

Solubilization of Inorganic Phosphate by Rhizospheric Fungi Isolated from Soil Cultivated with *Sorghum bicolor* L.

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Three zinc-phosphate-solubilizing fungi (ZPSF) were isolated from rhizospheric soil cultivated with *Sorghum bicolor* L. The fungal isolates were identified as *Aspergillus chevalieri*, *Fusarium moniliforme*, and *Trichoderma harzianum*. The results showed that halo zone formation by ZPSF on Pikovskaya (PVK) agar medium plates was an indicator of zinc phosphate (ZP) solubilization. The lowest in pH of the inoculated medium containing ZP was observed with *A. chevalieri*, followed by *T. harzianum*, followed by *F. moniliforme*, compared with the initial pH (6.5) of the non-inoculated medium. ZP solubilization processes at different temperatures (10 °C, 20 °C, 30 °C, and 40 °C) were conducted using ZPSF at different doses of ZP (0.5 g/L, 1 g/L, and 2 g/L). The released P, to P₂O₅, was monitored during the solubilization process. The released phosphorus increased as the temperature increased, with the greatest values of phosphorus obtained with *F. moniliforme*, *A. chevalieri*, and *T. harzianum* being 11.54 mg/L, 24.40 mg/L, and 28.40 mg/L, at 30 °C and a dose of 2 g/L of ZP, respectively. In contrast, the smallest values of phosphorus were 11.89 mg/L, 8.2 mg/L, and 7.97 mg/L, at 10 °C and a dose of 0.5 g/L of ZP, with *F. moniliforme*, *A. chevalieri*, and *T. harzianum*, respectively.

Keywords: Solubilization; Phosphate; Rhizospheric fungi; *Sorghum bicolor* L.

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INTRODUCTION

Phosphorus (P), along with nitrogen, is a vital ingredient of nucleic acids in all living things, including plants. Therefore, it plays an important role in the development of new tissue and the division of plant cells, leading to the evolution of plants (Hameeda *et al.* 2008; Sharma *et al.* 2013). Unfortunately, nearly 95% to 99% of P exists in insoluble forms, complicated with cations such as iron, aluminum, and calcium (Son *et al.* 2006). More than 80% of P becomes immobile and unavailable for plant nutrition for many reasons such as precipitation, adsorption, or transformation of P to organic form (Holford 1997; Bhattacharyya and Jain 2000).

As shown in several studies, rhizospheric microorganisms, particularly P-solubilizing microorganisms, play a critical function in the natural P cycle. Thus, these microorganisms can be exploited as low-input and cheaper technology for sustainable crop

production and ecosystem conservation (Vassilev *et al.* 2007; Abdel-Ghany *et al.* 2013, 2015, 2018a,b).

Fungi, compared to bacteria, have major capacities to liberate P from insoluble P form (Sharma *et al.* 2013). Bioavailability of soil P is increased by using P-solubilizing fungi, particularly some *Aspergillus* and *Penicillium* species (Richa *et al.* 2007). Several studies have demonstrated the capability of colonized soil fungi (including *Rhizoctonia* sp., *Rhizoctonia solani*, *Aspergillus niger*, *Aspergillus tubingensis*, *Aspergillus fumigatus*, *Aspergillus terreus*, *Aspergillus awamori*, *Penicillium italicum*, *Penicillium radicum*, *Penicillium rugulosum*, *Curvularia lunata*, *Humicola* sp., *Sclerotium rolfsii*, *Pythium* sp., *Aerothecium* sp., *Phoma* sp., *Cladosporium* sp, *Cunninghamella* spp., *Pseudogymnoascus* sp., *Rhodotorula* sp., *Oidiodendron* sp., *Schwanniomyces occidentalis*, *Candida* sp., and *Fusarium oxysporum*) to solubilize rock phosphate and insoluble mineral phosphates such as aluminum phosphate and tricalcium phosphate, suggesting the beneficial possibility of these fungi to enhance plant growth and productivity (Reyes *et al.* 1999; Goenadi *et al.* 2000; Jacobs *et al.* 2002a; Srivastava *et al.* 2004; Abdel-Ghany *et al.* 2013).

