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THE EFFECTS OF VERMICOMPOST MIXED WITH *TRICHODERMA ASPERELLUM* ON THE GROWTH AND PYTHIUM ROOT ROT OF LETTUCES

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ABSTRACT: Vermicompost products are efficient organic fertilizers. Therefore, vermicompost fortified with a biocontrol agent may have the potential to improve plant growth and also suppress plant disease. In this research, planting substrates (chopped coconut husk: coir dust; 50:30 % by volume) supplemented with vermicompost (VC) (20 % by volume) or VC fortified with *Trichoderma asperellum* CB-Pin-01 (VCT) (20 % by volume) were evaluated for the effects on lettuce growth and root rot caused by *Pythium aphanidermatum* (Pa). The 14-day-old lettuce (Butter Head) seedlings grown in a sowing substrate (perlite: vermiculite; 2:1 V/V) were transplanted into a 6-inch plastic pot containing prepared substrates. A plant nutrient solution with an electrical conductivity (EC) of 1.6 mS/cm and a pH of 5.5-6.0 was applied weekly to the substrate at 200 ml/pot. Results revealed that both VC and VCT increased plant growth after planting for 42 days as compared to a control (substrate without vermicompost). However, a substrate containing VCT significantly increased the height, leaf number, leaf area, basal stem cutting surface area, and plant fresh and dry weights when compared with the control. When the substrates were inoculated with Pa, the VCT (+Pa) treatment increased the height, leaf number, leaf area, basal stem cutting surface area, and plant fresh and dry weights when compared with a *Pythium* treated control (VC(+Pa)). After lettuce harvesting, *T. asperellum* CB-Pin-01 could be completely recovered from the planting substrate (100%) and the lettuce root (100%), while this fungus helped reduce substrate and lettuce root colonization by Pa when compared with a control (VC(+Pa)).

Keywords: Trichoderma, Vermicompost, Planting substrate, Root rot, Biocontrol

1. INTRODUCTION

Lettuce (*Lactuca sativa*) is the most important crop in the group of leafy vegetables [1]. Butter Head lettuce (*Lactuca sativa* L. var. *capitata* L.) is a heading type of lettuce with soft and tender leaves that are eaten raw. It is most popular in England, France, the Netherlands and other western and central European countries [2]. In recent decades, many cultivars have been bred and grown in the USA [3].

Soilless cultures can increase productivity whatever the climate conditions, but can also optimize the management of inputs (fertilizers or pesticides) within a given economic and environmental framework. They also aim at controlling diseases more efficiently [4]. For hydroponically and soilless substrate is grown lettuce, *Pythium* spp. particularly *P. aphanidermatum* (Pa) is a major fungal pathogen causing root rot disease. Zoospores produced by Pa are asexually motile biflagellate spores which can live in water and infect the roots, causing root rot disease on various kinds of lettuce [5]. This disease causes a significant reduction of lettuce growth, yield and quality, especially when the lettuce was grown during the high temperature (>35°C) season.

At present, biological control (biocontrol) is an alternative method for the control of plant diseases. The most widely used biocontrol agent (BCA) throughout the world belongs to the genus *Trichoderma* [6], [7]. *Trichoderma* is a well-known BCA which is highly effective in directly controlling fungal pathogens by antibiosis and mycoparasitism. However, efforts to induce resistance to plant disease and increase plant growth result in indirect benefits [6], [7], [8].

In Thailand, Chamswarng and Intanoo [9] prepared a *Trichoderma asperellum* strain CB-Pin-01 (Ta) (formerly identified as *T. harzianum*) as a pure stock culture in the form of coarse powder. This semi-finished bioproduct has been used for producing a fresh culture bioproduct (fungal colonized semi-cooked rice seeds) by using a simple technique [9]. Spore suspension derived from the fresh culture can be used for seed soaking, soil drenching and plant spraying for the control of fungal diseases on fruits, vegetables, cereals, ornamental crops and hydroponically grown lettuce [10]. Incorporation of Ta-spore suspension into mineral nutrient solutions used for growing lettuce (Green Cos) in the Nutrient Film Technique (NFT) system effectively reduced Pythium root rot and promoted lettuce growth [11]. Similar results were obtained by Kaensarn [12] who reported that sowing and planting Butter Head lettuce in bio substrates supplemented with *T. asperellum* CB-Pin-01 could enhance plant growth and control root rot disease caused by *Pythium aphanidermatum*.

