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Compost Teas for the Suppression of Gummy Stem Blight (*Didymella byroniae*) in Greenhouse Cucumbers

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ABSTRACT

Five vermicompost teas (four aerobic and one anaerobic) were tested for their ability to suppress gummy stem blight in conventionally and organically grown greenhouse cucumbers. Three aerobic teas were amended with clove, thyme or Japanese knott weed (JKW) while two were left un-amended. The fluorescent (*Pseudomonas)* and total microbial densities and physiological activities of all compost teas were determined. Teas containing clove, thyme and JKW had significantly higher fluorescent bacterial counts than un-amended teas. Aeration also enhanced fluorescent bacterial density. Total bacterial population density was greatest in clover -containing aerobic tea. However, physiological activity was lower in clove containing tea than in un-amended aerobic tea or aerobic tea amended with thyme. An *in vitro* plate test showed a significant reduction in the mycelia growth of *D. byroniae* on media containing clove, thyme or JKW. *D. byroniae* was completely inhibited when media contained 0.03 g/l clove. In conventionally grown cucumbers, lengths of stem lesions caused by *D. byroniae* were significantly reduced by all aerobic teas. Shortest stem lesion length was observed in plants treated with clove containing tea. The average lesion length in the control treatment for conventionally grown cucumber was 123 mm whereas that for organically grown cucumber was 77 mm. Stem lesion length did not differ significantly from the control in organically grown cucumber with respect to different compost tea treatments. In both conventionally and organically grown cucumbers, clove-amended teas resulted in the shortest stem lesions. Clove, because of its ability to suppress mycelial growth and enhance populations of fluorescent *Pseudomonas*, should be considered as a standard additive to compost teas, particularly those aimed at suppressing fungal pathogens.

Keywords: aerobic, anaerobic, clove, *Pseudomonas*, thyme

INTRODUCTION

Both aerobic and anaerobic compost teas prepared from composts made from a variety of feed stocks have been used to suppress a number of plant diseases (Weltzien 1991; Scheuerell and Mahaffe 2002). However, no documented evidence is available on the use of compost teas to control gummy stem blight. Gummy stem blight caused by *Didymella bryoniae* is a serious disease affecting cucumbers grown in several geographically diverse regions such as British Columbia, Canada (Utkhede and Koch 2002), Japan (Wako *et al*. 2000), the Netherlands (Van Steekelenburg 1982) and North Carolina, USA (St. Amand and Wehner 1995). Control of gummy stem blight is challenging since conditions that favor further infections are generated by frequent wounding of plants due to fruit harvesting, side shoot trimming and removal of senescent leaves during on-going crop management. The present study was undertaken to determine whether compost teas can be used to control gummy stem blight in greenhouse cucumbers grown using conventional and under organic practices. Since compost teas constitute non-chemical control, they are particularly useful to organic growers who are prohibited from using synthetic chemical pesticides. A further objective was to determine whether efficacies of compost teas can be augmented by supplementing with clove (*Syzygium aromaticum*), thyme (*Thymus vulgaris*) or japanese knott weed (*Polygonum cuspidatum*). Clove and thyme inhibit mycelial growth and the production of carcinogen aflatoxin by pathogen *Aspergillus parasiticus* (Bullerman *et al*. 1977; Buchanan and Shepherd 1981). Japanese knott weed (JKW) contains a natural phytoalexin resveratrol (Zhang *et al*. 2007) that inhibits the mycelial growth of *Botrytis cinerea* and *Eutypa lata* (Coutos-Thevenot *et al*. 2001). In view of the inherent anti-pathogen and anti-toxin activity of clove, thyme and JKW we hypothesized that incorporating these plants or their extracts in teas may further enhance the ability of compost teas to control fungal pathogens. In addition, these additives may discourage the growth of any enteric bacteria from the compost. Aqueous infusions of clove have been shown to inhibit several pathogens including *E. coli* and *Salmonella* (Banerjee *et al*. 2006). Conditions that favor the growth of *E. coli* in compost teas and precautionary measures including addition of carrot juice that contain antimicrobial phytoalexin 6-metoxymellein (Kurosaki and Nishi 1983) to minimize growth of such pathogens have been previously reported (Kannangara *et al*. 2006).

