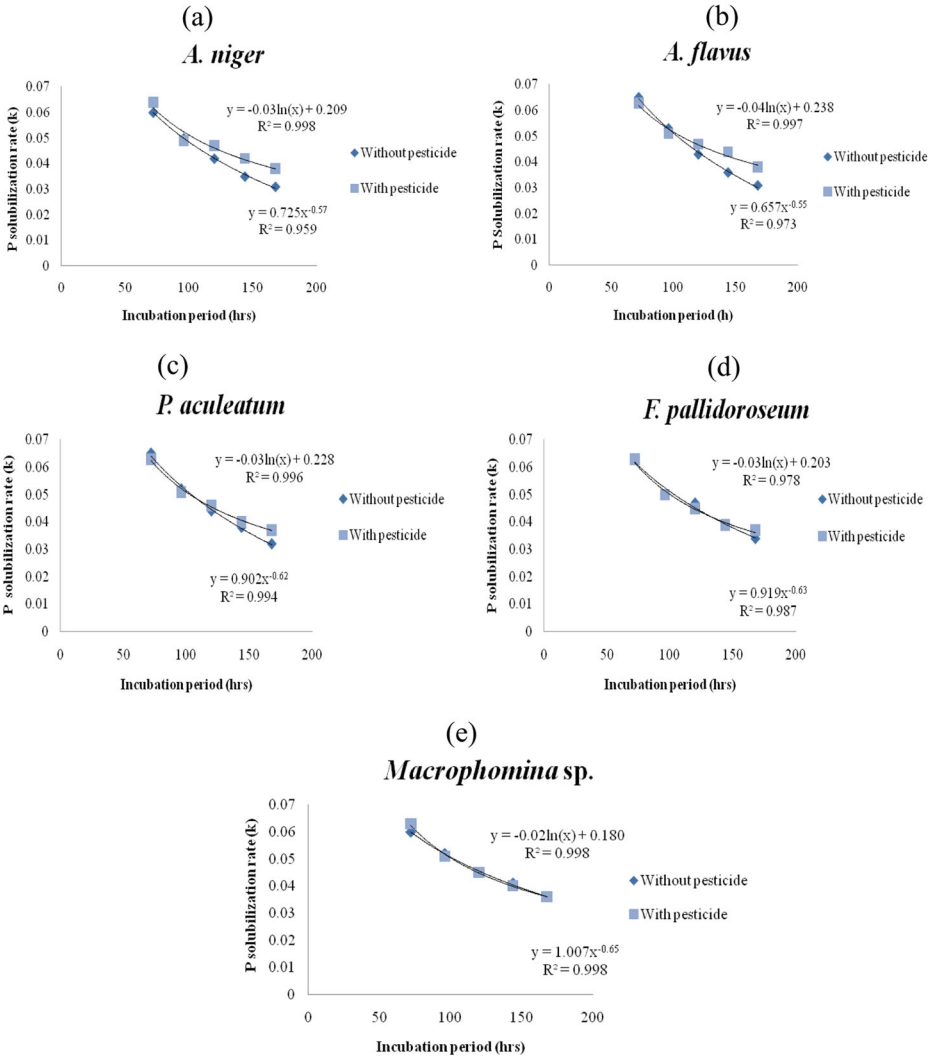

Fig. 4 Kinetics of phosphate solubilization by five fungal strains at different incubation periods (72, 96, 120, 144 and 168 h) in the absence and presence of 0.5 % monocrotophos

Table 2 Percent degradation of monocrotophos by isolated strains after 168 h of incubation

Sample no.	Strain	% Degradation of monocrotophos
1	*Aspergillus niger*	78±3.3*
2	*Aspergillus flavus*	67±2.33*
3	*Penicillium aculeatum*	75±1.34*
4	*Fusarium pallidoroseum*	56±3.4*
5	*Macrophomina* sp.	45±3.2*

*$p<0.05$ (significant)

[30, 31]. *N*-Methyl acetoacetamide remains persistent for some time and later converts into other small metabolites as methylamine, carbon dioxide, etc. In our study, the degradation product of monocrotophos *N*-methyl acetoacetamide was found to be persistent till 168 h of incubation, and afterwards, it decreased.

