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Research article

Solubilization by nematode-controlling *Trichoderma* of nutrients to promote rice growth, yield and phosphorus content

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Abstract

Importance of the work: Nematode-controlling *Trichoderma* spp. have various potential uses, one of which is the ability to dissolve minerals in the soil for RD31 rice growth promotion.

Objectives: To evaluate the efficacy of *Trichoderma* and the effects of solubilized phosphate by *Trichoderma* on the growth and yield of rice in the greenhouse.

Materials & Methods: The experiment used a complete randomized design with 12 treatments and 4 replications. The effect of *Trichoderma* was analyzed on the efficiency of acid production, phosphorus availability and potassium exchangeability. The growth, yield and phosphorus content of rice were examined.

Results: In total, 30 isolates produced acid. The isolate NTW1.1/1 produced the highest phosphorus availability (207.64 mg/L) and the isolate Sp1 had the highest potassium exchangeability (1.35 mg/L). At 120 d, the isolate NTW1.1/1 increased the height and tillering of rice (109.29 cm and 15.00 plants/hill, respectively). *Trichoderma* increased the rice yield by 18.17% compared to uninoculated rice. This isolate with a chemical fertilizer level of 100% produced the highest phosphorus content in the rice plants.

Main finding: The solubility effect of *Trichoderma* on phosphorus or potassium, or both, was appraised and NTW1.1/1 or chemical fertilizers were identified as influencing the growth and yield of rice in the Manorom soil series.

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Introduction

Khao Gor Kor 31 (RD31) is a Thai rice cultivar that is non-photosensitive and can be grown all year with high yields, good grain quality and disease resistance (Taprap et al., 2009). In Thailand, the off-season rice cultivation area is 1.17 million ha, with a total yield of 4.55 million t and a rice value of USD 1,062.61 million (Office of Agricultural Economics, 2020). Several main nutrients are needed by rice (Shrestha et al., 2020). Nitrogen stimulates plant growth and encourages plants to establish well in the early growth stages, as well as being necessary for the synthesis of various constituents (Cooper, 2000). Phosphorus accelerates leaf and stem growth, is used in the creation of cell membranes and is required for metabolic processes (Schachtman et al., 1998). Potassium accelerates flowering seed formation and is associated with osmotic pressure in the cell and the transportation of compounds in plants, as well as being required for metabolic processes and stress control (Hasanuzzaman et al., 2018). Application of biofertilizers has been used in soil and fertilizer management in rice cultivation to support an environmental-safe agriculture system (Atieno et al., 2020).

Trichoderma spp. are present naturally in plant residues, organic matter and soils and they are applied to combat antagonistic microorganisms as well as currently being very popular as a commercial biological regulator (Zin and Badaluddin, 2020). The hatching level of nematode eggs was significantly decreased by *T. harzianum* BI (Mukhtar et al., 2021). Several *Trichoderma* species have properties to control plant pathogenic fungi (Thambugala et al., 2020) and can be also used in promoting plant growth (Tančić-Živanov et al., 2020). *T. asprellum* SL2 promoted rice seedling growth by increasing germination, plant height, leaf count, root length and rice plant biomass (Doni et al., 2016). Various fungal mechanisms can increase nutrient absorption, carbohydrate metabolism and photosynthesis in plants and plant growth can be stimulated by affecting the balance of plant hormones (Stewart and Hill, 2014). The fungi were able to dissolve nutrients, especially phosphorus and potassium, making them useful for plants. The phosphate-solubilizing ability of *Trichoderma* strains has been reported to improve plant growth, phosphorus uptake and photosynthetic pigment contents in plant (Bedine et al., 2022). In total, 14 strains of *Trichoderma* have been isolated from the vegetative zone of forest trees. The maximum soluble phosphate content was 404.07 mg/L after 96 hr (Anil and Lakshmi, 2010) and *T. koningii* and *T. harzianum* were able to solubilize potassium (Nahidan et al., 2019). *T. koningiopsis* (NBRI-PR5) also produced phosphate solubilization under abiotic stress conditions (Tandon et al., 2020). Therefore, it is possible to use *Trichoderma* as a biofertilizer, which is effective both in controlling nematode diseases and promoting plant growth for rice cultivation.