Several mechanisms have been detected among many microorganisms for converting insoluble P to an available form (Reyes *et al.* 2002). Organic acids created by soil microorganisms are the main mechanism of mineral phosphate solubilization. Organic acids such as citric, oxalic, malic, succinic, and fumaric are secreted by numerous fungi, supplying both H⁺ and a metal complexing anion and mediating the release of mobile phosphate and metal species from insoluble sources (Gadd 1999; Bakri 2019). Patil *et al.* (2011), suggested that tricalcium phosphate is dissolved by acidification. Therefore, the acidification of surrounding media and soil particles by microorganisms will show some level of P solubilizing activity. Additionally (Sayer and Gadd 1997), certain fungi are able to immobilize and release P by precipitating insoluble metal. Now, it is important to identify better-functioning P-solubilizing fungi. Therefore, this study aimed to isolate zinc-phosphate-solubilizing fungi (ZPSF) from rhizospheric soil cultivated with economic crops.

EXPERIMENTAL

Fungal Isolates and Identification

The fungi used in this study were isolated from rhizospheric agriculture soil cultivated with *Sorghum bicolor* L. (Fig. 1) in the Jizan region, 16°40' 00.38" N and 42°44' 23.27" E in the south-west of Saudi Arabia. The Jazan region has a hot desert climate with an average annual temperature higher than 86 °F (30 °C)

Certain criteria were used for fungi identification, including macroscopic and microscopic studies such as surface color, texture, reverse color, exudates of colonies, conidiophores, and mycelia characteristics using Czapek-Dox agar and Potato dextrose Agar media (Raper and Fennell 1973; Gams and Bissett 1998; Nagamani *et al.* 2006; Samson *et al.* 2014).

Chemical Analysis of Rhizospheric Agriculture Soil

Total nitrogen of rhizospheric agriculture soil was measured by modified Kjeldahl method (Jackson 1973) while CO₂, pH, CO₃³⁻, Cl and Ca⁺⁺ % were measured according to standard procedure (Haluschak 2006). Estimation of soil phosphate was detected according to Olsen *et al.* (1954).

Phosphate-solubilizing Ability Test

Fungal isolates were checked for phosphate-solubilizing ability using modified Pikovskaya (PKV) agar medium that contained the following ingredients (g/L distilled water): 0.5 g of $(\text{NH}_4)_2\text{SO}_4$, 0.1 g of $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.02 g of KCl, 0.02 g of NaCl, 0.003 g of $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 0.003 g of $\text{MnSO}_4 \cdot \text{H}_2\text{O}$, 0.5 g of yeast extract, 10.0 g of glucose, and 20.0 g of agar (Pikovskaya 1948). Calcium phosphate was substituted by 5.0 g of zinc phosphate (ZP). Phosphate-solubilizing ability of the tested fungi was confirmed by the appearance of a clear halo zone around the colony development at 4 days of the incubation periods.

Zinc Phosphate Solubilization Index

The modified PKV agar medium supplemented with different doses (500 ppm, 1000 ppm, and 2000 ppm) of ZP was autoclaved and then poured into sterilized Petri plates. Fungal isolates (5-mm discs of fungal colonies) were inoculated on these plates' centers and incubated for 6 days at different temperatures ranging from 10 °C to 40 °C. The solubilization index was calculated according to Eq. 1,

$$SI = \left(\frac{C + H}{C} \right) \quad (1)$$

where SI is the solubilization index, C is the colony diameter (cm), and H is the halo zone diameter (cm).

pH Detection at Different Incubation Periods

The PKV broth medium (pH 6.5) was inoculated with fungal isolates and incubated for different incubation periods. At each period, the pH was recorded and compared with that of PKV broth medium (pH 6.5) without inoculation.

Determination of Released Phosphate during ZP Solubilization Process

Concentrations of phosphate during ZP solubilization process experiments were determined spectrophotometrically using a Shimadzu UV-Vis double-beam spectrophotometer (UV-1650-PC, Shimadzu Corp., Kyoto, Japan) equipped with a diode array detector. Its light sources were a deuterium lamp in the ultraviolet region and a tungsten iodine lamp in the visible to near infrared region. Estimation of phosphate was performed according to the modified molybdate method (Mahadevalah *et al.* 2007). The solution absorbance was measured at 327 nm.