MATERIALS AND METHODS

Preparation of vermicompost teas

Five different vermicompost teas (aerobic, aerobic containing clove, thyme, or JKW, and anaerobic) were prepared. Each compost tea contained 26 g of compost, 4 g of molasses, 4 g of kelp and 100 ml of carrot juice in 1litre of water. Carrot juice was prepared according to Kannangara *et al*. (2006) by homogenizing 235 g of chopped carrots in a glass jar blender and filtering the homogenate through 4 layers of cheese cloth. Final volume of the carrot juice was adjusted to 750 ml with distilled water and 100-ml aliquots were added to each of the compost teas. Clove-supplemented compost tea contained 2.5 g of dry clove powder while tea with thyme contained 50 ml of thyme extract. Thyme extract was prepared by homogenizing 45 g of thyme leaves and filtering the homogenate through cheese cloth and adjusting the volume to 400 ml with distilled water. Compost tea with JKW contained 50 ml of JKW root extract. JKW root extract was prepared by homogenizing 40 g of root bark in water and filtering the homogenate

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through cheese cloth and adjusting the volume to 300 ml. The final volume of each compost tea was adjusted to 1litre with distilled water. All teas except one (anaerobic) were aerated continuously at 0.8 l/min for 48 h using a single head oil-less Barnant diaphragm pump (Barnant Co., Illinois) as described previously (Kannangara *et al*. 2006).

Growing conditions of cucumber plants

All experiments were conducted in the greenhouses at the Pacific Agri-Food Research Centre in Agassiz, British Columbia, Canada. Cucumber (*Cucumis sativus* L. cv. 'Enigma') purchased from Terralink Horticulture Inc, Abbotsford, BC, Canada were seeded into rockwool cubes. Cucumber cultivar 'Enigma' was chosen as it has been used previously to study the effect of chemical and biological treatments on the control of gummy stem blight (Utkhede and Koch 2002). Each rock wool cube was seeded with a single cucumber seed, covered with vermiculite and watered with 2% solution of all purpose fertilizer (Technigro, 20: 20: 20 NPK) purchased from Sun Gro Horticulture, U.S.A. After 4 weeks from seeding, rockwool cubes bearing healthy seedlings were transplanted to 15-l bags of sawdust. Total of 192 plants divided into two groups of 96 were used in the experiment. The treatments were completely randomized within each group. One group was fed with conventional (inorganic) nutrient solution while the other was fed with organic nutrient solution. To dispense the nutrients, two drip tubes were placed in each sawdust bag. Conventional nutrient solution was prepared by dissolving macronutrients: Ca $(NO_3)_2$, 0.78; $MgSO_4$, 0.24; KH_2PO_4, 0.18; KNO_3, 0.546; K_2SO_4, 0.036; g/l in water and adding a commercial chelated micronutrient mix (Plant Products Co. Ltd, ON, Canada) at 0.15 ml/l to give final concentration of micronutrients in ppm: Fe, 1.05; Mn, 0.3; Zn, 0.06; Cu, 0.015; B, 0.195: Mo, 0.009. The pH of the nutrient solution was adjusted to 6.0 with 0.18 ml/l of 36% Sulfuric acid. Organic nutrient solution was prepared by dissolving 3.8 g each of Fishplus grow (3-1-1), Pacific Natural fish fertilizer (Great Pacific Bioproducts, Delta, BC, Canada), (2-3-0.3), 0.28 g of CalPril (Ca 36%), 0.23 g of DolPril (Ca 20%, Mg 11%), 0.62 g of OMRI certified mined Potassium Sulphate, 0.55 g of Food Grade Citric acid 1 l of water. CalPril, Dolpril, Potassium Sulphate and Food Grade Citric acid were purchased from Terralink Horticulture, Inc. All plants were supported using nylon cords attached to wires running horizontally 2 m above the greenhouse floor. Greenhouse temperature varied from 18 to 23°C, relative humidity maintained around 55% and a 16-h photoperiod with a light intensity of 684 watts per m^2.