The current study investigated phosphate and potassium solubility efficiency based on the acid production mechanism to release available phosphorus and exchangeable potassium and the nematode-controlling ability of *Trichoderma* (NTW1.1/1) with different rates of chemical fertilizers to promote rice growth and productivity. The results of this study should be useful in the development of commercial bio-products that can both resist nematodes and dissolve phosphates.

Materials and Methods

Trichoderma isolates, identification and efficiency

The *Trichoderma* isolates were collected along with their corresponding geographical information from soil samples in central Thailand. This was achieved through the serial dilution plate method. Subsequently, each fungal isolate was cultivated on potato dextrose agar (PDA), of analytical reagent (AR) grade and then was incubated at 25°C under a photoperiod of 12 hr light/12 hr dark for 5 d for further study. The 30 isolates of *Trichoderma* spp. were evaluated in other research (data unpublished) for *in vitro* effects against the juveniles 2 (J2) stage of the root knot nematode, *Meloidogyne incognita*. *Trichoderma* spp. were kept on culture collection by the Department of Plant Pathology, Faculty of Agriculture at Kamphaeng Saen, Kasetsart University, Kamphaeng Saen campus, Nakhon Pathom, Thailand. *Trichoderma* NTW1.1/1 identification used morphological characteristics and a molecular technique based on polymerase chain reaction (PCR). The internal transcribed spacer (ITS) region (ITS-PCR) was analyzed.

Trichoderma acid production test in solid medium

In total, 30 isolates of *Trichoderma* sp. were subjected to laboratory testing, with 10 replications each. A cork borer was used to remove agar plugs of approximately 5 mm in diameter containing mycelia; the separate samples were placed on either Aleksandrov medium (Zhang and Kong, 2014) or the National Botanical Research Institute's phosphate growth medium, NBRIP (Nautiyal, 1999). The Aleksandrov medium contained 0.5% glucose, 0.05% $MgSO_4$ $7H_2O$, 0.0005% $FeCl_3$, 0.01% $CaCO_3$, 0.2%$Ca_3(PO_4)_2$, 0.2% $KAlSi_3O_8$ and 15 g agar that was adjusted to a pH range of 7.0–7.5 and modified by adding bromocresol purple (100.0 mg/L). The NBRIP medium contained 10 g glucose, 10 g $Ca_3(PO_4)_2$, 5 g $MgCl_2.6H_2O$, $MgSO_4.7H_2O$ 0.25 g, 0.2 g KCl, 0.1 g $(NH_4)_2SO_4$ and 15 g of agar, adjusted to pH 7 and was modified by adding bromocresol purple (100.0 mg/L). All the chemicals used were analytical AR grade. The diameters of clear zones and colonies were measured after 3 d of incubation. At 30°C, the size of the clear zone was calculated from the diameter of the clear zone per colony.

Ability of Trichoderma spp. to solubilize potassium and phosphate in liquid media

Phosphate and potassium solubilization of the 30 *Trichoderma* sp. isolates were evaluated in the laboratory. The *Trichoderma* sp. were cultivated in a 150 mL flask with 4 replications containing 100 mL of the Aleksandrov or NBRIP broth containing $Ca_3(PO_4)_2$ as a phosphate source and $KAlSi_3O_8$ as a potassium source. A cork borer was used to remove an agar plug approximately 5 mm in diameter containing mycelia and this was placed in a liquid medium. *Trichoderma* sp. Spores (1×10^6 colony forming units (CFU)/mL were incubated for 5 d at 30°C, shaken at 120 revolutions per minute (rpm)/min and then centrifuged at 10,000 rpm for 10 min, after which the clear part was analyzed for potassium and phosphate solubilization. The exchangeable potassium content was analyzed according to the method described by Parmar and Sindhu (2013), based on a pipetted amount of 1 mL of *Trichoderma* sp. The potassium content was analyzed using an atomic absorption spectrometer (Agilent Technologies, USA). The potassium concentration was determined based on the standard KCl curves, showing the potassium content in milligrams per milliliter. Evaluation of the phosphate released in liquid NBRIP was performed by analyzing the available phosphate (Fiske and Subbarow, 1925) in a 2 mL pipette amount of the transparent *Trichoderma* sp. with 4 mL of ammonium molybdate-ascorbic acid, measured using a spectrophotometer (Thermo Scientific; USA) at a wavelength of 882 nm. The phosphate concentration was determined based on the standard curve of KH_2PO_4, showing the phosphate content in milligrams per milliliter.

