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Short note

EFFECT OF NATIVE *TRICHODERMA ASPERELLUM* ISOLATE ON PHOSPHATE SOLUBILIZATION AND GROWTH OF PEA

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ABSTRACT

Application of phosphorus solubilizing microbes is a promising tool for enhancing P uptake in plant. The aim of this study was to evaluate the phosphate solubilization potential and growth promotion of pea (*Pisum sativum* L.) by native *Trichoderma asperellum* isolate G3. Phosphate solubilization by *T. asperellum* strain was assessed in National Botanical Research Institute's Phosphate (NBRIP) broth medium. Pea plants were inoculated by *Trichoderma* strain and grown in pot in phosphate deficit condition. The fungal strain was able to solubilize phosphate (from 188.95±2.04 to 262.50±3.80 mgL^{-1}) in broth at different time periods and decreased solution pH. The *Trichoderma* inoculated pea plant increased the root growth, shoot growth, leaf number, shoot biomass, root biomass, total dry weight, chlorophyll a, chlorophyll b and carotenoid by 23.9%, 33.3%, 33%, 37.1%, 32.7%, 28.4%, 24.5%, 17.4% and 14.7% respectively over control after 5 weeks of post inoculation. The results showed that the native *T. asperellum* isolate G3 has great potential in the phosphorus solubilization.

Keywords: *Trichoderma*, phosphorus, solubilization, pea.

Phosphorus is required in relatively large amounts by plants for optimal growth. Although, most agricultural soils contain high concentrations of phosphorus, in both inorganic and organic forms, but that is poorly available for plant uptake due to its immobilization and fixation with other soil minerals as insoluble forms. Plant growth promoting microorganisms mobilize P in plant rhizosphere by hydrolysis and solubilization of insoluble phosphates and increase P availability for plant uptake (Oliveira *et al.*, 2009). In addition, plant growth promoting microorganisms increase plant growth by stimulating plant growth promotion hormones like auxins, reducing plant disease and abiotic stresses (García-López *et al.*, 2016). Thus, application of plant growth promoting microorganisms in the plant rhizosphere would be an effective approach for nutrient management and growth promotion of crop plants.

The *Trichoderma* belongs to Ascomycota, subdivision Pezizomycotina, class Sordariomycetes, order Hypocreales and family Hypocreaceae are found in most soils. Many of *Trichoderma* isolates are also well known for solubilizing complex form

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of soil phosphates to readily available form for plants (Bononi *et al.*, 2020). In a survey work, a novel strain of *Trichoderma asperellum* (NCBI accession MW052549) was isolated from agricultural soil of Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur. The present study was undertaken to investigate effect of native *T. asperellum* on the phosphorus solubilization and subsequent growth promoting effect on pea plant under phosphate deficit condition.

The *in vitro* phosphate solubilizing capacity of *Trichoderma* isolate was assayed in National Botanical Research Institute Phosphate (NBRIP) broth medium (Nautiyal, 1999) and analyzed for pH over a period of time. The phosphate concentration (ppm) was measured by the method described by Fiske and Subbarow (1925). Sterilized pea seed were planted in potting mixture (sand: vermiculite, 1:1 v:v) treated with either *Trichoderma* or agar plug (control). Plants were watered with low phosphate Long Ashton Nutrient Solution (LANS) medium where source of phosphate was replaced by $Ca_3(PO_4)_2$ twice in a week (Hewitt, 1966). After five weeks, whole plants were removed from the potting mixture and total shoot length, root length, plant height, number of leaves, chlorophyll, carotenoid contents and dry weight were recorded.

The *T. asperellum* isolate G3 significantly reduced $Ca_3\ (PO_4)_2$ and increased soluble phosphate concentration 188.95±2.04 to 262.50±3.80 mg/L in NBRIP broth (Table 1). The soluble phosphate concentration was increased in 24 h to 72 h of incubation and decreased later. The *T. asperellum* isolate G3 dropped the pH neutral to acidic condition, approximately 7.15 to 5.05 (Fig. 1). *Trichoderma* isolates studied by Bader *et al.*, (2020) also showed the pH decreasing properties in broth.