Vermicomposting is the non-thermophilic biodegradation of organic material through the interaction between earthworms and microorganisms. This is an environmentally friendly, highly cost-effective conversion method of biodegradable organic waste into valuable products. Vermicompost products have been proved to be effective as organic fertilizers and biological control agents providing suppression of many plant disease incidences caused by soil-borne or foliar plant pathogens and pests [13], [14]. The application of compost not only suppresses disease severity but also involves induced systemic resistance [15], [16]. Compost fortified with plant growth promoting microorganisms (PGPM) has shown that it can increase the suppression of diseases against fungal pathogens, plant growth and soil health [17].

Therefore, this study was conducted to evaluate the efficacy of planting substrates supplemented with vermicompost and vermicompost fortified with *T. asperellum* CB-Pin-01 (Ta) for promoting plant growth and yield of Butter Head lettuce and for reducing lettuce root and substrate colonization by Pa.

2. MATERIALS AND METHODS

2.1 *Trichoderma* Fresh Culture

For fresh culture preparation, four parts of broken milled rice were added with two parts of tap water (v/v) in an automatic rice cooker. After the switch turned off, 250 g of semi-cooked rice was placed into an 8x12 inch heat tolerant plastic bag. The bags were folded and left to cool down with the ends still open. Warm, semi-cooked rice was inoculated with an aliquot (0.3-0.5 g/bag) of pure Ta stock culture. All bags were sealed with a stapler or a plastic sealer and then each bag was punched along the sealed line with a fine needle making 20-30 holes. The Ta- inoculated bags were incubated at 25-30^{o}C for 6-7 days (d). To prepare the spore suspension, 50 g of Ta fresh culture was added into 10 L of clean water contained in a bottle. The bottle was vigorously shaken in order to remove dark greenish Ta spores from the surface of the semi-cooked rice. The spore suspension was filtered through a double layer of cheesecloth [18].

2.2 *Pythium* Inoculation

The plant pathogenic isolate *Pythium aphanidermatum* (Pa) was isolated from rotted roots of lettuce (Green Cos). The Pa fungus was cultured on potato dextrose agar (PDA) for 48 hr at room temperature (25-30 °C). The agar contained Pa mycelia in each plate and was cut into small pieces before adding 100 ml of sterile water. To prepare the mycelial suspension, agar pieces were homogenized at 9500 rpm for 15 sec [11]. For Pa inoculation, 7 ml/pot of mycelial suspension were applied on to the planting substrate at 14 d after planting.

2.3 Preparation of Basal Nutrient Solution

Mineral nutrient solution stocks modified from Cooper [19] were prepared according to the methods provided by Chiemchaisri [20]. In this preparation, 1 L of each concentrated nutrient solution stock was comprised of 220.0 g of calcium nitrate for stock A; 53.0 g of potassium dihydrogen phosphate, 117.0 g of potassium nitrate and 112.8 g of magnesium sulfate for stock B; 10.0 g of Fe DTPA, 5.0 g of Fe DDHA, 2.0 g of Mn EDTA, 0.1 g of ammonium molybdate, 0.06 g of nickel sulfate and 10.0 g of Nic-Spray® (7.0% Mg, 1.8% Fe, 1.8% Mn, 1.9% Ca, 1.7% Zn, 1.7% B, 0.01% Mo) as a micronutrient fertilizer for stock C. To prepare the basal nutrient solution, 200 ml of the concentrated stock solutions of A, B and C were mixed with 100 L of water. The pH and EC in the nutrient solution were adjusted at 5.5-6.5 and 1.6-1.8 mS/cm respectively.