Soil properties analysis before and after plantation

Samples were randomly collected at a depth of 0–30 cm in the Manorom soil series in Tha Phaeng sub-district, Manorom district, Chainat province, Thailand (15.3637161°N, 100.1529319°E). Some of the soil properties were analyzed in terms of nutrient contents for planting rice. The basic soil information collected was the pH and electrical conductivity (EC). Soil samples were digested in a H_2SO_4-Na_2SO_4-Se mixture as specified to analyze the total nitrogen content based on the Kjeldahl method, the soil organic matter (Walkley and Black, 1934), the phosphorus content (Bray and Kurtz, 1945) and potassium using atomic absorption (Pratt, 1965).

Effect of phosphate solubilization of Trichoderma sp. on growth and rice yield

Trichoderma isolate NTW1.1/1 was used to study the growth and rice yield. Rice grown in a square plastic tray was established based on a completely randomized design with 12 treatments, each with 4 replications, where T1 = no microorganisms and chemical fertilizers; T2 = no microorganisms + 100% chemical fertilizers; T3 = no microorganisms + 50% chemical fertilizers; T4 = no microorganisms + 25% chemical fertilizers; T5 = *Trichoderma* without chemical fertilizers; T6 = *Trichoderma* + 100% chemical fertilizers; T7 = *Trichoderma* + 50% chemical fertilizers; and T8 = *Trichoderma* + 25% chemical fertilizers. *Trichoderma* was applied at 1×10^6 CFU/ml and soaked with 30 g of rice seeds for 24 hr. The seeds were wrapped in a thin white cloth for 36 hr until they germinated. Five seedlings of the rice cultivar Khao Gor Kor 31 were planted in a square plastic tray (width 37 cm length 57 cm × height 20 cm). Fertilizer based on soil analysis (37.5-18.75-0 of N:P_2O_2:K_2O kg/ha) was applied after planting at 36 d, 60 d and 90 d and a water level at 10 cm was maintained throughout. The growth and yield of the rice were recorded at 120 d. The phosphorus content in the rice was analyzed.

Data analysis

The statistics were analyzed using the R program v.3.5.1 (R Core Team, 2018). The data were normally distributed and confirmed using the Shapiro-Wilk test (Shapiro and Wilk, 1965). Analysis of variance was employed for data analysis. Then difference between treatments was tested using Duncan's multiple range test with significance probability at <0.05.

Results

Isolate, identification and nematode destroying ability of Trichoderma NTW1.1/1

Trichoderma NTW1.1/1 was identified as *Trichoderma asperellum* based on its morphological characteristics (Fig. 1) and a molecular technique using ITS-PCR. High efficiency was identified for infection of the egg mass stage of the root-knot nematode by eight isolates: NTW1.1/1, NTW1.1/2, DR3.2, Sp4, Sp6.3/2, Ay10.1, SPB04 and Sp1. The rate of infection was 100% in each case and egg hatching was reduced by isolate NTW1.1/1 to 19.0% at 96 hr after incubation. Infestation of nematode eggs by the hyphae of isolate NTW1.1/1 is shown in Fig. 2.

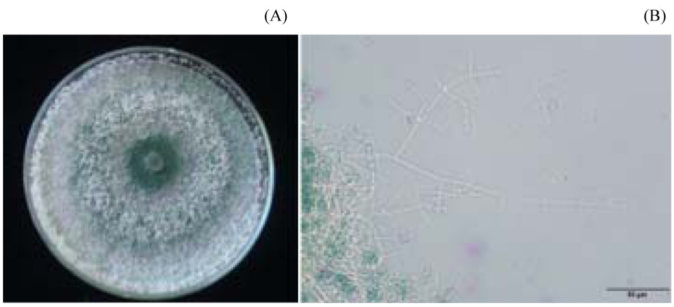

Fig. 1 *Trichoderma asperellum* (NTW1.1/1 isolate): (A) on potato dextrose agar incubated at 25°C under a photoperiod of 12 hr light/12 hr dark for 5 d; (B) under compound microscope at 40×