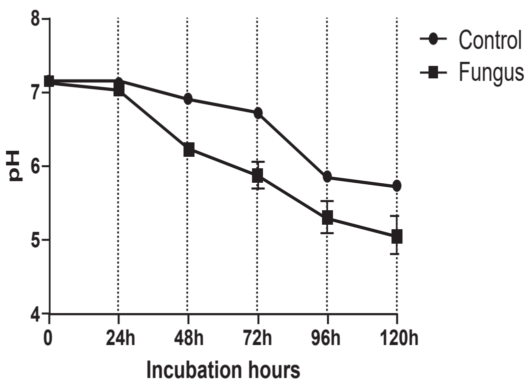

Fig. 1. The pH changes (mean±SEM) by *T. asperellum* isolate G3 in NBRIP broth at different time intervals.

Table 1. Solubilization of tri-calcium phosphate in NBRIP broth by *T. asperellum* isolate G3 at different hours of incubation (The mean values were compared by paired t$^-$ test, p < 0.01 at individual time point)

Incubation period	Control (Soluble P mgL^{-1})	With fungus (Soluble P mgL^{-1})
24h	67.56±1.22	188.95±2.04***
48h	76.40±2.33	212.47±1.41***
72h	90.36±1.65	262.50±3.80***
96h	93.60±1.47	241.46±2.03***
120h	81.93±2.10	228.96±3.36***

*** Significant at p < 0.01

Table 2. Effect of the *T. asperellum* isolate G3 on pea (The means were compared by paired t⁻ test)

Plant parameter	Control	With Trichoderma	% over control
Shoot height (cm)	58.6±1.21	72.6±2.46***	23.9
Root length (cm)	19.2±0.92	25.6±1.29***	33.3
Leaves number (pairs)	21.8±1.07	29±1.14***	33.0
Shoot biomass (g/plant)	1.332±0.05	1.826±0.09***	37.1
Root biomass (g/plant)	0.808±0.07	1.072±0.06***	32.7
Total dry wt (g/plant)	0.232±0.15	0.298±0.007***	28.4
Chlorphyll a (mg/g FW)	0.106±0.002	0.132±0.004***	24.5
Chlorphyll b (mg/g FW)	0.0596±0.0002	0.07±0.002***	17.4
Carotenoid (mg/g FW)	6.13±0.13	7.03±0.49***	14.7

*** Significant at $p < 0.01$

The effect of *T. asperellum* isolate G3 on pea plant in phosphate deficit condition was determined by measuring growth parameters after five weeks of treatment (Table 2). Pea plants inoculated with *T. asperellum* increased plant growth. The fungus inoculated plants increased shoot and root length by 23.9% and 33.3% over control, respectively. The inoculated plants showed higher proliferation (29±1.14) of pair leaves number than control (21.8±1.07). All inoculated plants showed an increase in the fresh shoot and root weight which were 37.1% and 32.7% higher in comparison with the control, resultantly also increased the total biomass (28.4% over control). The inoculation of pea plants with *Trichoderma* strains significantly increased both the chlorophyll a and chlorophyll b and carotenoid over control by 24.5%, 17.4% and 14.7%, respectively.

In pot experiment, it was noted that the *Trichoderma* inoculated plants showed higher shoot and root growth as compared to non-inoculated plants in phosphate deficit condition. Similar result was obtained by Roksana *et al.* (2019), where *Trichoderma* fortified poultry manure showed 55% increase of plant height as compare to control. The increased root growth further extended the root architecture results in increase of fungus colonization area in root and expanded root system for nutrient uptake (Berg, 2009). The strain of *Trichoderma* also increased the number of leaf and chlorophyll contents in inoculated plants (Table 2). This data indicates more photosynthesis and more plant anabolism process (Bader *et al.*, 2020).

The results suggest that the *T. asperellum* isolate G3 isolated from our native soil increases the growth of pea plants in phosphate deficit condition by increasing availability of phosphate in rhizosphere and increasing the roots for more nutrient uptake enhancing more shoot and leaf for more photosynthesis. However, further study is needed in field condition using the same strain.

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