Statistical Analysis

The results are reported as mean \pm standard deviation S.D. of three independent replicates. Statistical analyses of data were carried out by computer using SPSS ver. 22.0 software.

RESULTS AND DISCUSSION

The contents of total N, CO_3^{3-} , CO_2 , CL, Ca^{++} and available phosphorus of the *S. bicolor* L. rhizosphere soil were detected as 25.95%, 6.39%, 2.55%, 0.075%, 0.089%, and 1.98 $\mu\text{g/g}$ soil, respectively. Physical properties were also measured, including moisture content (2.05%), soil electrical conductivity (280 ppm), and pH (7.44). The physico-chemical properties of the rhizosphere soil play an important role in soil community of microorganisms (Lauber *et al.* 2008).

In the current study, three fungal isolates from rhizosphere soil cultivated with *S. bicolor* L. were identified based on the macroscopic and microscopic characteristics, where the colony growth of *Fusarium moniliforme* on potato dextrose agar media, initially appeared as white aerial mycelium, then became pale pink, salmon pink on the under surface. Abundant unicellular oval to club-shaped microconidia (5-10 x 1.2-3.2 μm) were present, but chlamydospores were not found. Hyaline and curved/straight 3 to 7 septate macroconidia (22-55 x 2.2-4.2 μm) were present. Colony growth of *Aspergillus chevalieri* on potato dextrose agar media were flat, bluish to grey, but on Czapek-Dox's solution agar with 20% sucrose they were characterized by abundant radiate conidial heads (125 to 175 μm diameter) in gray-green shades. Hyaline conidiophores (700 to 800 μm in length), spherical vesicles (20 to 33 μm diameter), uniseriate conidiogenous cells (5.3 to 7.2 x 3.0 to 3.5 μm) and ovoidal to ellipsoidal hyaline conidia (5.2 to 5.4 μm in length). Colony growth of *Trichoderma harzianum* showed dark green with dull yellowish reverse color. Branching and verticillate conidiophores, subglobose to obovoid conidia (8.7 μm), and convergent phialides (29.75 μm) were found.

The three fungal isolates *A. chevalieri*, *F. moniliforme*, and *T. harzianum* were tested for ZP solubilization. After 4 d of incubation on solidified PVK medium supplemented with 1000 ppm of ZP, a clear halo zone had appeared around the fungal colonies, indicating phosphate-solubilizing ability of the fungal isolates (Fig. 2). Recent scientific papers in agricultural fields deal with P-solubilizing fungi for enhancement of soil fertility and crop productivity (Abdel-Ghany and Alawlaqi 2018). The ability of filamentous fungi isolated from forest soils, especially *Aspergillus* spp. and *Penicillium* spp., to solubilize inorganic phosphates was reported earlier (Illmer and Schinner 1992). Additionally, in Canada, Cunningham and Kuiack (1992) registered a commercial formulation of *Penicillium bilaiae* Chalabuda as a safe enhancer of plant nutrition. The greatest decrease in pH of the growth medium containing different doses of ZP was observed with *A. chevalieri*, followed by *T. harzianum*, followed by *F. moniliforme* (Fig. 3), compared with the initial pH (6.5) of the growth medium amended by ZP but not inoculated by fungal isolates. This may be due to production of acidic metabolites.

According to Gaind (2016), different mechanisms were employed by *T. harzianum* to reduce medium pH for release of P from tri-calcium phosphate, aluminium phosphate and ferric phosphate through the production of citric, succinic, propionic, malic and acetic acid. Other mechanisms of phosphate solubilization by fungi are the production of inorganic acids such as sulphuric and nitric acids. Previously, Whitelaw (1999) reported that P-solubilizing microorganisms are able to dissolve insoluble phosphates by the production of inorganic or organic acids and/or by reduction of pH. Also, liberation of enzymes or enzymolysis was reported as a mechanism of phosphate solubilization (Zhu *et al.* 2011). Chelating mediated mechanism also was reported during phosphorous solubilization by a large number of fungi (Rathore *et al.* 2014; Whitelaw 2000).

