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Research article

Efficacy of *Trichoderma asperellum* CB-Pin-01 and potassium dihydrogen phosphate to enhance growth and yield and reduce Pythium root rot of hydroponically grown lettuce

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Abstract

Trichoderma asperellum CB-Pin-01 (Ta) prepared as a fresh culture (Ta-F) and four levels of potassium dihydrogen phosphate (KH_2PO_4, KP) in concentrated stock nutrient solution B at the normal basal rate (0%, KP-0, 53 g/L) and at 5% (KP-5, 55.65 g/L), 10% (KP-10, 58.3 g/L) and 15% (KP-15, 60.95 g/L) were evaluated for their efficacy to enhance the growth and fresh weight yield of 'Green Cos' lettuce grown in a nutrient solution. The results revealed that compared with KP-0, the levels KP-10 and KP-15 significantly increased the fresh weights of the aerial parts (leaves and stem) by 7.45% and 18.21%, respectively, the roots by 38.68% and 22.68%, respectively, and the total plant (aerial parts and roots) by 10.05% and 18.58%, respectively. Treatment with KP-15 supplemented with a spore suspension derived from Ta-F produced the significantly highest plant fresh weight. The two finished bioproducts of Ta (Ta No.1 and Ta No.2) were compared with using Ta-F to control root rot and enhance the growth of 'Green Cos' lettuce grown in a nutrient solution infested with *Pythium aphanidermatum* (Pa). Compared to the non-supplemented control, the KP-15 supplemented with spore suspensions of Ta-F or with the bioproducts Ta No.1 or Ta No.2 significantly reduced the root rot disease index by 50.01%, 47.50% and 22.99%, respectively. The efficacy of Ta No.1 to reduce root rot and increase plant fresh weight, leaf area and root mass volume was comparable to the use of the Ta-F spore suspension. This beneficial fungus colonized lettuce roots, survived at high populations in nutrient solutions and reduced the nitrate accumulation in leaves of hydroponically grown lettuce.

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Introduction

Hydroponic systems have been extensively used for the production of fruits, vegetables and fresh-cut herbs in the greenhouse (Stapleton and Hochmuth, 2001). Hydroponic techniques generally involve growing plants in a water-based, nutrient rich solution. Compared to soil-based systems, hydroponic systems support better nutritional control to ensure sufficient availability of nutrients for plant growth (Shinohara et al., 2011). In addition, they usually provide a higher crop yield and a better growth rate of plants compared to the use of soil-based systems (Gashgari et al., 2018). High quality, clean and safe products are usually obtained from hydroponic culture.

Lactuca sativa L. (lettuce) is well known as a home-garden, leafy vegetable or as a commercial product worldwide. It is commonly used in the preparation of salads, soups and vegetable curries (Rubatzky and Yamaguchi, 1997).

Among the diseases found on commercially grown lettuces, root rot caused by *Pythium aphanidermatum* (Pa) is the most important. This disease is usually found in many nutrient film technique (NFT) growing systems either in greenhouses or outdoor planting areas. This pathogenic fungus produces asexually motile biflagellate zoospores which can move toward the root system of lettuce and cause root rot infection (Lamool, 2006). This disease reduces the lettuce growth and yield in most hydroponic farms, especially during periods of high temperatures (> 35°C).

Trichoderma spp. are widely used as biocontrol agents against plant pathogenic fungi through several mechanisms (Kumar et al., 2014) that may be direct which include competition for nutrients and ecological niches, mycoparasitism and antibiosis or indirect which include host-induced systemic resistance and increased nutrient uptake through solubilization and sequestration of nutrients (Harman, 2000; Sharma, 2018). The promotion of plant growth from using *Trichoderma* spp. may also be due to the production of growth hormones (Contreras-Cornejo et al., 2014) or the increase in phosphate solubilization or both, as well as other plant promotion substances such as pentyl pyrone, harzianic acid and harzianic acid isomers (Intana et al., 2003a; Promwee et al., 2014). Yedidia et al. (2001) reported that *T. harzianum* significantly increased the concentration of Cu, P, Fe, Zn, Mn and Na around the root area of hydroponically grown cucumbers and these minerals were then utilized by plants. This bioavailability of various minerals through solubilization may improve plant active-uptake mechanisms which result in increased plant growth (Menezes-Blackburn et al., 2014).