Inoculum preparation and infection of the wounds

D. *bryoniae* isolated from the greenhouse cucumber plants was cultured on cucumber dextrose agar (CDA). CDA medium contained dextrose (0.6%) and agar (2%) in cucumber leaf broth. Cucumber leaf broth was prepared by boiling chopped cucumber leaves in water (250 g/l) for 10 mins and then filtering through cheesecloth. The CDA plates were incubated at room temperature until spore producing pycnidia were formed. Conidia were collected by washing the plates with sterile water and scraping the surface with a rubber spatula. The washings were filtered through four layers of sterile cheesecloth to remove mycelial fragments, pycnidia and any pieces of dislodged agar. The spore density of the filtrate was determined using a hematocytometer and adjusted to 1×10^6 per ml with sterile distilled water. The plants were inoculated through wounds created at 2nd, 4th and 6th nodes from the cotyledon node. Wounds were created by removing the leaves at nodes 2, 4 and 6 while leaving approximately 1cm petiole stubs from the stem, and then crushing the petiole stubs with a pair of pliers. One ml of inoculum was pipetted into each crushed petiole stub. Approximately two hours after inoculation, 4ml of water or compost teas were pipetted into each inoculated site. The lengths of stem lesions at the nodes caused by *D. bryoniae* were measured at six weeks after inoculation.

Characterization of compost teas

All compost teas were characterized with respect to pH, EC, total and fluorescent microbial density, and physiological activity. Total bacterial counts and the fluorescent *Pseudomonas* counts were determined by plating appropriate dilutions of compost teas on nutrient agar and on King's B medium (King *et al*. 1954) respectively. In addition, the presence of any fluorescent bacteria in clove powder, thyme and JKW extracts were determined by plating on King's B medium suspensions of clove powder in water, diluted extracts of thyme and JKW equivalent to that added to compost tea. Physiological activities of compost teas were determined using Biolog Ecoplates (Biolog Inc., Hayward, CA, USA). Each Ecoplate contained 96 wells out of which 93 were coated with 31 different tetrazolium containing carbon sources each replicated three times and three uncoated wells which served as controls. Aliquots (150 µl) of compost tea were dispensed into each well and the plates were incubated at room temperature (25°C). Three plates were used for the evaluation of each compost tea. Respiration from the utilization of carbon sources by microbes in compost tea resulted in reduction of tetrazolium leading to color development in wells. Color development in the wells was read at 590 nm for five consecutive days using a microplate reader (Beckman Coulter, AD 340). The intensity of color development in the wells (summed over all substrates) was used as an indicator of microbial activity of compost teas.

The growth of *D. bryoniae* on cucumber dextrose agar (CDA) containing clove, thyme or Japanese knott weed

Inhibitory effects of clove, thyme and JKW on the growth of *D. bryoniae* mycelium in vitro were determined using CDA media amended with clove, thyme or JKW. Ten different CDA media containing varying concentrations of clove powder, thyme or JKW were prepared. Clove powder amended media contained 0.1, 0.3 or 0.5 g dry clove powder per 100 ml of the cucumber broth. Thyme amended media contained 50, 25 or 5 ml thyme in 100 ml cucumber broth while JKW amended media contained 50, 25 or 10 ml of JKW in 100 ml cucumber broth. The thyme and JKW solutions used in the preparation of the CDA media were the same as those used in compost tea preparations. All media contained 0.6% dextrose and 2% agar. *D. bryoniae* growth on un-amended CDA medium served as the control. Four Petri dishes were prepared from each medium. Discs measuring 6 mm in diameter were cut from the perimeter of actively growing *D. bryoniae* on CDA and placed, one per dish, at the centre of each Petri dish. The diameters of the discs were measured at 120 h to determine the effect of clove, thyme and JKW on mycelial growth of *D. bryoniae*.

All data were analyzed by ANOVA using SigmaStat (SPSS Inc, Chicago, Illinois) software package and the means were compared using Duncan's multiple range test.