Recently, Paul and Sinha (2017) explained that the halo zone formed around the fungal colonies could be because of the production of polysaccharides or the activity of phosphatase enzymes of phosphate-solubilizing fungal strains. However, Jacobs *et al.* (2002b) stated that the uptake of ZP by *Rhizoctonia solani* mycelia was unaffected by the pH of the medium or the growth temperature.



Fig. 1. Site of fungal isolation around roots (R) of *Sorghum bicolor* L and from soil (S) of rhizospheric region (A) cultivated with *Sorghum bicolor* L. (B)

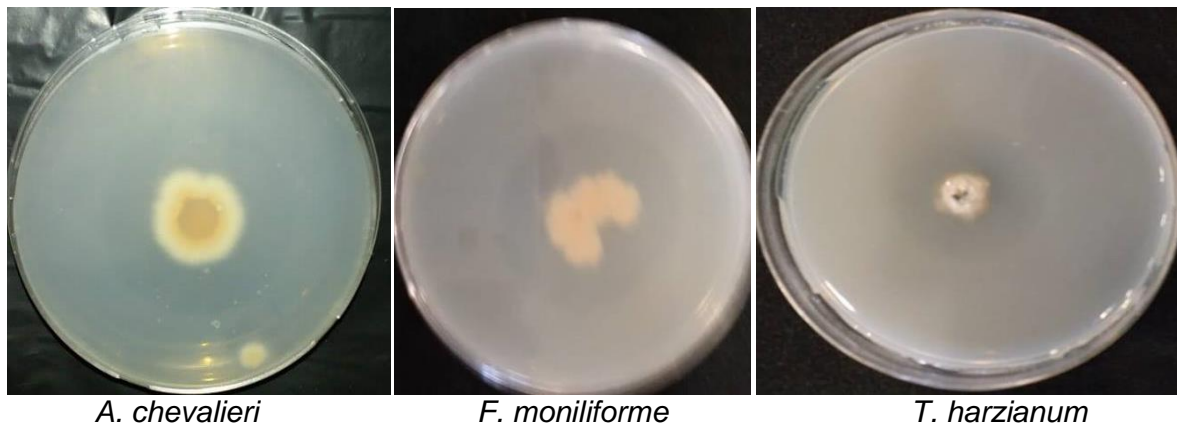


Fig. 2. Halo zones around fungal colonies

Zinc phosphate solubilization by fungal isolates at different temperatures ranging from 10 °C to 40 °C was determined through solubilization index calculation. The ZP solubilization by all fungal isolates at different temperatures was recorded but differed depending on fungal species. The solubilization index increased with increasing temperature for *A. chevalieri*, for which the maximum temperature of 40 °C maximized the ZP solubilization (Table 1). The present results were agreement with Xiao *et al.* (2011), who found that fungi isolated from wheat rhizospheric soil were differed in their abilities to release soluble P from rock phosphate at temperature stress ranged from 10 to 45 °C. In Table 2, the maximum solubility index is reached at 2000 ppm at 30 °C, although 20 °C is superior at 500 ppm and 1000 ppm with *F. moniliforme*. In Table 3, the solubility index is

greatest at 40 °C for all concentrations with *T. harzianum*. These results indicated that there was no relationship between the growth and the solubility index. Altomare *et al.* (1999) investigated the capability of the plant-growth-promoting fungus *T. harzianum* to solubilize *in vitro* insoluble phosphate. The following were recorded as phosphate solubilizers *Fusarium* sp., *F. oxysporum*, *Aspergillus* sp., *A. niger*, *A. tubingensis*, *A. fumigatus*, *A. terreus*, and *A. awamori* (Nopparat *et al.* 2009; Morales *et al.* 2011; Abdel-Ghany *et al.* 2018b).