In Thailand, *T. asperellum* CB-Pin-01 (Ta), formerly identified as *T. harzianum* CB-Pin-01 (Unartngam et al., 2020), has been prepared as a pure powder stock culture used for producing a fresh culture bioproduct (fungal-colonized rice seeds, Ta-F) by using a simple technique developed by Chamswarng and Intanoo (2002). The spore suspension is prepared by washing spores from 100 g Ta-F with 20 L of sterile water. This spore suspension has been used for soaking seeds and spraying plants to control fungal diseases of cereals, fruits, vegetables, ornamentals and hydroponic lettuces (Unartngam et al., 2020). Lamool (2006) proved that spore suspensions derived from Ta-F (100g/200L) could reduce Pythium root rot and promote the growth of lettuce ('Green Cos') grown using the nutrient film technique (NFT) hydroponic system. This fungus colonizes and survives on the root surface of lettuce plants.

The nutrient solution is the most important factor for the success of most lettuce production in hydroponic cultures. Potassium dihydrogen phosphate (KH_2PO_4, KP) is an essential nutrient source of phosphorus (P) and potassium (K) in solution. Niu et al. (2015) evaluated plant responses to different P concentrations in one-quarter strength modified Hoagland solution. They found that phosphorus nutrition significantly enhanced the root and shoot growth of hydroponically grown eucalyptus seedlings. Chiemchaisri (2016) reported that the use of a modified Cooper (1979) nutrient solution with an increased rate of KP in the concentrated stock nutrient solution effectively enhanced plant growth and root development in 'Red Oak' lettuce. However, the effects have not been reported of KP used in combination with *Trichoderma* spp. on the growth and disease control in lettuce. Therefore, the current study aimed to determine the effects of Ta-F used in combination with increased rates of KP on the fresh weight yield of 'Green Cos' lettuce. The most effective rate of KP was selected and supplemented with either Ta-F or finished bioproducts to evaluate their efficacy for the control of Pythium root rot as well as to increase the plant growth and fresh weight yield of 'Green Cos' lettuce grown in an NFT hydroponic system.

Materials and Methods

Trichoderma fresh culture

A fresh culture of *T. asperellum* CB-Pin-01 (Ta-F) was prepared according to the procedure developed by Chamswarng and Intanoo (2002). In brief, four parts of broken milled

rice and two parts of tap water (volume per volume, v/v) were combined in an automatic rice cooker. After the cooker had been shut off, 250 g of semi-cooked broken milled rice were put into heat-tolerant plastic bags (20 cm × 30 cm). The open-end was folded closed during the cooling period. An aliquot (0.3–0.5 g) of pure stock culture powder of *T. asperellum* CB-Pin-01 (Ta) was added into the nearly-cool, semi-cooked broken milled rice and sealed using a plastic sealer. Each bag was punched 20–30 times near the sealed line with a fine needle. All inoculated bags were incubated at 25–30°C for 6–7 d. To prepare the concentrated spore suspension, 50 g of fresh culture were placed into a bottle containing 1 L of sterile water and shaken for several minutes to remove the darkish green *Trichoderma* spores from the surface of the semi-cooked broken milled rice in water. The spore suspension was filtered through a layer of cheesecloth. The concentrated green spore suspension (1 L) was added to 100 L of the nutrient solution. The concentration of Ta in the nutrient solution was 1×10^4 colony forming units (CFU)/mL.

Trichoderma bioproducts

Two finished bioproducts of *T. asperellum* CB-Pin-01 (Ta), namely Ta No.1 and Ta No.2 that had been formulated as a fine powder by Associate Professor Dr Chiradej Chamswarng of Kasetsart University, Nakhon Pathom, Thailand were used in this experiment. The bioproducts Ta No.1 (7.9×10^9 CFU/g) and Ta No.2 (9.0×10^8 CFU/g) were prepared as sachets (7 cm×7.5 cm) containing granular formulations of *T. asperellum* CB-Pin-01 (Ta) at a rate of 10 g per sachet. Spore suspensions of each bioproduct were prepared by placing two sachets (20 g in total) into a bottle containing 500 mL of sterile water, shaking it for several minutes and then adding it into 100 L of the nutrient solution. The nutrient solutions containing Ta No.1 or Ta No.2 (1×10^4 CFU/mL) were used throughout the planting period.

Pythium aphanidermatum inoculum

The pure culture of *P. aphanidermatum* (Pa), a causal agent of lettuce root rot, was obtained from the Plant Disease Biocontrol Laboratory, Department of Plant Pathology, Kasetsart University, Kamphaeng Saen campus, Thailand. The Pa fungus was cultured on potato dextrose agar and incubated at room temperature (25–30°C) for 48 hr. Agar containing fresh mycelia on each plate was cut into small pieces using a sterile blade; then, 100 mL of sterile water were added. For producing Pa-inoculum, all agar pieces were homogenized (IKA Ultra-Turrax® T25; Germany) at 9,500 revolutions per minute for 15 s (Lamool, 2006). This inoculum was kept in refrigerator at 5°C until used.