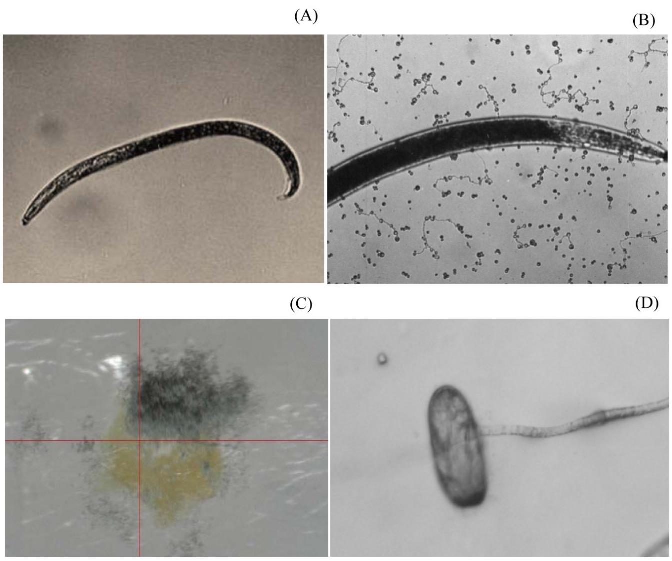

Fig. 2 Nematodes infested by isolate NTW 1.1/1 under a microscope: (A) *Meloidogyne incognita* at 200×: (B) spore suspensions of *Trichoderma* isolate NTW1.1/1 germinated and starting to infect and colonize nematodes at 400× on water agar culture; (C) fungal mycelia destroying *M. incognita* J2 egg mass (yellow color) at 200×; (D) egg penetrated by hypha at 400×

Acid production by Trichoderma spp. for phosphate or potassium solubilization

The bromocresol purple indicator has a color-pH range of yellow 5.2 to purple 6.8. All the isolates of *Trichoderma* tested were acidic, as indicated by the color change from purple to yellow. The fungi growing in Aleksandrov medium were used in the potassium solubility assay (Fig. 3A) and the fungi growing in the NBRIP were used in the phosphate solubility assay (Fig. 3B). The discolored zone diameter is shown in Fig. 3C. All isolates secreted organic acids and dissolved minerals. The highest acidic zones of Sp6.3/2 and NTP11 were 6.83cm ($p < 0.05$) and 8.38 cm at 3 d in the Aleksandrov and NBRIP media, respectively, that were significantly ($p < 0.05$) different from other isolates.

Solubilization by Trichoderma spp. of phosphate or potassium in liquid medium

The phosphate and potassium solubility assays of *Trichoderma* spp. were incubated for 5 d at 30°C in the NBRIP and Aleksandrov media. The potassium exchangeability (Fig. 4) and the phosphorus availability (Fig. 5) were analyzed and the results were significantly ($p < 0.05$) different. All isolates were phosphate-soluble. The maximum phosphorus availability of the NTW1.1/1 isolate was 207.64 mg/L ($p < 0.05$), while the highest potassium exchangeability levels of the sp1 and sp3.3 isolates were 1.35 and 1.30 mg/L, respectively ($p < 0.05$).

Properties of Manorom soil series before planting

The Manorom soil series was a clay loam. Reaction was moderately acidic (pH 5.92), the EC at 0.65 dS/m was not salty, organic matter was quite high (2.95%), total nitrogen was high (0.13%) and total phosphorus was high (0.04% or 400 mg/kg), while the phosphorus availability was low (8.74 mg/kg). The total potassium was 0.32% or 3,200 mg/kg and the potassium exchangeability and calcium and magnesium levels were 131.97 mg/kg, 1,054.78 mg.kg and 253.74 mg/kg, respectively. The fertilizer levels based on soil analysis for N, P_2O_2 and K_2O were 37.5 kg/ha, 18.75 kg/ha and 0 kg/ha, respectively.

Effect of phosphate solubilizing Trichoderma on growth and yield of rice in experimental greenhouse

The NTW1.1/1 isolate of *Trichoderma* was selected to investigate the cultivation of the RD31 rice cultivar in the