2.4 Lettuce Planting and Experimental Design

The formula for the planting substrate was developed by Associate Prof. Dr. Chiradej Chamswarng. This includes chopped coconut husks: coir dust; (50:30 % by volume) supplemented with vermicompost (VC) (20 % by volume) or VC fortified with *T. asperellum* CB-Pin-01 (VC+T and VCT) (20 % by volume) to be used for lettuce planting. Butter Head seedlings were grown in a small cup (36 ml) containing perlite: vermiculite (2:1 v/v) for 14 d using a nutrient solution. The seedlings were transplanted into a 6-inch plastic pot (1,000 ml) containing prepared substrates. A plant nutrient solution of

200 ml was added weekly adjustments of pH (5.5-6.5) and EC (1.6-1.8 mS/cm) during plant growth.

The completely randomized design (CRD) was performed in seven treatments with four replications for each treatment, four pots per replication and one plant per pot. Treatments 1-4 were comprised of (1) substrate only (control), (2) substrate supplemented with VC (20% v/v) (VC), (3) substrate supplemented with VC (20 % v/v) and 200 ml/pot of Ta spore suspension prepared from Ta-fresh culture at 14 d after sowing (VC+T) and (4) substrate supplemented with VCT (20% v/v) (VCT). All four treatments were not inoculated with Pa (-Pa). Treatments 5-7 were the same as treatments 2-4 but were inoculated with Pa (+Pa) as previously described.

2.5 Promotion of Plant Growth

After harvesting 42-d-old lettuce (Butter Head) plants, plant growth characteristics were recorded. These included the height, width, fresh and dry weights of lettuce leaves, leaf numbers, leaf area (measured by Li-3100 Area Meter) and the basal stem cutting surface area of each plant.

2.6 Fungal Colonization of Roots

Colonization of lettuce roots by Pa and Ta were detected on modified BNPRA [21] and Martin's medium respectively. The samples of roots collected from 42-d-old lettuce plants were disinfested in 0.525% sodium hypochlorite for 3 min, washed three times with sterile distilled water, blotted dry and cut into 1.0 cm pieces before being placed on the selective media. Mycelial growth from the lettuce root samples was detected and recorded.

2.7 Substrate Colonization by *Pythium* and *Trichoderma*

Substrate samples from all treatments were collected and enumerated for surviving populations of Pa and Ta using modified BNPRA and Martin's medium respectively. All plates were incubated for 3-5 days at room temperature (25-30 °C). Colonies developed from substrate samples were observed and counted.

2.8 Statistical Analyses

All data were statistically analyzed using analysis of variance (ANOVA). The significance of the difference between the treatment means was determined by the Least Significant Difference (LSD) using the statistical programs R [22]. The significance level was set at P≤0.05.

3. RESULTS

3.1 Effects of VCT and VC+T on Plant Growth Promotion

Both of the treatments without Pa inoculation, VCT (-Pa) and VC+T (-Pa) significantly enhanced the heights of Butter Head lettuce by 22.13 and 15.07 % respectively as compared to the control (-Pa). Treatments inoculated with Pa, VCT (+Pa) and VC+T (+Pa) significantly increased the heights of Butter Head lettuce by 9.43 and 9.43 % respectively when compared with the control (VC (+Pa)) (substrate supplemented with vermicompost and Pa- inoculation). Similar results were obtained for the width of Butter Head lettuce. The VCT and VC+T treatments without Pa inoculation (-Pa) significantly increased the width of Butter Head lettuce by 12.16 and 18.56 % respectively. Only the VCT treatment with Pa inoculation (VCT (+Pa)) significantly increased the width of butter head lettuce by 6.27 % when compared with the control (VC (+Pa)) (Table 1).