Nutrient solution

To prepare the nutrient solution for hydroponic culture, three types of concentrated basal stock nutrient solutions were prepared as stock A, stock B and stock C as described by Chiemchaisri (2016). Each 1 L of concentrated basal stock nutrient solution was prepared as follows: stock A comprised 220.0 g of calcium nitrate ($Ca(NO_3)_2.4H_2O$), while stock B comprised 53.0 g of potassium dihydrogen phosphate (KH_2PO_4), 117.0 g of potassium nitrate (KNO_3) and 112.8 g of magnesium sulfate ($MgSO_4.7H_2O$) and stock C contained 10.0 g of Fe DTPA ($C_{14}H_{19}FeN_3NaO_{10}$), 5.0 g of FeEDDHA ($C_{18}H_{16}N_2O_6FeNa$), 2.0 g of MnEDTA ($C_{10}H_{12}N_2O_8MnNa_2.4H_2O$), 0.1 g of ammonium molybdate ($(NH_4)_6\ Mo_7\ O_{24}.4H_2O$), 0.06 g of nickel sulfate ($NiSO_4$) and 10.0 g of Nic-Spray® (7.0% Mg, 1.8% Fe, 1.8% Mn, 1.9% Cu, 1.7% Zn, 1.7% B, 0.01% Mo) as the micronutrient fertilizer. To prepare the basal nutrient solution for planting, 100 L of water were combined with 200 mL each of the concentrated basal nutrient stock solutions A, B or C. The pH in the nutrient solutions was adjusted to 5.5–6.5, while the electrical conductivity (EC) was 1.6–1.8 mS/cm.

Lettuce planting and experimental design

The nutrient film technique (NFT) system was used for lettuce planting with a water flow rate of 21.6 L/min and a 5 mm water film. 'Green Cos' seedlings (Tiberius R2) were grown in small cups (36 mL) containing a perlite-to-vermiculite ratio of 2:1 (v/v) for 14 d, using the NFT system supplied with the full strength basal nutrient solution. The seedlings were transplanted into the NFT system with daily adjustments of pH (5.5–6.5) and EC (1.6–1.8 mS/cm) of the nutrient solution during plant growth. The nutrient solution of all treatments was replaced three times weekly. Two separate experiments were conducted.

The first experiment was performed to study the effects of Ta-F and KP in the concentrated nutrient stock solution B on the growth and yield of 'Green Cos' lettuce. The experiment was conducted in a nylon screenhouse at the Department of Plant Pathology, Kasetsart University at the Kamphaeng Saen Campus in Nakhon Pathom province, Thailand. A 2×4 factorial

in a completely randomized design (CRD) was used with eight treatments, five replications for each treatment and five plants per replication. The two levels of factor A were: A-1 = with (+) Ta-F and A-2= without (-) Ta-F. Four levels of KP fertilizer rates constituted factor B, where B-1 = the normal basal rate of KP (KP-0 = 53.00 g/L), B-2 = an increased rate of KP by 5% (KP-5 = 55.65 g/L), B-3 = an increased rate of KP by 10% (KP-10 = 58.30 g/L) and B-4 = an increased rate of KP by 15% (KP-15 = 60.95 g/L). Plants were grown hydroponically in a double (connected)-trough (DT) NFT system and nutrient solution, according to the treatments released throughout the DT system. Each trough contained 16 plants, with a total of 32 plants per treatment. For the treatments with factor A-1, 1 L of spore suspension prepared from 50 g of the Ta-F as previously described was added to 100 L of the nutrient solutions. These four treatments of factor A-1 were: KP-0+Ta-F, KP-5+Ta-F, KP-10+Ta-F and KP-15+Ta-F.

The second experiment was conducted to determine the efficacy of KP-15 in combination with each of the bioproducts Ta No.1, Ta No.2 or Ta-F on the plant growth, yield and root rot disease control of lettuce. The lettuces were grown in nutrient solutions infested with Pa (+Pa). The Pa- inoculation was performed by adding 400 mL of Pa- inoculum (mycelial preparation) as previously described into 100 L of nutrient solution at 14 d, 21 d, 28 d and 35 d after the lettuce seedlings were transplanted into the planting system. The experiment was conducted in a nylon screenhouse and arranged in a CRD with four treatments, five replications for each treatment and five plants per replication. These four treatments were: KP-15 (+Pa), KP-15+Ta-F (+Pa), KP-15+Ta No.1 (+Pa) and KP-15+Ta No.2 (+Pa). Plants in the second experiment were hydroponically grown using a DT-NFT system as previously described in the first experiment.