RESULTS AND DISCUSSION

Characterization of compost teas

The pH of all aerated compost teas ranged from 7.1 to 7.5 while that of non-aerated compost tea remained acidic at 4.62. The production of acids under anaerobic conditions during the brewing of compost teas and decomposition of solid organic waste is well known (Lynch 1977; Kannangara *et al*. 2006). The EC values of the aerated and non-aerated compost teas did not differ significantly and ranged from 1.8 to 1.9 ms/cm^2. On nutrient agar, the clove containing compost tea had significantly higher bacterial colony forming units (CFU) than other teas (**Fig. 1A**). The lowest CFU was noted for anaerobic tea, but the CFU counts in anaerobic tea was not significantly different from those of aerobic, thyme, or JKW containing teas. The fluorescent bacterial CFU in compost teas containing clove, JKW or thyme were significantly higher than those of un-amended aerated, and non-aerated compost teas (**Fig. 1B**). The fluorescent CFU was significantly higher in clove- containing compost tea compared to other teas. JKW-containing compost tea had a significantly higher fluorescent CFU than thyme-containing compost tea while the aerobic compost tea had a significantly higher fluorescent CFU than

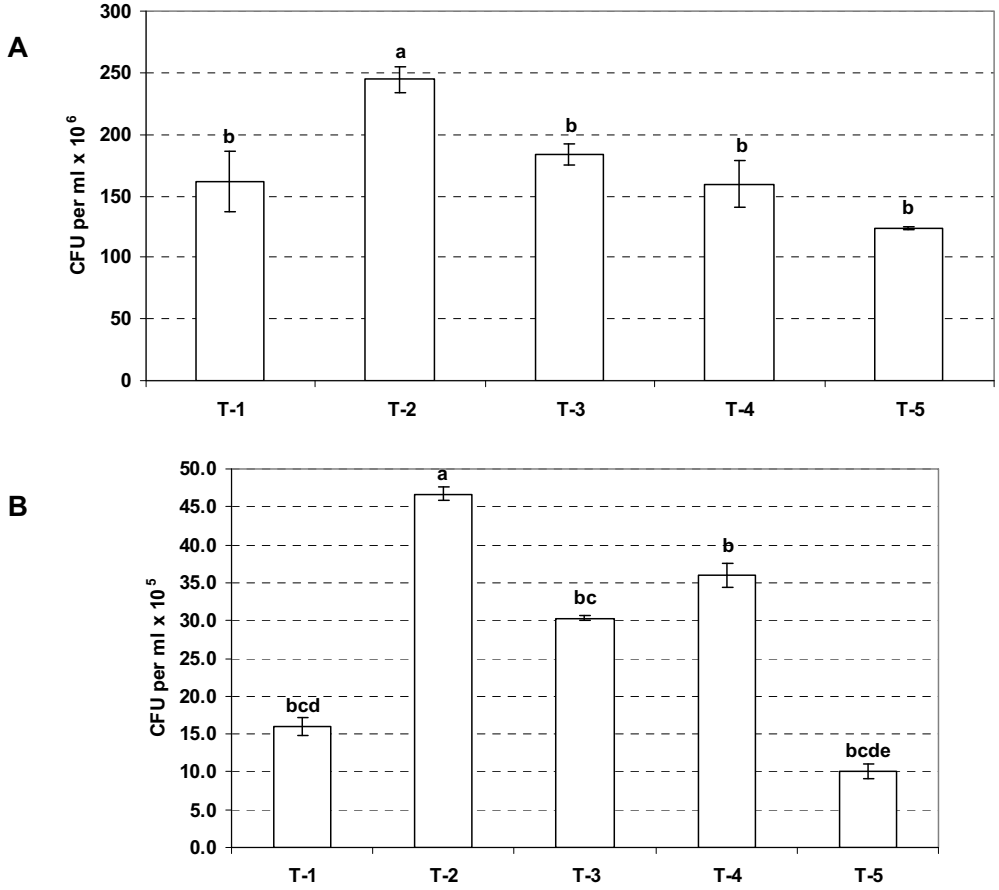

Fig. 1 (**A**) Colony forming units (CFU) of bacteria in compost teas plated on nutrient agar. Un-amended aerobic compost tea (T1), aerobic compost tea amended with clove (T2), thyme (T3), Japanese knott weed (T4) and un-amended an-aerobic compost tea (T5). Values represent mean ± standard error. Bars with different letters indicate a significant difference ($P < 0.05$). (**B**) Colony forming units of fluorescent bacteria in compost teas plated on King's B medium. Un-amended aerobic compost tea (T1), aerobic compost tea amended with clove (T2), thyme (T3), Japanese knott weed (T4) and un-amended an-aerobic compost tea (T5). Values represent mean ± standard error. Bars with different letters indicate a significant difference ($P < 0.05$).