Table 1 Effects of planting substrates supplemented with vermicompost (VC) and vermicompost fortified with *Trichoderma asperellum* CB-Pin-01 (VCT and VC+T) on the height and width of 42-d-old Butter Head lettuce

Treatment	Height (cm)	Width (cm)
1.Control (-Pa)	16.58 c[1/]	24.67 cd
2.VC (-Pa)	18.83 b (+13.57%)[2/]	24.75 cd (+0.32%)
3.VC+T (-Pa)	19.08 b (+15.07%)	29.25 a (+18.56%)
4.VCT (-Pa)	20.25 a (+22.13%)	27.67 b (+12.16%)
5.VC (+Pa)	16.75 c	23.92 d
6. VC+T (+Pa)	18.33 b (+9.43%)[3/]	24.33 cd (+1.71%)
7. VCT (+Pa)	18.33 b (+9.43%)	25.42 c (+6.27%)
C.V. (%)	1.98	1.97

[1/] Means in each column followed by the same letter (s) are not significantly different according to the Least Significant Difference (LSD) (P≤0.05).
[2/] Percentage of increment (+) of each treatment mean when compared with the control (-Pa) (substrate without VC, Pa-uninoculation).
[3/] Percentage of increment (+) of each treatment mean when compared with the control (VC (+Pa)) (substrate with VC and inoculated with Pa).

Two *Trichoderma* treatments (VC+T and VCT) increased leaf number, leaf area and basal stem cutting surface area by 13.06 and 21.05 %, 8.44 and 16.97% and 10.56 and 32.54 % respectively when compared with the control (-Pa). When treatments were inoculated with Pa (+Pa), the *Trichoderma* treatments, VC+T (+Pa) and VCT (+Pa), could also increase leaf number, leaf area and basal stem cutting surface area by 10.73 and 11.18 %, 7.19 and 20.16% and 27.62 and 39.15 % respectively when compared with the control (VC (+Pa)) (Table 2).

Table 2 Effects of planting substrates supplemented with vermicompost (VC) and vermicompost fortified with *Trichoderma asperellum* CB-Pin-01 (VCT, VC+T) on leaf number, leaf area and basal stem cutting surface area of 42-d-old Butter Head lettuce

Treatment	Leaf number	Leaf area (cm^2)	Basal stem cutting surface area (cm^2)
1.Control (-Pa)	25.5 cd[1/]	64.27 ab	1.84 e
2.VC (-Pa)	26.33 c (+3.35%)[2/]	71.48 ab (+11.21%)	2.44 bcd (+32.54%)
3.VC+T (-Pa)	28.83 b (+13.06%)	75.57 a (+16.97%)	2.04 cd (+10.56%)
4.VCT (-Pa)	30.92 a (+21.25%)	69.70 ab (+8.44%)	3.01 a (+63.61%)
5.VC (+Pa)	24.13 d	61.02 c	1.99 de
6.VC+T (+Pa)	26.72 c (+10.73%)[3/]	65.41 bc (+7.19%)	2.55 bc (+27.62%)
7.VCT (+Pa)	26.83 c (+11.18%)	73.36 a (+20.16%)	2.78 ab (+39.15%)
C.V. (%)	1.97	1.96	2.10

[1/] Means in each column followed by the same letter (s) are not significantly different according to the Least Significant Difference (LSD) ($P \leq 0.05$).
[2/] Percentage of increment (+) of each treatment mean when compared with the control (-Pa) (substrate without VC, Pa-uninoculation).
[3/] Percentage of increment (+) of each treatment mean when compared with the control (VC (+Pa)) (substrate with VC and inoculated with Pa).

3.2 Effects of VCT and VC+T on Plant Weight

The treatments of VC+T and VCT significantly increased fresh and dry weights of 42-d-old Butter Head lettuce by 12.51, 28.60 and 29.11, 35.58 % respectively when compared with the control (-Pa). The treatments inoculated with Pa, VC+T (+Pa) and VCT (+Pa) increased fresh and dry weights of Butter Head lettuce by 2.33, 9.81 and 7.36, 15.07 % respectively. The VC treatment (substrate supplemented with only vermicompost and Pa-uninoculation) increased Butter Head lettuce fresh and dry weights by 0.61 and 15.88 % when compared with the control (-Pa) (Table 3).