Evaluation of plant growth and yield

The lettuce plants were harvested at 42 d after sowing. The fresh weights of the aerial parts (leaves and stem) and roots for each lettuce plant from the first experiment were separately recorded. The lettuce plants from the second experiment were evaluated for leaf area using a leaf area meter (Li-3100c; USA.), and plant fresh weights were also recorded. The root mass volume was determined by recording the increased volume of water after placing the lettuce root system into a known volume of water.

Determination of root disease and root colonization

Root rot of lettuce caused by *P. aphanidermatum* in the second experiment was evaluated after harvest using the disease index described by Cirulii and Alexander (1966). The disease rating was assessed based on five disease levels: 0 = white root system with normal growth and no disease symptoms; 1 = very light brown roots with 1–25% of disease symptoms; 2 = light brown roots with 26–50% of disease symptoms; 3 = normal brown roots with 51–75% of disease symptoms; and 4 = dark brown roots with 76–100% of disease symptoms. The disease index was calculated according to Equation 1:

$$\text{Disease index (\%)} = \frac{\Sigma\ (\text{Disease level} \times \text{Number of roots}) \times 100}{\text{Total number of roots} \times \text{Maximum disease level}} \quad (1)$$

Lettuce roots colonized by Pa or Ta were determined using a modified BNPRA medium (Chamswarng et al., 1985) and Martin's medium (Martin, 1950), respectively. Root samples obtained from lettuce plants aged 42 d were immersed in 0.525% sodium hypochlorite for 3 min, washed with sterile distilled water three times, blotted dry and cut into 1.0 cm pieces before being placed on the selective media. Root colonization was recorded by observing the fungal growth that developed from root pieces on the surface of the media.

Enumeration of Pythium and Trichoderma populations

After the lettuce plants in the second experiment were harvested, a nutrient solution sample from each treatment was collected and enumerated for surviving populations of Pa and Ta on a modified BNPRA medium (Chamswarng et al., 1985) and Martin's medium (Martin, 1950), respectively. A nutrient solution sample was serially diluted (1×10^{-1}–1×10^{-6}) and 0.1 mL of diluted sample was dropped on the selective media. The droplets were spread over the surface of the agar; then, all plates were incubated at room temperature for 3 d. Colonies that developed on the surface of the agar were recorded as colony forming units (CFU) per 1 mL of nutrient solution.

Nitrate analysis

Analysis of nitrate in the lettuce leaves harvested from the second experiment was performed by Central Laboratory (Thailand) Co., Ltd. The nitrate concentration (in milligrams

per kilogram) was determined using the brucine colorimetric method and the changes in colors were measured using a spectrophotometer (Latimer, 2012).

Statistical analyses

All data were analysed using analysis of variance. The significance of differences between the treatment means was determined based on least significant differences using the statistical program R (R Core Team, 2014). The significance level was set at $p < 0.05$.

Results

Effects of increased KP and Ta on plant growth

Increasing the level of KP in the normal concentrated basal nutrient stock solution B (KP-0) by 5%, 10% and 15% (KP-5, KP-10 and KP-15, respectively) significantly enhanced the total plant fresh weight (aerial parts and roots) by 7.41%, 10.05% and 18.58%, respectively, compared to the control (KP-0). The treatments KP-10 and KP-15 significantly increased the fresh weights of the aerial parts and roots compared to the control (KP-0). The use of KP-15 most effectively increased the total fresh weight of the lettuce plants (aerial parts plus roots) to 357.33 g/plant which was significantly higher than the total plant fresh weights derived from the use of KP-5 (323.66 g/plant) or KP-10 (331.60 g/plant) or the control (KP-0, 301.33 g/plant), as shown in Table 1 and Fig. 1A.

The total plant fresh weights in all treatments combined with Ta-F were significantly increased compared to treatments with the same KP levels but without combining with Ta-F. The treatment KP-15 combined with Ta-F produced the highest total plant fresh weight (388.67 g/plant) which was significantly higher than for the control (KP-0, 301.33 g/plant) and all the increased KP treatments (KP-5, KP-10, KP-15) without the addition of the Ta-F, but was not significantly different compared with the treatments of KP-5+Ta-F (371.33 g/plant) and KP-10+Ta-F (379.67 g/plant). By adding Ta-F into the nutrient solutions of the treatments KP-0, KP-5, KP-10 and KP-15, the total plant fresh weights increased by 21.24%, 23.23%, 26.00% and 28.98%, respectively, compared to the control (KP-0), as shown in Table 1 and Fig. 1B.