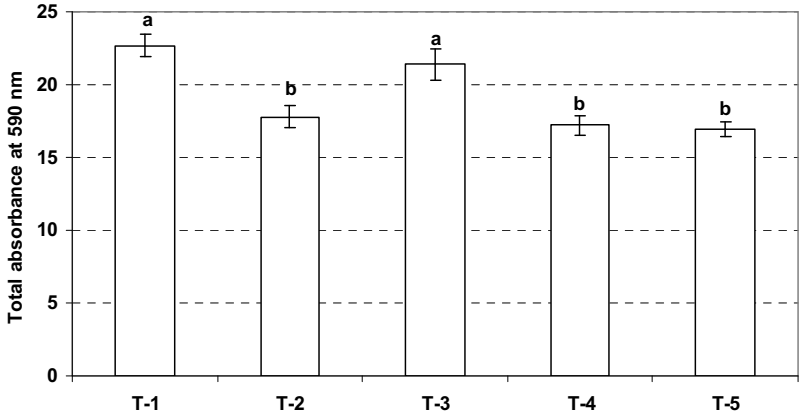

Fig. 2 Physiological activity of compost teas. Un-amended aerobic compost tea (T1), aerobic compost tea amended with clove (T2), thyme (T3), Japanese knott weed (T4) and un-amended an-aerobic compost tea (T5). Values represent mean ± standard error. Bars with different letters indicate a significant difference ($P < 0.05$).

anaerobic compost tea. The lowest fluorescent CFUs were found in anaerobic compost tea. Fluorescent Pseudomonad bacteria were not detected in aqueous suspensions of clove powder or extracts of thyme and JKW, suggesting that they originated from the compost and that clove, thyme and JKW enhanced their proliferation during the brewing of compost teas. Fluorescent Pseudomonads (*Pseudomonas fluorescens* E118) have been shown to utilize eugenol, a major ingredient of clove, as an enrichment substrate (Furukawa *et al*. 1998). The involvement of fluorescent Pseudomonads in disease suppression through the production of iron chelating siderophores (Kloepper *et al*. 1980; Scher and Baker 1982) and the production of antifungal compounds by fluorescent bacteria is well known (Baehler *et al*. 2005). Furthermore, in a previous study we observed that potting mixes amended with compost resulted in greater rhizosphere populations of fluorescent Pseudomonads that suppressed the fungal pathogen *Fusarium oxysporum* (Kannangara *et al*. 2000) in cucumber seedlings grown in compost amended potting mixes.

The physiological activities of compost teas as measured by the utilization of substrates in Biolog Ecoplates indicated that un-amended aerobic tea and thyme containing tea had significantly higher physiological activity than other teas (**Fig. 2**). The bacterial CFU on nutrient agar was highest in clove containing tea. However, total light absorbance

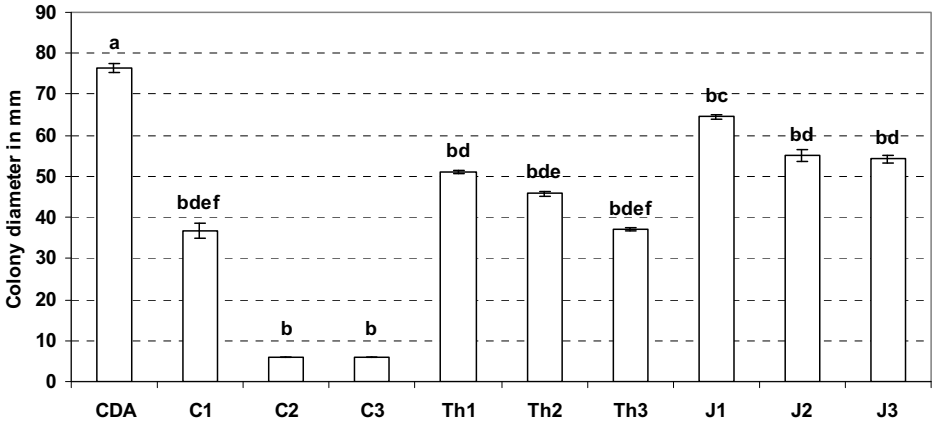

Fig. 3 The colony diameter of *Didymella bryoniae* after 120 h of culture on cucumber dextrose agar (CDA) amended with clove, thyme or Japanese knott weed. Amended CDA contained clove 0.1 g (C1), 0.3 g (C2), 0.5 g (C3), aqueous extracts of thyme 5 ml (Th1), 25 ml (Th2), 50 ml (Th3), Japanese knott weed 10 ml (J1), 25 ml (J2), 50 ml (J3) per 100 ml of the medium. Values represent mean ± standard error. Bars with different letters indicate a significant difference ($P < 0.05$).