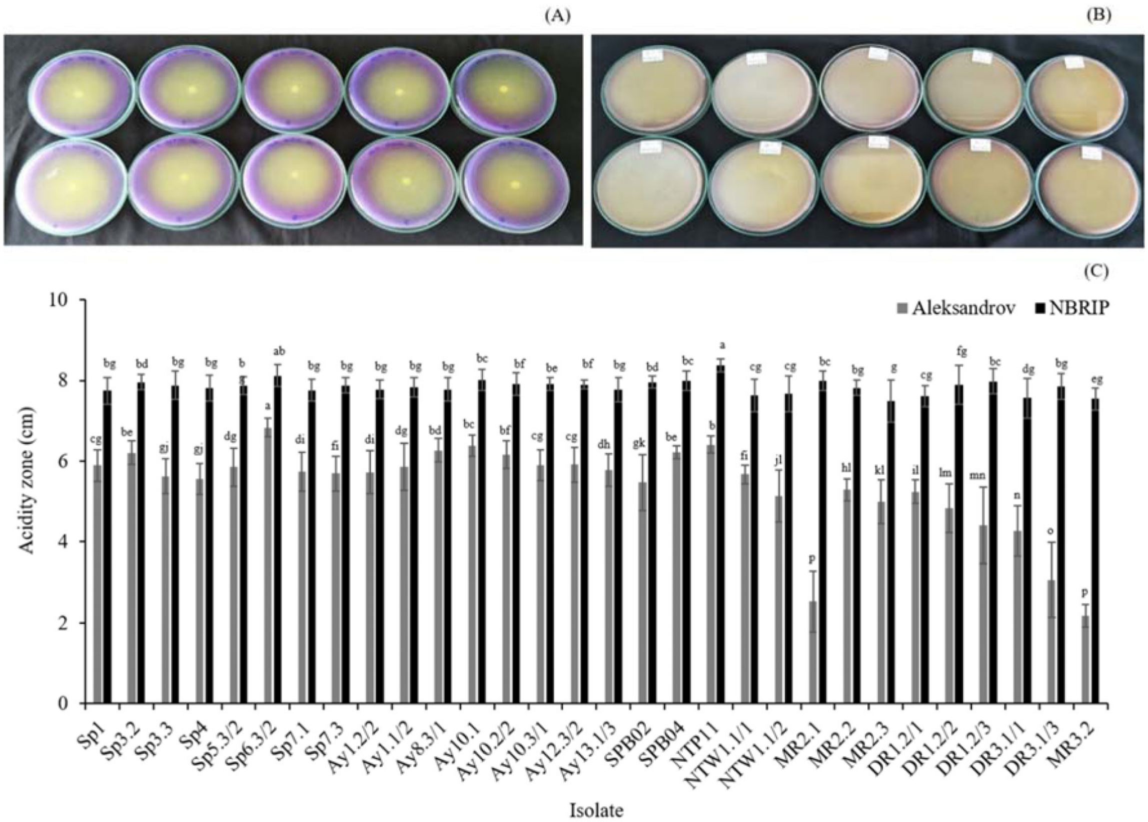

Fig. 3 *Trichoderma* isolates cultured: (A) on Aleksandrov medium at 3 d for potassium solubility assay; (B) on National Botanical Research Institute phosphate growth medium for phosphate solubility assay; (C) diameters of acidity zones, where different lowercase letters above columns are significantly ($p < 0.05$) different and error bars represent SD of 10 samples.