Table 1 Effects of *Trichoderma asperellum* CB-Pin-01 fresh culture (Ta-F) and four levels of potassium dihydrogen phosphate (KP) on fresh weights of aerial parts (leaves and stem), roots and total plant (aerial parts plus roots) of hydroponically grown 'Green Cos' lettuce aged 42 d, using nutrient film technique system

Treatment	Fresh weight (g/plant)		
	Aerial part	Roots	Total plant
Control KP-0	276.33±29.06^{d}	25.00±5.00^{e}	301.33±31.88^{e}
	-	-	-
KP-5	294.33±21.62^{cd}	29.33±7.04^{de}	323.66±24.24^{d}
	(+6.51%)†	(+17.32%)†	(+7.41%)†
KP-10	296.93±27.09^{c}	34.67±7.43^{bc}	331.60±31.87^{d}
	(+7.45%)†	(+38.68%)†	(+10.05%)†
KP-15	326.66±26.37^{b}	30.67±7.04^{cd}	357.33±31.27^{c}
	(+18.21%)†	(+22.68%)†	(+18.58%)†
KP-0+Ta-F	326.00±31.35^{b}	39.33±8.84^{ab}	365.33±36.42^{bc}
	(+17.97%)†	(+57.32%)†	(+21.24%)†
KP-5+Ta-F	335.33±18.23^{ab}	36.00±5.07^{ab}	371.33±19.95^{abc}
	(+21.35%)†	(+44.00%)†	(+23.23%)†
KP-10+Ta-F	341.00±14.37^{ab}	38.67±5.16^{ab}	379.67±15.26^{ab}
	(+23.40%)†	(+54.68%)†	(+26.00%)†
KP-15+Ta-F	348.00±33.85^{a}	40.67±7.99^{a}	388.67±38.15^{a}
	(+25.94%)†	(+62.68%)†	(+28.98%)†
CV (%)	8.14	19.85	8.33

KP = potassium dihydrogen phosphate in the concentrated nutrient solution B, KP-0 = 53.00 g/L, KP-5 = 55.65 g/L, KP-10 = 58.30 g/L, KP-15 = 60.95 g/L.; CV = coefficient of variation;

mean values (±SD) within each column superscripted with different lowercase letters are significantly ($p < 0.05$) different.

†percentage increment (+) respective to each aerial part, root and total plant fresh weight relative to the untreated control (KP-0).

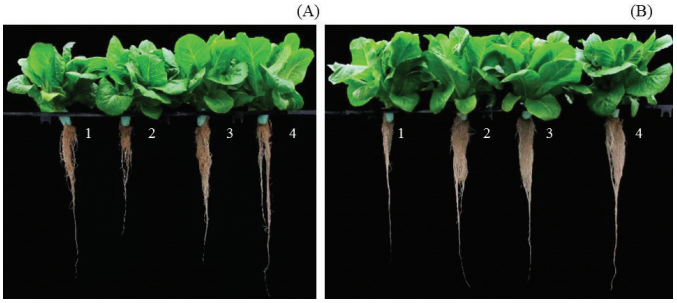

Fig. 1 Growth of 'Green Cos' lettuce aged 42 d grown in nutrient solutions prepared from concentrated nutrient stock solution B with four levels of potassium dihydrogen phosphate (KP) of 1) normal basal rate (KP-0), 2) increased rate of KP by 5% (KP-5), 3) by 10% (KP-10) and 4) by 15% (KP-15): (A) without any supplementation in nutrient solution; (B) with supplementation of *Trichoderma asperellum* CB-Pin-01 fresh culture in nutrient solution

In the nutrient solution inoculated with a mycelial suspension of Pa (+Pa), there were no significant differences in the effects of Ta among the treatments using the Ta-F. bioproducts Ta No.1 and Ta No.2 on leaf area, aerial parts and total plant fresh weights of 'Green Cos' lettuce. Nevertheless, Ta-F and bioproducts Ta-No.1 and Ta-No.2 significantly increased leaf area by 12.85%, 11.84% and 12.71%, respectively, aerial part fresh weight by 25.64%, 25.64% and 19.08%, respectively, total plant fresh weight by 24.79%, 24.51% and 18.67%, respectively, and root mass volume by 33.72%, 49.37% and 30.10%, respectively, compared with the Pa-inoculated control [KP-15(+Pa)]. The highest increase in root mass volume (49.37%) compared to the Pa-inoculated control was produced by the treatment using bioproduct Ta-No.1 (Table 2).