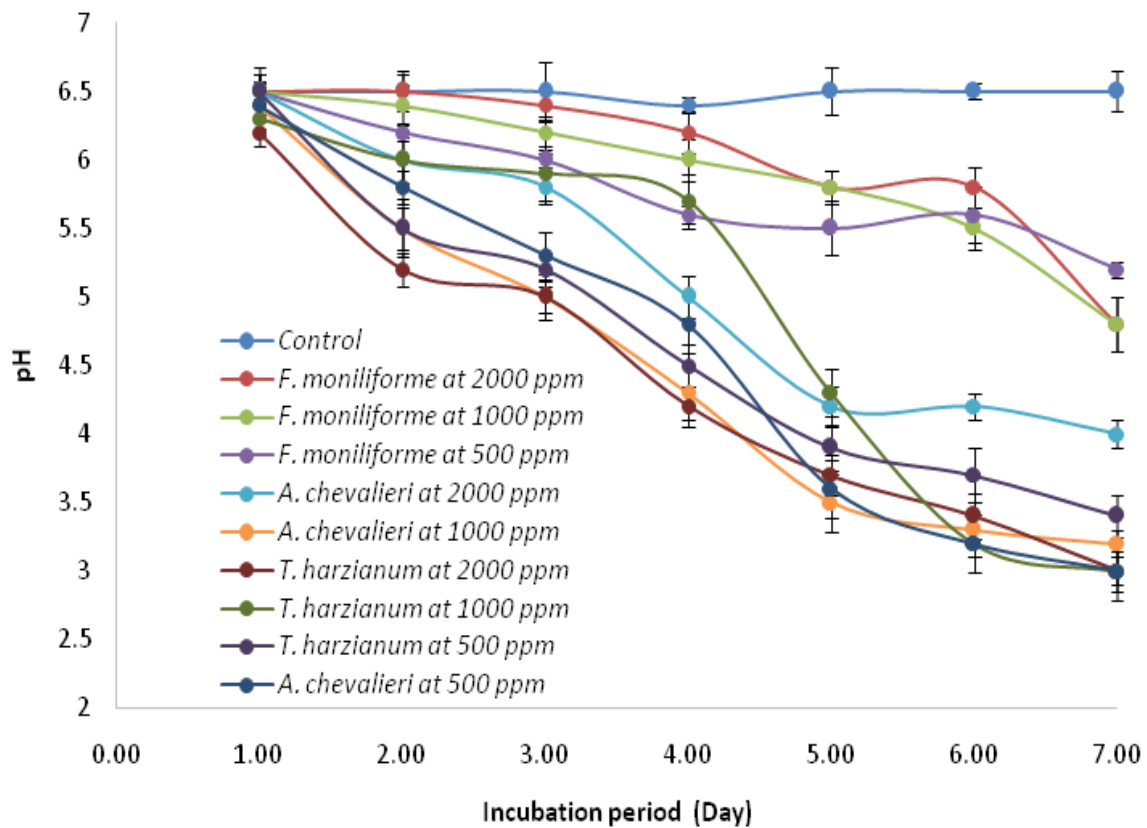


Fig. 3. pH detection at different incubation periods of fungal growth medium containing different doses (ppm) of ZP

Table 1. Solubilization Index of the ZP at Different Temperatures with *A. chevalieri*

ZP (ppm)	Temperature (°C)							
	10		20		30		40	
	Growth ± SD* (mm)	SI	Growth ± SD* (mm)	SI	Growth ± SD* (mm)	SI	Growth ± SD* (mm)	SI
500	7.33±0.58	2.02	19.67±0.58	2.22	24.67±1.53	2.54	22.67±0.58	2.82
1000	7.67±0.58	2.17	22.67±1.15	2.28	25.33±0.58	2.66	23.33±0.58	3.13
2000	5.67±1.15	2.15	20.33±0.58	2.33	20.33±0.58	2.87	18.67±0.58	3.11