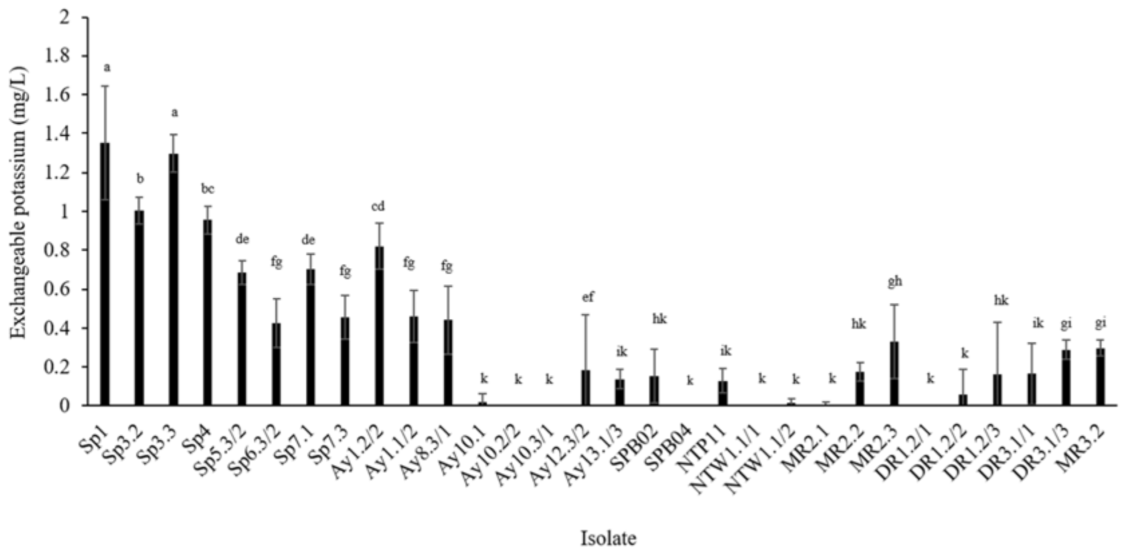

Fig. 4 Potassium exchangeability analysis using atomic absorption spectrometry for *Trichoderma* isolates incubated for 5 d at 30°C in Aleksandrov broth, where different lowercase letters above columns are significantly ($p < 0.05$) different and error bars represent SD of five samples.

greenhouse for 120 d. Illustration of the step-by-step process in using *Trichoderma* in rice cultivation and harvesting are provided in Fig. 6 and the results are shown in Tables 1 and 2. The rice height and tillering at 120 d were significantly ($p < 0.05$) different from the control (Table 1). The use of *Trichoderma* resulted in the highest height and tillering of rice plants (109.29 cm and 15.00 plants/hill, respectively) compared to the uninoculated control. The use of chemical fertilizers increased the height of rice compared to without chemical fertilizers. The rice yield and phosphorus contents at 120 d were significantly ($p < 0.05$) different (Table 2). The highest yield of the rice was 7,358.23 kg/ha in the treatment with 100% chemical fertilizer based on soil analysis. Adding *Trichoderma* increased the productivity by 18.17% compared to the uninoculated plot. However, no synergistic effect was found between the application of *Trichoderma* and chemical fertilizers, except for the phosphorus component in the rice plant. The use of *Trichoderma* only influenced the phosphorus contents, with 0.14% compared to 0.12% in the uninoculated plot. The *Trichoderma* with 100% chemical fertilizers had the highest phosphorus content level (0.17%).

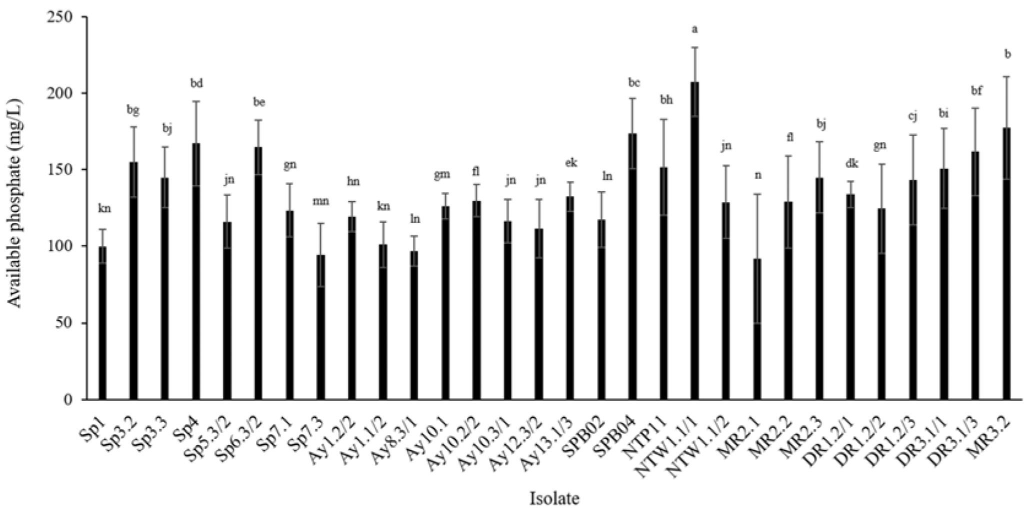

Fig. 5 Phosphorus availability analysis using spectrophotometry of *Trichoderma* isolates incubated for 5 d at 30°C in National Botanical Research Institute phosphate growth broth, where different lowercase letters above columns are significantly ($p < 0.05$) different and error bars represent SD of five samples.

