Effect of Fungicides on Phosphate Solubilization by *Klebsiella oxytoca* and *Enterobacter ludwigii*

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The aim of the present study was to isolate phosphate solubilizing bacteria (PSB) and to assess their potential tolerance to fungicides. Out of thirty PSB, two strains *Klebsiella oxytoca* and *Enterobacter ludwigii* were selected on the basis of their tolerance to fungicides. Both strains were assessed for their phosphate solubilizing ability using three different fungicides (difenoconazole, fluazinam and streptomycin) each with the concentrations of 0, 1, 2 or 3 times of the recommended rate. Both strains showed increased phosphate solubilization with difenoconazole at 1, 2 and 3 times of the recommended rate as compared to the phosphate solubilization of the control. The phosphate solubilization in *Klebsiella oxytoca* was recorded as 326, 538, 518 and 481 µg mL\(^{-1}\) at 0, 1, 2 and 3 times of the recommended rate respectively, whereas in *Enterobacter ludwigii* it was recorded as 395, 499, 529 and 533 µg mL\(^{-1}\) respectively at various doses. Based on the present findings, it may be concluded that both strains have the potential to be used as bio-inoculants which can solubilize phosphate even at the higher doses as compared to the recommended rate of fungicides.

Key words: Phosphate solubilization, Fungicides, *Klebsiella oxytoca*, *Enterobacter ludwigii*, Difenoconazole, Fluazinam, Streptomycin

**Introduction**

Use of agrochemicals (such as fertilizers, pesticides, soil conditioners and phytohormones) aiming at optimizing crop production has become very common in agriculture (Ahemad and Khan, 2011). Pesticides, the major plant protection agents include a wide range of compounds including insecticides, fungicides, bactericides, herbicides, rodenticides, molluscicides and nematicides (Aktar et al., 2009). Use of pesticides increased by 3-fold over the past several decades due to intensified agricultural systems (Fox et al., 2007).

Many pesticides showed no detectable effects on soil microorganisms at the recommended application rates. However, application at increased rates is often reported as most of the farmers decide based on their own experience of the effective pest control. Repeated and overuse of pesticides in agriculture is a matter of concern because these chemicals are recognized as a source of potential adverse impacts on the metabolic activities of soil microorganisms as well as their plant growth promoting characteristics (Wani et al., 2005; Ahemad et al., 2009).

Some microorganisms (called as phosphate solubilizing microorganisms-PSMs) perform phosphate solubilization. Their growth and phosphate solubilizing activity may also be affected by the pesticides leading to the imbalance phosphorous nutrition for the crop plants. However, the effect of pesticides on microbial growth and their activity especially phosphate solubilization can only be assessed using microorganisms which are tolerant to the pesticide of concern (Oves et al., 2009). Under this background, in the present investigation, an attempt has been made to examine the effects of different fungicides applied at various rates on *Klebsiella oxytoca* and *Enterobacter ludwigii*, the two phosphate solubilizing bacteria.

**Materials and Methods**

Isolation of phosphate solubilizing microorganisms

Phosphate solubilizing microorganisms used in this study were isolated from the soils collected from green houses in Chungcheongnam-do, Gongju-gun area and abounded mines of Boryeong area in South Korea. In order to isolate, a serial dilution assay was carried out in 0.85% NaCl solution and 10 µl of
Table 1. Fungicides used in the study.

<table>
<thead>
<tr>
<th>Common name</th>
<th>Chemical name</th>
<th>Chemical family</th>
<th>Recommended dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Difenoconazole</td>
<td>Cis-trans-3-chloro-4-[4-methyl-2-(1H-1, 2, 4-triazol-1-ylmethyl)-1, 3-dioxolan-2-yl]phenyl 4-chlorophenyl ether</td>
<td>Triazole</td>
<td>0.5 mL L(^{-1})</td>
</tr>
<tr>
<td>Fluazinam</td>
<td>3-chloro-N-(3 chloro-5-trifluoromethyl-2-pyridyl)-α α-α-trifluoro-2,6-dinitro-p-toluidine</td>
<td>Dintroaniline</td>
<td>0.5 g L(^{-1})</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>O-2-deoxy-2-methylamino-α-L-glucopyranosyl-(1 to 2)-O-5-deoxy-3-C-formyl-α-L-lyxofuranosyl-(1 to 4)-N3-diamidino-D-streptamine-sulfate</td>
<td>Antibiotics</td>
<td>0.5 g L(^{-1})</td>
</tr>
</tbody>
</table>
zation with difenoconazole at various doses of the pesticide (Fig. 1), higher as compared to the control. The values for phosphate solubilization by *Klebsiella oxytoca* were recorded as 326, 538, 518 and 481 µg mL\(^{-1}\) for 0, 1, 2 and 3 times of the recommended rate respectively, whereas for *Enterobacter ludwigii* it was recorded as 395, 499, 529 and 533 µg mL\(^{-1}\) respectively for the different doses. The results were in agreement with Ramani (2011) who reported enhancement in phosphate solubilization by *Bacillus sp* and *Pseudomonas cepacia* respectively with chlorpyriphos and monocrotophos at 1X and 2X of the recommended rate. Narison (1995) also reported similar results with chlorpyriphos and quinalphos by *Aspergillus aculeatus*.