of clove containing tea was significantly less than that of un-amended aerobic tea. This suggests that clove is interfering with the utilization of carbon sources by bacteria in aerated compost teas. Eugenol, a major ingredient of clove has been shown to inhibit color development in tetrazolium based assays (Kasugai *et al*. 1990). Even though there was no significant difference between aerobic and anaerobic compost teas in CFU counts on nutrient agar, there was a significant difference in total color development in aerobic tea compared to anaerobic tea. This may indicate anaerobic conditions may reduce the diversity of bacteria such that the number of substrates utilized by the community is reduced.

Growth of *D. bryoniae* on cucumber dextrose agar media containing clove and extracts of thyme and Japanese knott weed

Clove, thyme and JKW significantly reduced mycelial growth of *D. bryoniae in vitro* with clove being the most inhibitory to growth (**Fig. 3**). Clove at 0.1 g/l inhibited the growth of mycelia by 50% and at 0.3 g/l mycelial growth was completely inhibited. Thyme was more effective than JKW in inhibiting the growth of the mycelia even with the extract of lower concentration (0.112 g fresh wt/ml for thyme Vs 0.133 g fresh wt/ml for JKW). All amended and un-amended CDA media on which *D. bryoniae* were grown contained same amount (0.6%) of dextrose and hence the impact on mycelia growth that resulted from dilution of cucumber leaf broth by the addition of thyme and JKW extracts was expected to be minimal. Mycelial growth of *D. bryoniae* was similar in CDA and Potato dextrose agar except that the former is more conducive to pycnidia formation (unpublished observations). Significantly lower growth of *D. bryoniae* mycelium on CDA that contained clove, thyme and JKW (**Fig. 3**) and significantly higher fluorescent bacterial counts (**Fig. 1B**) in compost teas that contained clove, thyme and JKW suggest that clove, thyme and JKW are beneficial additives to compost teas.

Suppression of *D. bryoniae* stem lesions by application of compost teas

All aerobic tea applications to conventionally grown cucumber plants significantly reduced lesion size (**Fig. 4A**) compared to water treated control. Lesions on plants treated with anaerobic tea were smaller but not significantly different from the control. The smallest lesions were recorded for clove- containing compost tea which also had the highest fluorescent bacterial counts (**Fig. 1B**). All additives (clove, thyme and JKW) reduced lesion lengths, but were not significantly different from un-amended aerobic tea. In organically grown cucumbers, the teas containing clove, thyme and JKW did not significantly reduce the stem lesion lengths. In both conventional and organically grown crops clove amended tea resulted in the lowest lesion length. Overall, lesions caused by *D. bryoniae* in organically grown cucumbers were smaller than lesions on conventionally grown cucumbers (compare **Fig. 4A, 4B**). Since the crops grown under two nutrient regimes (conventional and organic) were kept as two separate groups, statistical comparisons of lesion lengths between the two groups were not attempted. Nevertheless, the data suggest that organically grown cucumber may be less susceptible damage by *D. bryoniae*.