Molecular Characterization of Efficient Phosphate-Solubilizing Fungal Isolate

Two distinct fungal isolates, viz. *A. niger and P. aculeatum*, were found to be simultaneously efficient for phosphate solubilization as well as monocrotophos degradation. Therefore, their DNA was isolated by the CTAB method. PCR product of the amplified DNA appeared as intense bands on agarose gels. The approximate size of the amplified PCR product was 617 and 609 bp for *A. niger* and *P. aculeatum*, respectively. The sequences were subjected to BLAST analysis to find out the nearest homology (Table 3). The sequence and comparative phylogenetic analysis of PCR-amplified 18S rDNA gene fragment confirmed *A. niger* and *P. aculeatum* as the closest homologue of *A. niger* NCBI GenBank accession no. JQ660373 and *P. aculeatum* JQ 660374.

Conclusion

It can be concluded from the study that the five strains, viz. *A. niger*, *A. flavus*, *P. aculeatum*, *F. pallidoroseum* and *Macrophomina* sp. are potential phosphate solubilizers and can be best utilized in the agriculture fields supplied with monocrotophos pesticide for the solubilization or mineralization of inorganic/organic phosphates. It can also be inferred from the results that

Table 3 BLAST analysis of the two most competent phosphate-solubilizing fungi

Sample no.	Strain	GenBank accession no.	Homologous sequence similarity (%)	Strain	GenBank accession no.	Homologous sequence similarity (%)
	Aspergillus (MCP1)			*Penicillium* (MCP3)		
1	*Aspergillus sp.*LZ11	GU258410	100	*Penicillium aculeatum*	HQ392497	98
2	*Aspergillus niger*	AB369898	100	*Penicillium aculeatum*	HQ392496	100
3	*Aspergillus niger* strain WCZM0.74	HQ014690	100	*Penicillium pinophilum*	HQ392503	97
4	*Aspergillus niger* strainWCZM0.74	HQ014696	100	*Penicillium verruculosum*	HQ607791	95
5	*Aspergillus niger* strain MUM05.13	JF838357	100	*Penicillium primulinum*	JN899317	94

monocrotophos exerts a positive effect on the growth and phosphate-solubilizing activity of selected strains.

The overall order of fungal efficiency was found to be *A. niger* > *P. aculeatum* > *A. flavus* > *F. pallidoroseum* > *Macrophomina* sp. The greenhouse studies with the inoculation of these strains to improve the growth and yield of crops are in offing. Further, there could be a possibility that P solubilization may be a mechanism for the degradation of monocrotophos; however, more studies are required to prove it.

Acknowledgments Authors gratefully acknowledge Prof. Aditya Shastri, Vice Chancellor, Banasthali University for the financial support through the Department of Bioscience and Biotechnology, Banasthali University.