There are some reports about microorganisms which could degrade complex compounds in pesticides. Microbes used these compounds as their carbon and energy sources. Sangodkar et al. (1998) and MacLoughlin et al. (1992) reported that *P. cepacia* had the ability to degrade chlorinated aromatic substances present in complex pesticides and herbicides. Sopid (2012) isolated novel atrazine degrading *Klebsiella sp.* from a long term atrazine treated sugarcane field.

Fig. 2 shows the effect of various concentrations of fluazinam on phosphate solubilization by *Klebsiella oxytoca* and *Enterobacter ludwigii*. Phosphate solubilization by *Klebsiella oxytoca* decreased with increasing concentrations of fluazinam from 1 to 3 times of the recommended rate. It was decreased by 19%, 29% and 32% respectively for the fluazinam concentrations of 1, 2 and 3 times of the recommended rate compared to the control. However, there was no significant reduction (P ≤ 0.05) in phosphate solubilization by *Enterobacter ludwigii* with increasing fluazinam concentrations and it ranged from 395 µg mL\(^{-1}\) for the control to 384 µg mL\(^{-1}\) for 3 times of the recommended rate of fluazinam.

Fig. 3 shows the effect of various concentrations of
streptomycin on phosphate solubilization by *Klebsiella oxytoca* and *Enterobacter ludwigi*. Similar to fluazinam, phosphate solubilization by *Klebsiella oxytoca* decreased with increasing concentrations of streptomycin from 1 to 3 times of the recommended rate. However, there was no significant reduction (P ≤ 0.05) in phosphate solubilization at the recommended rate compared to the control. However, phosphate solubilization by *Enterobacter ludwigi* decreased by 40%, 50% and 52% respectively for the streptomycin concentrations at 1X, 2X and 3X of the recommended rate compared to the control. The decrease in phosphate solubilization with fluazinam and streptomycin probably may be due to the impairment of various metabolic activities of the organisms (Kumar et al., 2010).

However, comparatively to the present values, Ahemad and Khan (2012a) reported severe reductions in phosphate solubilization (94%, 91%, 89% and 83% at 3X of the recommended rates of tebuconazole, hexaconazole, metalaxyl and kitazin respectively) by *Klebsiella sp.* isolated from mustard rhizosphere. In a separate study, the same authors (Ahemad and Khan, 2012b) observed more or less similar reduction in phosphate solubilization (95%, 94%, 94% and 82% at 3X of the recommended rate of metalaxyl, tebuconazole, hexaconazole, and kitazin respectively) by *Pseudomonas putida* isolated from mustard rhizosphere. Therefore, in contrast, both strains of the present study showed considerably high phosphate solubilizing potential even at the higher doses of the fungicides.

The negative relationship between pH and phosphate solubilization was observed in all the experiments. Decrease in medium pH accompanied by phosphate solubilization may indicate that lowering pH of the medium through organic acid production may be the possible mechanism for phosphate solubilization as reported by other researchers as well (Yasmin and Banu, 2011; Yu et al., 2011).

**Conclusion**

Both strains showed phosphate solubilizing potential even at the higher doses over the recommended rates of fungicides. Furthermore, strains showed relatively higher phosphate solubilization with difenoconazole than the other pesticides tested. Therefore, these fungicide tolerant strains as bio-inoculants would have attractive beneficial impacts on sustainable agricultural practices. Field studies with an assessment on the effect of fungicides on the other plant growth promoting activities of the strains would still be needed in exploring the potential agricultural significance of the strains further.

**References**


Ahemad, M. and M.S. Khan. 2012b. Effect of fungicides on plant growth promoting activities of phosphate solubilizing
Phosphate solubilizing bacteria