Table 3 Effects of planting substrates supplemented with vermicompost (VC) and vermicompost fortified with *Trichoderma asperellum* CB-Pin-01 (VCT, VC+T) on the fresh and dry weights of 42-d-old Butter Head lettuce

Treatment	Fresh weight (g)	Dry weight (g)
1.Control (-Pa)	98.83 b[1/]	3.40 d
2.VC (-Pa)	99.44 b (+0.61%)[2/]	3.94 bc (+15.88%)
3.VC+T (-Pa)	111.00 a (+12.51%)	4.39 ab (+29.11%)
4.VCT (-Pa)	127.10 a (+28.60%)	4.61 a (+35.58%)
5.VC (+Pa)	89.17 c	3.08 d
6.VC+T (+Pa)	91.25 c (+2.33%)[3/]	3.31 d (+7.36%)
7.VCT (+Pa)	97.92 c (+9.81%)	3.55 cd (+15.07%)
C.V. (%)	1.96	2.08

[1/] Means in each column followed by the same letter (s) are not significantly different according to the Least Significant Difference (LSD) ($P \leq 0.05$).
[2/] Percentage of increment (+) of each treatment mean when compared with the control (-Pa) (substrate without VC, Pa-uninoculation).
[3/] Percentage of increment (+) of each treatment mean when compared with the control (VC (+Pa)) (substrate with VC and inoculated with Pa).

3.3 Effects of VCT and VC+T on Root and Substrate Colonization by Pa and T

Lettuce roots and planting substrates from all *Trichoderma* treatments were found to be highly colonized by *T. asperellum* CB-Pin-01 by 30-100 and 100 % respectively. Colonization of roots and planting substrates by Pa in treatments using VCT (+Pa) and VC+T (+Pa) were 0, 53.33 and 0, 80.00 % respectively, whereas 100 % of colonization was found from the treatment of VC (+Pa) without T (Table 4 and 5).

Table 4 Colonization percentages of *Pythium aphanidermatum* (Pa) and *Trichoderma asperellum* (T) on 42-d-old lettuce roots planted in substrates supplemented with vermicompost (VC) and vermicompost fortified with *T. asperellum* CB-Pin-01 (VCT, VC+T)

Treatment	Root colonization (%)	
	Pa	T
1.Control (-Pa)	nd[1/]	nd
2.VC (-Pa)	nd	nd
3.VC+T (-Pa)	nd	100
4.VCT (-Pa)	nd	35
5.VC (+Pa)	100	nd
6.VC+T (+Pa)	53.33	100
7.VCT (+Pa)	0	30

[1/] nd = Non determined

Table 5 Colonization percentages of *Pythium aphanidermatum* and *Trichoderma asperellum* on planting substrates supplemented with vermicompost and vermicompost fortified with *T. asperellum* CB-Pin-01 (T) at 42 d after sowing

Treatment	Planting substrates colonization (%)	
	Pa	T
1.Control (-Pa)	nd[1/]	nd
2.VC (-Pa)	nd	nd
3.VC+T (-Pa)	nd	100
4.VCT (-Pa)	nd	100
5.VC (+Pa)	100	nd
6.VC+T (+Pa)	80	100
7.VCT (+Pa)	0	100

[1/] nd = Non determined

4. DISCUSSION

Enhancement of plant growth induced by *Trichoderma* species has been reported for a large number of different groups of plants including cereals, vegetables and ornamental and forestry crops. Various mechanisms have been proposed to explain plant growth promotion associated with *Trichoderma* species. These include synthesis of phytohormones, either by microbes or plants; production of vitamins; increased uptake and translocation of nutrients; enhanced solubilization of soil nutrients; enhanced root development and an increased rate of carbohydrate metabolism; photosynthesis and plant defense mechanisms [6],[7]. Benitez *et al*. [23] reported that *Trichoderma* strains were able to produce plant growth hormones such as cytokinin-like molecules, *e.g.* zeatin and gibberellin GA3 or GA3-related which directly affect plant growth.