References

1. Pandey, S., & Singh, D. K. (2004). *Chemistry, 55*(2), 197–205.
2. Bakalivanov, D. (1990). *Pochvoznanie-i-Agrokhimiya, 25*(5), 56–61.
3. Cernakova, M. (1993). *Folia, 38*(4), 331–334.
4. Binner, R., Berendes, K. H., Felgentreu, D., Friesland, H., & Glitschka, M. (1999). *Nachrichtenblatt-des-Deutschen-Pflanzenschutzdienstes, 51*(9), 227–237.
5. Das, A. C., & Mukherjee, D. (1998). *World Journal of Microbiology, 14*(6), 903–906.
6. Dordevic, S., Sestovic, M., Raicevic, V., & Dordevic, A. (1998). *Pesticide, 13*(4), 281–288.
7. Lee, P. W., Fukuto, J. M., Hernandez, H., & Stearns, S. M. (1990). *Journal of Agricultural and Food Chemistry, 38*, 567–573.
8. Kucey, R. M. N. (1988). *Canadian Journal of Soil Science, 68*, 261–270.
9. Goldstein, A. H., & Liu, S. T. (1987). *Bioresource Technology, 5*, 72–74.
10. Goldstein, A. H., Rogers, R. D., & Mead, G. (1993). *Bioresource Technology, 11*, 1250–1254.
11. Halder, A. K., Mishra, A. K., & Chakarbarthy, P. K. (1991). *Indian Journal of Experimental Biology, 29*, 28–31.
12. Abd-Alla, M. H. (1994). *Letters in Applied Microbiology, 18*, 294–296.
13. Whitelaw, M. A. (2000). *Advances in Agronomy, 69*, 99–151.
14. Minaxi, & Saxena, J. (2010). *BioControl, 55*(6), 799–810.
15. Banik, S. (1983). *Zentralblatt für Mikrobiologie, 138*, 209–216.
16. Berthelin, F., Leyval, C., Laheurte, F., & De-Giudici, P. (1991). *Special publication series of the British Ecological Society, No. 10*. Oxford: Blackwell Scientific.
17. Rodriguez, H., & Fraga, R. (1999). *Biotechnol Adv, 17*, 319–339.
18. Barroso, C. V., Pereira, G. T., & Nahas, E. (2006). *Brazilian Journal of Microbiology, 37*, 434–438.
19. Son, H. J., Park, G. T., Cha, M. S., & Heo, M. S. (2006). *Bioresource Technology, 97*, 204–210.
20. Rangaswamy, V., & Venkateswarlu, K. (1992). *Bulletin of Environmental Contamination and Toxicology, 49*(6), 797–804.
21. Edi-Premono, M., Moawad, A. M., & Vlek, P. L. G. (1996). *Indonesian Journal of Agricultural Science, 11*, 13–23.
22. Murphy, J., & Riley, J. P. (1962). *Analytica Chimica Acta, 27*, 31–36.
23. Tham, S.-J., Chang, C.-D., Huang, H. J., & Lee, Y.-F. (2010). *Bioscience, Biotechnology, and Biochemistry, 74*(4), 727–735.
24. Panhwar, Q. A., Radziah, O., Sariah, M., & Ismail, M. R. (2009). *International Journal of Agriculture and Biology, 6*, 667–673.
25. Janghel, E. K., Rai, J. K., Rai, M. K., & Gupta, V. K. (2006). *Journal of the Chinese Chemical Society, 53*, 343–347.
26. Doyle, J. J., & Doyle, J. L. (1987). *Phytochemical Bulletin, 19*, 11–15.
27. Gomashe, V. A., Sabina, S. S., & Dharmik, G. P. (2012). *International Journal of Science Innovations and Discovery, 2*(2), 310–315.
28. Pradhan, N., & Sukla, L. B. (2005). *African Journal of Biotechnology, 5*(10), 850–854.
29. Sanjotha, P., Mahantesh, P., & Patil, C. S. (2011). *International Journal of Microbiology Research, 3*(1), 56–58.
30. Bhadbhade, B. J., Sarnaik, S. S., & Kanekar, P. P. (2002). *Journal of Applied Microbiology, 93*, 224–234.
31. Bhalerao, S. T., & Puranik, R. P. (2009). *International Biodeterioration and Biodegradation, 63*, 503–508.
32. Cunningham, J. E., & Kuiack, C. (1992). *Applied and Environmental Microbiology, 58*(5), 1451–1458.

33. Dutton, V. M., & Evans, C. S. (1996). *Canadian Journal of Microbiology, 42*(9), 881–895.
34. Nahas, E. (1996). *World Journal of Microbiology and Biotechnology, 12*(6), 567–572.
35. Jones, D. L. (1998). *Plant and Soil, 205*(1), 25–44.
36. Richardson, A. E., Hadobas, P. A., & Hayes, J. E. (2000). *Plant, Cell and Environment, 23*(4), 397–405.
37. Tarafdar, J. C., Bareja, M., & Panwar, J. (2003). *Indian Journal of Microbiology, 43*(1), 27–32.
38. Aseri, G. K., Jain, N., & Tarafdar, J. C. (2009). *American-Eurasian Journal of Agricultural and Environmental Sciences, 5*(4), 564–570.
39. Goldstein, A. H. (1986). *American Journal of Alternative Agriculture, 1*, 51–57.
40. Rodriguez, H., Gonzalez, T., Selman, G. (2000) *Journal of Biotechnology, 84*: 155–161.
41. George, T., Gregory, P., Wood, M., Read, D., & Buresh, R. (2002). *Soil Biology and Biochemistry, 34*, 1487–1494.
42. Nenwani, V., Doshi, P., Saha, T., & Rajkumar, S. (2010). *Journal of Yeast and Fungal Research, 1*(1), 009–014.
43. Ohtake, H., Kato, J., Kuroda, A., Taguchi, K., & Sakai, Y. (1996). In T. Nakazawa, K. Furukawa, D. Haas, & S. Silver (Eds.), *Molecular biology and biotechnology* (pp. 188–194). Washington DC: American Society for Microbiology.