Effects of bioproducts on disease incidence, root colonization and fungal population

The Pythium root rot disease indices on 'Green Cos' lettuce in all Ta treatments (33.33–46.67%) were significantly lower compared to the Pa-inoculated control (66.67%). The disease index value obtained using bioproduct Ta No. 1 (35.00%) was comparable to the use of the Ta-F (33.33%) and both disease indices were significantly lower than those derived from using bioproduct Ta No.2 (46.67%), as shown in Table 3. The percentage root colonization by Pa in all Ta treatments (20.00–53.33%) was significantly lower than those in the Pa-inoculated control (80.00%), while 100% of roots were colonized by Ta (Table 3). Populations of Pa in nutrient solutions from the bioproduct treatments Ta No.1 (6.6 CFU/mL), Ta No.2 (10.0 CFU/mL) and Ta-F (6.6 CFU/mL) were significantly lower than in the control (13.0 CFU/mL). The Ta populations in nutrient solutions of all treatments after plant harvesting were in the range 8.0×10^3–4.0×10^4 CFU/mL (Table 3).

Effects of bioproducts on nitrate accumulation in lettuce leaf

Analysis of nitrates in 'Green Cos' leaves grown in a Pa-inoculated nutrient solution indicated a lower nitrate accumulation in the treatments using the Ta-F (1,029.23 mg/kg), bioproduct Ta No.1 (917.22 mg/kg) and bioproduct Ta No.2 (1,150.41 mg/kg). The results showed that the application of Ta-F, Ta No.1 or Ta No.2 in nutrient solutions reduced nitrate accumulation in 'Green Cos' leaves by 13.52%, 22.95% and 3.34%, respectively, compared with the control (Table 4).

Table 2 Effects of *Trichoderma asperellum* CB-Pin-01 fresh culture (Ta-F) and bioproducts No.1 (Ta No.1) and No.2 (Ta No.2) on the growth of 'Green Cos' lettuce aged 42 d grown in nutrient solution using 15% increased potassium dihydrogen phosphate (KP-15) and infested with a mycelial suspension of *Pythium aphanidermatum* (+Pa)

Treatment	Leaf area (cm^2/leaf)	Fresh weight (g/plant)		Root mass volume/ (mL/plant)
		Aerial part	Total plant	
Control KP-15 (+Pa)	167.96±18.38^b	208.27±18.84^b	239.60±20.14^b	27.67±3.72^c
	–	–	–	–
KP-15+Ta-F (+Pa)	189.54±19.90^a (+12.85%)†	261.67±36.24^a (+25.64%)†	299.00±37.85^a (+24.79%)†	37.00±5.28^b (+33.72%)†
KP-15+Ta No.1 (+Pa)	187.85±19.63^a (+11.84%)†	261.67±36.24^a (+25.64%)†	298.33±36.29^a (+24.51%)†	41.33±4.81^a (+49.37%)†
KP-15+Ta No.2 (+Pa)	189.31±19.34^a (+12.71%)†	248.00±28.59^a (+19.08%)†	284.33±32.23^a (+18.67%)†	36.00±4.71^b (+30.10%)†
CV (%)	10.08	12.24	11.30	13.00

Pa = *Pythium aphanidermatum*; KP = potassium dihydrogen phosphate in the concentrated nutrient stock solution B, KP-15 = 60.95 g/L.; CV = coefficient of variation; mean values (±SD) within each column superscripted with different lowercase letters are significantly ($p < 0.05$) different.
†percentage increment (+) of each treatment mean relative to the Pa-inoculated control.

Table 3 Effects of *Trichoderma asperellum* CB-Pin-01 (Ta) fresh culture (Ta-F) and bioproducts No.1 (Ta No.1) and No.2 (Ta No.2) on the root rot disease index, fungal colonization of roots and fungal populations in nutrient solution infested with *Pythium aphanidermatum* (+Pa) after harvesting of 'Green Cos' lettuce aged 42 d

Treatment	Disease index (%)	Root colonization (%)		Populations in nutrient solution (CFU/mL)	
		Pa	Ta	Pa	Ta
Control KP-15 (+Pa)	66.67±1.73[a]	80.00±4.47[a]	nd	13.0±1.11[a]	nd
KP-15+Ta-F (+Pa)	33.33±0.71[c] (50.01%)[†]	20.00±7.07[d]	100±0.00[a]	6.6±1.37[c]	3.9×10^4
KP-15+Ta No.1 (+Pa)	35.00±0.78[c] (47.50%)[†]	26.67±2.60[c]	100±0.00[a]	6.6±1.36[c]	4.0×10^4
KP-15+Ta No.2 (+Pa)	46.67±0.61[b] (29.99%)[†]	53.33±4.08[b]	100±0.00[a]	10.0±2.61[b]	8.0×10^3
C.V. (%)	2.37	11.05	1.42	15.09	