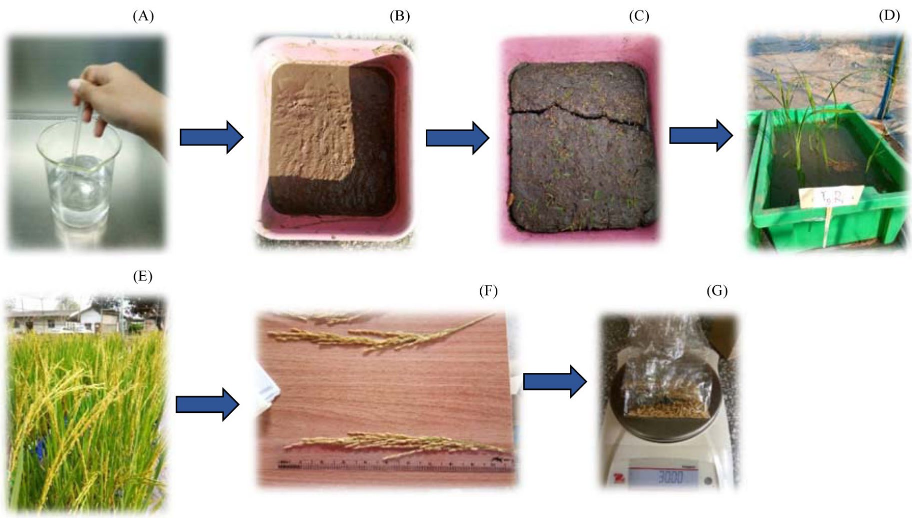

Fig. 6 Illustration of step-by-step process using *Trichoderma* in rice cultivation and harvesting: (A) soaking rice seeds in *Trichoderma* solution overnight; (B) covering with a thin white cloth for germination; (C) prepared rice seeds sowed in seedling tray; (D) seedlings aged 12 d transplanted; (E) rice plants harvested at 120 d; (F) ears measured for length; (G) seeds weighed

Table 1 Mean (± SD) of plant height and plants per hill for Khao Gor Kor 31 rice after applying with and without *Trichoderma* and four levels of chemical fertilizer treatments after 120 d where statistical analysis results (2 way ANOVA) were shown

Fertilizer level	Average plant height (cm)		Average for fertilizer level
	Microorganism application		
	Without *Trichoderma*	*Trichoderma*	
0%	102.13±2.27	107.93±2.24	105.03±2.26^{b}
100%	108.13±3.19	110.40±1.20	109.27±2.20^{a}
50%	106.30±0.62	108.93±2.88	107.62±1.75^{a}
25%	105.47±3.14	109.90±0.57	107.68±1.86^{a}
Average for microorganism	105.51±2.31^{b}	109.29±1.72^{a}	
Microorganism (M)	**		
Fertilizer level (F)	**		
M F	ns		
Coefficient of variation (%)	1.59		
Fertilizer level	Average tillering (plants/hill)		Average for fertilizer level
	Microorganism		
	Without *Trichoderma*	*Trichoderma*	
0%	12.75±1.15	16.00±1.43	14.83±1.29
100%	14.25±0.82	15.75±1.46	14.50±1.14
50%	13.25±0.94	14.25±0.90	14.33±0.92
25%	13.75±1.83	13.00±0.90	13.17±1.37
Average of microorganism	13.42±1.19^{b}	15.00±1.17^{a}	
Microorganism (M)	**		
Fertilizer level (F)	ns		
M F	ns		
Coefficient of variation (%)	7.04		

** = significant ($p < 0.01$); ns = not significant; $n = 4$ replicates; ^{a,b} = different lowercase letters above means in each category are significantly ($p < 0.05$) different.

Table 2 Mean (± SD) of yield and phosphorus contents for Khao Gor Kor 31 rice after applying with and without *Trichoderma* and four levels of chemical fertilizer treatments after 120 d where statistical analysis results (2 way ANOVA) were shown