Many researchers have reported the abilities of root colonization by *T. harzianum* to increase nutrient uptake and fertilizer utilization efficiency resulting in plant growth promotion. *T. harzianum* T22 treated maize seeds showed an increase in the efficacy of nitrogen use [6], [7]. This is similar to Lamool [11] who reported that the application of spore suspension of *T. asperellum* CB-Pin-01 (1 kg-fresh culture 2,000 L^{-1}) into the nutrient solution of lettuce grown in the NFT hydroponic system effectively enhanced not only the reduction of Pythium root rot but also the promotion of lettuce growth. Root colonization by *T. asperellum* CB-Pin-01 also resulted in the enhancement of phosphorus, potassium and nitrogen in lettuce leaves by 70, 18 and 6% as compared to untreated controls respectively. This is similar to Kansarn [12] who reported that the transplanting of lettuce seedlings sown in a sowing substrate supplemented with spore suspension of *T. harzianum* CB-Pin-01 into a planting substrate with a ratio of 70:20:10 (percent by volume) of *T. harzianum* CB-Pin-01 – soaked, chopped coconut husks, compost and ash powder, reduced the percentage of *P. aphanidermatum* infected roots and promoted the growth of lettuce when compared with the *Pythium* inoculated control. Buasuwan [24] reported that the treatment used *Bacillus mycoides* FL17 bacteria bioproduct-vermicompost combination could promote plant growth by giving the highest fresh weight of Butter Head lettuce plants (149.19 g/plant).

The application of microbial enriched vermicompost has been reported by Chaohem [25] who found that *T. asperellum* CB-Pin-01 (Ta)-fortified vermicompost (VCT) and cow manure (CM+Ta) promoted the growth of yard-long beans by increasing plant height and root length. These treatments also increased the percentages of seed germination and seedling survival of yard-long beans grown in a planting substrate inoculated with *Rhizoctonia solani*. Baliah and Muthulakshmi [26] reported that Okra growth in vermicompost enriched with microbial, *Pseudomonas fluorescens*, *Azospirillum brasilense* or *Bacillus megaterium* has shown growth in such characteristics as seed germination, the seedling vigor index, shoot lengths, root lengths, and plant fresh and dry weights. These characteristics were significantly higher when compared with a control or only vermicompost. Moreover, biochemical factors such as total chlorophyll, carotenoid, protein, and amino acids were higher in *A. brasilense* enriched vermicompost whereas in the case of glucose and NR activity, results were higher with *P. fluorescens* enrichment. These findings were similar to Khare *et al*. [27] who reported aonla and bael seedlings grown in microbial enriched vermicompost showed a significant increase in plant growth over the seedlings grown in unammended vermicompost.

Our studies obviously indicated that the vermicompost (VC) was an effective organic fertilizer while the vermicompost fortified with *T. asperellum* CB-Pin-01 (VCT) was effective as both an organic fertilizer and a biocontrol agent promoting growth and yield and also suppression of Pythium infection on the roots of lettuce. This finding was supported by the previous report by Joshi *et al*. [28]

5. CONCLUSIONS

This study revealed that a planting substrate supplemented with VCT provided the highest growth promotion of lettuce by increasing the height, leaf number, leaf area, basal stem cutting surface area, and plant fresh and dry weights when compared with a control (substrate without vermicompost). When the substrates were inoculated with *P. aphanidermatum* (+Pa), the treatment VCT (+Pa) completely reduced Pa-colonization on lettuce roots and planting substrates. Moreover, *T. asperellum* CB-Pin-01 could be completely recovered from planting substrates (100%) and lettuce roots (100%). The effects of VCT on the nutrient quality of lettuce plants should be investigated further.

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