Pa = *Pythium aphanidermatum*; KP = potassium dihydrogen phosphate in the concentrated nutrient stock solution B, KP-15 = 60.95 g/L.; CFU = colony forming units per milliliter; CV = coefficient of variation;
mean values (±SD) within each column superscripted with different lowercase letters are significantly ($p < 0.05$) different.
nd = not determined;
[†]percentage of disease index reduction of each treatment mean relative to the control.

Table 4 Effects of *Trichoderma asperellum* CB-Pin-01 fresh culture (Ta-F) and bioproducts No.1 (Ta No.1) and No.2 (Ta No.2) on nitrate accumulation in leaves of hydroponically grown 'Green Cos' lettuce aged 42 d in nutrient solution using 15% increased potassium dihydrogen phosphate (KP-15) and infested with *Pythium aphanidermatum* (+Pa)

Treatment	Nitrate accumulation in leaves (mg/kg)
Control KP-15 (+Pa)	1,190.10
KP-15+Ta-F (+Pa)	1,029.23 (-13.52%)[†]
KP-15+Ta No.1(+Pa)	917.22 (-22.95%)[†]
KP-15+Ta No.2 (+Pa)	1,150.41 (-3.34%)[†]

Pa = *Pythium aphanidermatum*; KP = potassium dihydrogen phosphate in the concentrated nutrient stock solution B, KP-15 = 60.95 g/L;
[†]percentage decrement (-) of each treatment mean relative to the control.

Discussion

The results revealed that the total plant fresh weight (aerial parts and roots) increased (7.41–18.58%) when the normal rate of KP in the concentrated basal nutrient stock solution B (53g/L) increased by 5–15%. These results were in agreement with Niu et al. (2015) who reported a promotion of plant and root growth of hydroponically grown eucalyptus when the level of phosphorus increased. In addition, application of Ta-F into the nutrient solution containing the normal rate of KP in the concentrated basal nutrient stock solution B as KP-0 increased the total plant fresh weight by 21.24% and revealed the effectiveness of the Ta-F in promoting plant growth and yield which was consistent Lamool (2006). The use of Ta-F in combination with nutrient solutions containing a 15% increased rate of KP in stock B as KP-15 provided a significantly higher total plant fresh weight compared to treatment KP-0 combined with Ta-F and the treatment using KP-15 alone (Table 1). These results revealed the additive effect of Ta-F and of increased KP on the growth and yield of 'Green Cos' lettuce. Enhancement of phosphate solubilization and phosphorus uptake by plants after using Ta might be the reason for the increase in plant growth and yield (Yedidia et al., 2001).

These results suggested that increasing the phosphorus contained in KP by 5–15% compared to the normal concentrated basal nutrient stock solution stock B effectively enhanced the growth and yield of 'Green Cos' lettuce. This finding indicated that the most suitable levels of phosphorus in the concentrated nutrient solution stock B for producing a higher or maximum yield of lettuce need to be further considered. Zhao and Zhang (2015) reported a close connection between the P content and the growth rate of cucumber plants by comparing the size of leaves, shoots and the root length of plants grown in water cultures, probably due to the transition of both inorganic and organic insoluble phosphates into available phosphorus by *T. asperellum* Q1, which could be easily utilized by cucumber seedlings.

One of the various properties of microorganisms is their phosphorus-solubilizing activity which results in the promotion

of plant growth and nutrition (Vassilev et al., 2006). Hence, solubilized phosphorus contained in the *Trichoderma* biomass which colonized the roots could be released from around the roots after lysis of the mycelia with age. This may have partially explained the ability of the Ta to increase plant growth. Phosphate solubilization by the *Trichoderma* species has been widely reported by several researchers (Kapri and Tewari, 2010; Saravanakumar et al., 2013; Promwee et al., 2014). Under the conditions used for the infestation of the nutrient solution with Pa, another mechanism for plant growth and yield promotion resulting from Ta in the current study may have been due to an indirect effect on the plant via induction of resistance to root rot infection caused by Pa (Shoresh et al., 2010; Malmierca et al., 2012; Yoshioka et al., 2012). Furthermore, the direct stimulation of plant growth may compensate for the root damage by Pythium infection.