*Standard deviation

Table 2. Solubilization Index of the ZP vs. Temperature with *F. moniliforme*

ZP(pp m)	Temperature (°C)							
	10		20		30		40	
	Growth ± SD* (mm)	SI	Growth ± SD* (mm)	SI	Growth ± SD* (mm)	SI	Growth ± SD* (mm)	SI
500	8.65±0.58	2.30	26.67±1.15	3.04	22.33±1.15	2.75	7.67±0.58	2.25
1000	8.67±0.58	2.66	27.67±0.58	3.14	25.00±1.00	3.08	8.33±0.58	2.10
2000	10.33±1.15	2.58	29.33±0.58	3.14	24.67±1.53	3.20	8.67±0.58	2.50

*Standard deviation

Table 3. Solubilization Index of the ZP vs. Temperature with *T. harzianum*

ZP (ppm)	Temperature (°C)							
	10		20		30		40	
	Growth ± SD* (mm)	SI	Growth ± SD* (mm)	SI	Growth ± SD* (mm)	SI	Growth ± SD* (mm)	SI
500	5.67±0.58	2.10	17.33±1.15	2.22	30.67±1.15	2.20	27.00±1.73	2.31
1000	7.00±1.00	2.10	19.33±1.15	2.40	31.33±1.15	2.31	29.76±0.58	2.50
2000	6.67±1.15	2.00	19.67±0.58	2.35	31.67±0.58	2.41	29.00±1.00	2.50

*Standard deviation

To investigate the effect of temperature on the ZP solubilization process, the processes were conducted using *F. moniliforme*, *A. chevalieri*, and *T. harzianum* at different doses of ZP (0.5 g/L, 1 g/L, and 2 g/L) and different temperatures (10 °C, 20 °C, 30 °C, and 40 °C) for ZP solubilization. The released P was monitored during the solubilization process under the aforementioned conditions. The results indicated that the released concentration of P (taken as solubilization efficiency indicator) was increased as the temperature increased. As shown in Table 4 and Fig. 4, the greatest P values with *F. moniliforme*, *A. chevalieri*, and *T. harzianum* were 5.04 mg/L, 10.65 mg/L, and 13.18 mg/L, at 30 °C and a dose of 2 g/L of ZP, respectively. In contrast, the smallest P values were 4.98 mg/L, 6.44 mg/L, and 5.19 mg/L, at 10 °C and a dose of 0.5 g/L of ZP, with *F. moniliforme*, *A. chevalieri*, and *T. harzianum*, respectively. This result may be because increasing the temperature increased the production of organic acids which decrease the pH of the solubilization medium, increasing ZP solubilization (Whitelaw 1999; Barroso *et al.* 2006). Upon increasing temperature from 30 °C to 40 °C, the released P either decreased or showed no appreciable increase. This result may be because of cell death of the microorganisms (Rinu and Pandey 2010).

Table 4. P Releasing (mg/L) at Different Temperatures Using Fungal Isolates

Fungal Isolate	ZP Dose (g/L)	Temperature (°C)			
		10	20	30	40
Without inoculation	2.0	2.72	2.72	2.72	2.72
<i>F. moniliforme</i>	0.5	4.98	5.01	5.04	2.17
	1.0	6.44	10.30	10.65	3.13
	2.0	5.19	13.36	13.18	4.74
<i>A. chevalieri</i>	0.5	2.11	3.76	4.91	5.08
	1.0	3.22	8.33	8.38	9.74
	2.0	3.58	8.33	8.43	8.79
<i>T. harzianum</i>	0.5	2.72	4.08	4.42	5.10
	1.0	3.44	10.03	10.15	10.51
	2.0	3.48	9.86	11.41	12.43