Fertilizer level	Average yield (kg/ha)		Average of fertilizer level
	Microorganism		
	Without *Trichoderma*	*Trichoderma*	
0%	4,632.62±107.11	5,106.60±125.28	4,869.61±116.20^{c}
100%	6,371.45±109.90	8,345.00±191.17	7,358.23±150.53^{a}
50%	6,103.18±183.27	6,497.06±106.71	6,300.12±144.99^{b}
25%	5,611.09±161.16	6,898.43±185.06	6,254.76±173.11^{b}
Average of microorganism	5,679.59±140.36^{b}	6,711.77±152.06^{a}	
Microorganism (M)	**		
Fertilizer level (F)	**		
M F	ns		
Coefficient of variation (%)	13.26		
Fertilizer level	Average phosphorus contents (%)		Average of fertilizer level
	Microorganism		
	Without *Trichoderma*	*Trichoderma*	
0%	0.13±0.02^{bc}	0.13±0.02^{bc}	0.13±0.02
100%	0.11±0.02^{bc}	0.17±0.01^{a}	0.14±0.02
50%	0.10±0.00^{c}	0.12±0.00^{bc}	0.11±0.00
25%	0.13±0.01^{bc}	0.14±0.00^{ab}	0.13±0.01
Average of microorganism	0.12±0.01^{b}	0.14±0.01^{a}	
Microorganism (M)	*		
Fertilizer level (F)	ns		
M F	*		
Coefficient of variation (%)	10.48		

* = significant ($p < 0.05$); ** = highly significant ($p < 0.01$); ns = not significant; $n = 4$ replicates; ^{a,b,c} = different lowercase letters above means in each category are significantly ($p < 0.05$) different.

Discussion

All 30 tested *Trichoderma* fungal isolates had the ability to produce acids as was reported for *Trichoderma* spp. in an Amazon soil where different organic acids were produced during the phosphate dissolution process (Bononi et al., 2020). Some isolates in the current research dissolved both phosphate and potassium, similar to a study that reported *Trichoderma koningii*, *T. harzianum*, *T. citrinoviride* and *T. viridescens* had phosphate solubility, while some species were also capable of increased soluble potassium from the biotite (Nahidan et al., 2019). The isolate NTW1.1/1 had the highest phosphorus availability (207.64 mg/L) and the sp1 isolate had the maximum potassium exchangeability (1.35 mg/L). However, only the NTW1.1/1 isolate was selected for further experiment. The Sp1 isolate produced less potassium solubility compared to *T. atroviride* LX-7 which dissolved potassium at 2.05 mg/L (Chen et al., 2021). In the greenhouse, *Trichoderma* NTW1.1/1 was identified as *Trichoderma asperellum* and produced the highest rice height and tillering number at 120 d. This genus has been reported as being able to increase physiological processes in rice, such as the net photosynthetic rate, transpiration, stomatal conductance, internal carbon dioxide concentration and water use efficiency (Doni et al., 2014) and chemical fertilizer-support of plant nutrients (Khrueakham et al., 2015). The use of *Trichoderma* resulted in a higher rice yield (6,711.77 kg/ha) than for the un-inoculated plant. The use of *Trichoderma* not only dissolved the phosphate or potassium in a form that was beneficial to plants but also improved the absorption of plant nutrients. Several *Trichoderma* strains have been reported to reduce plant stress due to environmental stresses (Cai et al., 2013) and to induce resistance to nematodes and fungi (Poveda et al., 2020; Thambugala et al., 2020), as well as producing phytohormones to promote rice growth (Jaroszuk-Ściseł et al., 2019). The current results showed that *Trichoderma* NTW1.1/1 increased the phosphorus content in rice plants, which was consistent with other *Trichoderma* research that reported phosphates solubilization, growth promotion and rice productivity (Chagas et al., 2017).

The 30 *Trichoderma* isolates were acid-producing and differed in their efficacy of phosphate or potassium solubilization, or both. The NTW1.1/1 (*Trichoderma asperellum*) isolate promoted the growth and yield of the RD31 rice variety and increased efficiency in rice cultivation in the Manorom soil series. Applying *Trichoderma* with 100% chemical fertilizers resulted in the highest efficiency in the RD31 rice cultivation, with a yield increase of about 60% compared to only using *Trichoderma*. On the other hand, if chemical fertilizer usage were decreased by 50–75%, combined with *Trichoderma*, RD31 rice could still be planted; however, the yield increase would only be about 30-35% compared to using solely *Trichoderma*. These results supported the important role of *Trichoderma* sp. to increase soil nutrient efficiency. The results of the present investigation showed that chemical fertilization treatments had a strong effect on the absorption of nutrients. The low microbial concentration in the crop production soil and/or low organic matter content in the soil were probably the limiting factors controlling microbial activities and their metabolites in the soil. Future studies must be to determine the impact of these treatments on soil quality and productivity in the short and long-term during the growth cycle of rice. Moreover, application of chemical fertilizers supplemented with *Trichoderma* sp. could increase plant nutrient solubilizing efficiency resulting in cost saving for rice cultivation.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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