The current study showed that Ta increased root mass volumes both in the absence and the presence of Pa. This might be attributed to the promotion of root growth by the indole-3-acetic acid produced by *Trichoderma* spp., as reported by Hexon et al. (2009). An abundant root system is important for optimal plant health since it facilitates the efficient use of water and enhances the utilization of various nutrients from mineral solutions in the hydroponic system. *Trichoderma* spp. contribute to the increase in nutrient availability, nutrient use efficiency and uptake by the plant roots (Harman, 2000; Yedidia et al., 2001) resulting in the development of an abundant root system. Therefore, enhancement of the root mass volume of lettuce plants in all *Trichoderma* treatments using either fresh culture (Ta-F) or the finished bioproducts (Ta No.1 and Ta No.2) in the current study could have contributed to the better plant growth and higher fresh weight yield.

The current results showed the complete colonization of lettuce roots by Ta from both the use of fresh culture (Ta-F) and finished bioproducts (Ta No.1 and Ta No.2), regardless of the presence of Pa in the nutrient solution. This evidence revealed the capability of this strain to grow along the roots during their growth and development, thus colonizing the whole root system and benefiting the crop for its entire life. This finding was in agreement with the capability for rhizosphere competence of the *T. harzianum* strain T-22 described by Sivan and Harman (1991). The colonization of Ta on lettuce roots might play important roles in reducing the root rot disease index, root colonization and populations of Pa in nutrient solutions (Table 3), as well as enhancing plant growth, root mass volume and fresh weight yield (Table 2). The high percentages of Ta recovered from the roots of harvested lettuce indicated the capability of this fungal isolate to penetrate and survive in lettuce roots as a symbiont similar to mycorrhizal fungi. In addition, there is a possibility that this fungus was restricted to the epidermis and outer cortex of the roots in a manner similar to that reported in cucumber plants treated with the *T. asperellum* isolate T 203 in hydroponic systems (Yedidia et al., 2003) and in NFT-hydroponic lettuce ('Green Cos') roots treated with Ta (Lamool, 2006).

The significant reductions in the *Pythium* populations in the nutrient solutions after harvest was probably due to the direct parasitism of Pa by Ta, resulting in preventing the establishment or delaying the build-up and spread of any secondary inocula of Pa to surrounding roots (Utkhede et al., 2000; Intana et al., 2003b). From the current study, reduced populations of Pa in nutrient solution also resulted in significantly lower percentages of root colonization compared to the control.

Analysis of the nitrate accumulation in the lettuce leaves indicated that the application of Ta as a fresh culture (Ta-F) or finished bioproducts (Ta No.1 or Ta No.2) in the nutrient solutions using KP-15 effectively reduced the accumulation of nitrates in 'Green Cos' leaves. This finding was consistent with Brendon (2003) who described the ability of *Trichoderma* to function as a mycorrhizal fungus and increase the availability of ammonium for plant uptake. Increased available ammonium in the root tissues corresponded with a decrease in the nitrate accumulation in leaves, while the levels of Cu, Na, Fe and P increased in leaf tissues. Significantly increased nitrate reductase activity was a possible reason for diminished nitrate accumulation in plants (Kohler et al., 2008). The maximum acceptable level of nitrate concentration in vegetables has been set at 3,500 mg/kg (fresh weight) for winter-grown crops in Germany (Anonymous, 1993) and at 3,000 mg/kg (fresh weight) for leafy vegetables in China (Anonymous, 2003). From the current study, the nitrate accumulation in 'Green Cos' leaves in all treatments was less than 1,200 mg/kg (fresh weight) which was over twice as low as the maximum acceptable nitrate levels previously mentioned.

The results from the current study indicated that the overall efficacies of bioproduct Ta No.1 for promoting lettuce growth, reducing root rot disease and nitrate accumulation were comparable to the use of Ta-F. Therefore, the future development of this bioproduct as an effective, environmental-friendly, cheap and ready-to-use product should be developed to the commercial level, both in hydroponic culture and organic agriculture.

In conclusion, the data presented here revealed that the use of concentrated nutrient stock solution B as KP-15 alone or combined with Ta-F increased the plant growth and fresh weight yield of 'Green Cos' lettuce. When the nutrient solution was infested with Pa, the use of bioproduct Ta No.1 in combination with KP-15 significantly reduced root rot disease, while increasing the leaf area, the fresh weights of aerial parts and total plant, and the root mass volume compared to the control. All treatments of KP-15 combined with the *Trichoderma* fungus led to effective colonization of lettuce roots by the beneficial fungus, reduced populations of Pa in the nutrient solution and reduced nitrate accumulation in the lettuce leaves.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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