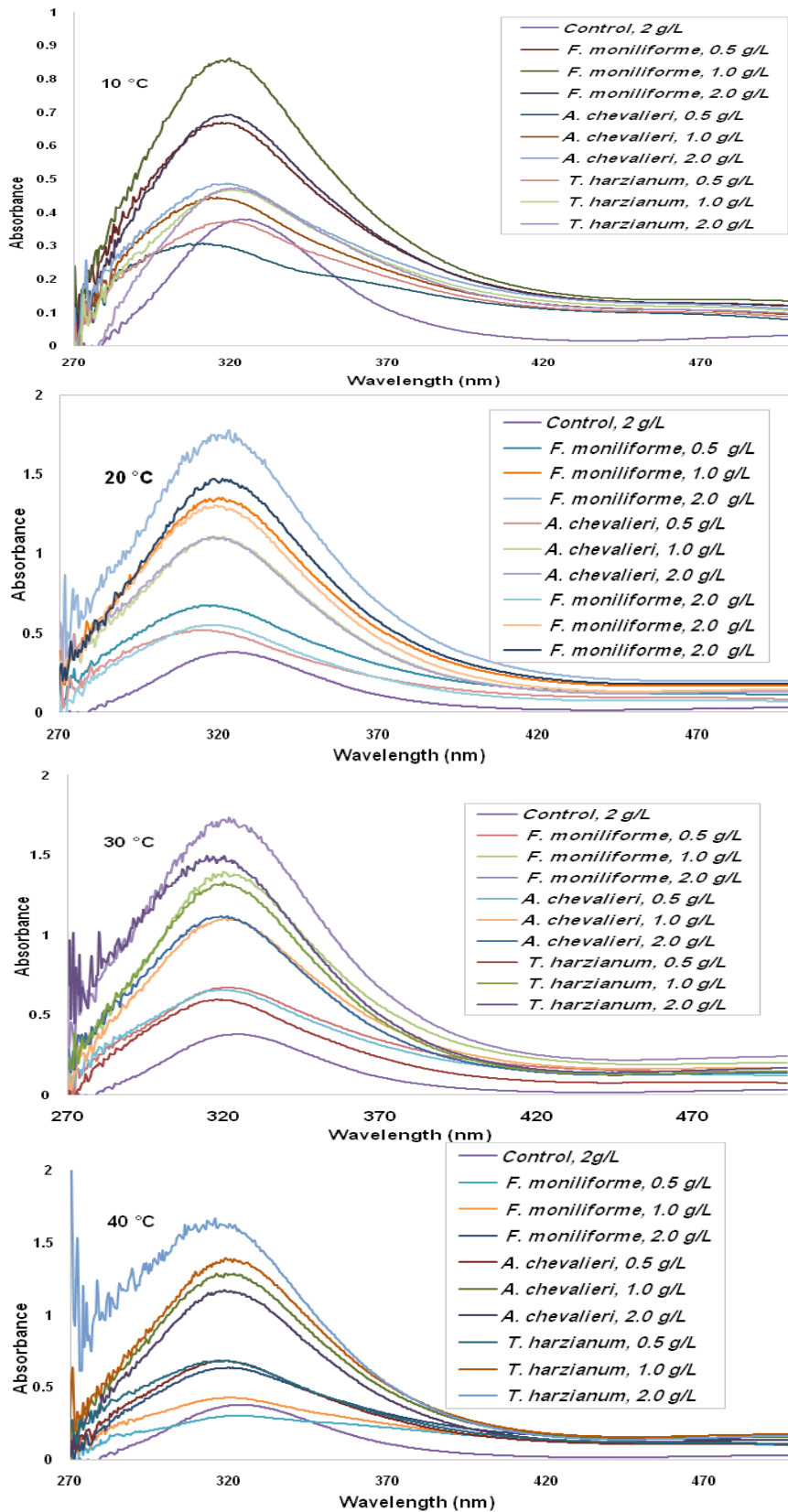


Fig. 4. UV spectra of P releasing (mg/L) at different temperatures using fungal isolates; Control (without fungal inoculation)

CONCLUSIONS

1. Rhizospheric fungi including *F. moniliforme*, *A. chevalieri*, and *T. harzianum* were effective for ZP solubilization.
2. The results indicate that co-application of fungal isolates with insoluble phosphate sources has a positive impact on plant growth. However, this result needs to be tested under field conditions.
3. Evidence from the study proved that a decrease in final pH occurred during solubilization of phosphorus in the medium.
4. Factors such as pH, temperature, and fungal species may affect the efficacy of phosphate solubilization.
5. More studies are needed to understand the mechanisms underlying the liberation of soluble phosphate by fungal isolates and its benefits as bio-inoculants.

Conflict of Interest: The authors declare no conflicts of interest.

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