CONCLUSIONS

Compost teas can be used to suppress gummy stem blight caused by *D. bryoniae* in greenhouse grown cucumbers. Aerobic compost teas are significantly better than anaerobic compost teas in suppressing *D. bryoniae*. The densities of fluorescent bacteria in compost teas can be increased by natural additives of clove, thyme and JKW that are known to posses anti pathogenic and anti toxic activity. Clove was the most effective of the three additives in suppressing gummy stem blight. Clove-containing compost tea had greatest bacterial CFU counts on nutrient agar, the greatest fluorescent bacterial counts on King's medium and gave the smallest lesion length. Furthermore, in Petri dishes, mycelial growth of *D. bryoniae* was lowest when CDA contained clove. Since it has been shown that aqueous infusions of clove inhibit *E. coli* and *Salmonella* (Banerjee *et al*. 2006), clove could also be used as a beneficial additive to compost teas to suppress fungal plant pathogens. A wider range of concentrations of clove besides that has been used in the present investigation needed to be tested to choose a suitable concentration to be used in a given compost tea. Besides its anti pathogenic activity, clove will counter any offensive odors that might emanate from anaerobic compost teas or from a given feedstock used in the preparation of compost teas. A reduction in physiological activity as measured by the tetrazolium color development in Biolog Ecoplates in clove containing compost tea had no bearing on the suppression of *D. bryoniae* suppression. In general the lengths of stem lesions caused by *D. bryoniae* were shorter in cucumbers grown under organic conditions compared to stem lesions of cucumbers grown using inorganic nutrients. On average shorter stem lesion lengths were noted in cucumber plants treated with clove containing compost tea.

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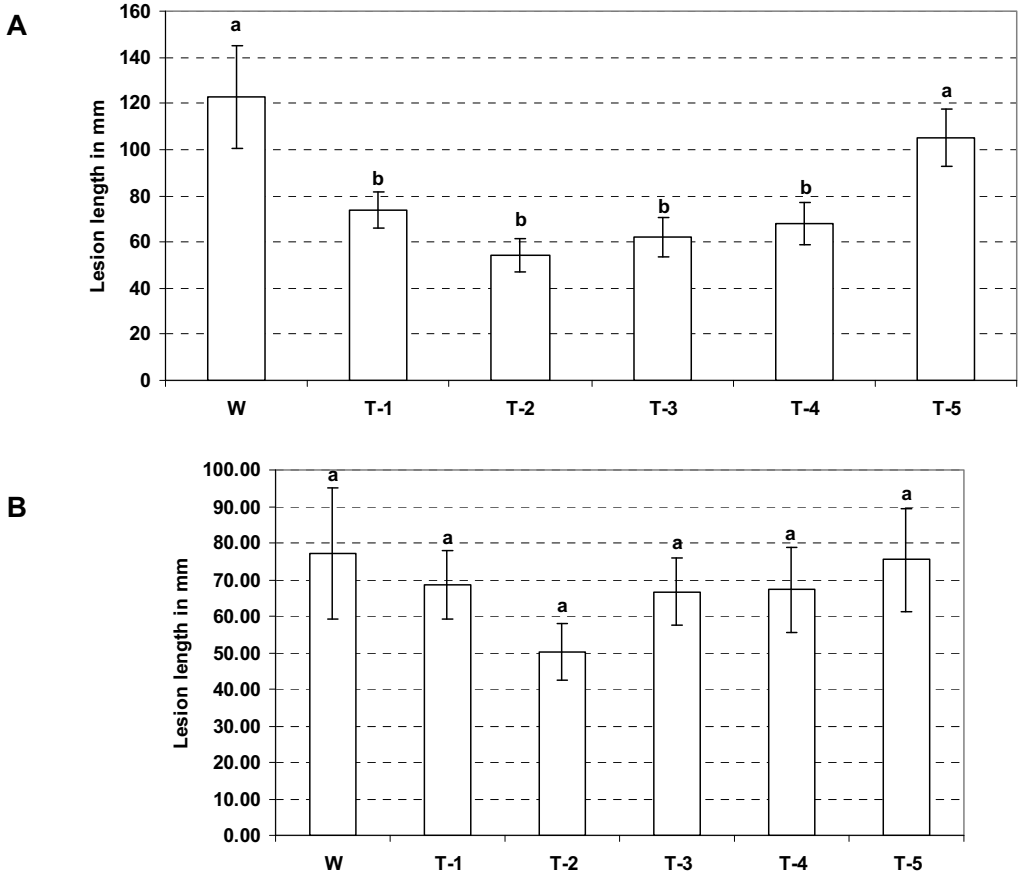

Fig. 4 (**A**) Effect of compost teas on the stem lesions caused by *Didymella bryoniae* in conventionally grown greenhouse cucumbers. Water (W), Un-amended aerobic compost tea (T1), aerobic compost tea amended with clove (T2), thyme (T3), Japanese knott weed (T4) and un-amended an-aerobic compost tea (T5). Values represent mean ± standard error. Bars with different letters indicate a significant difference ($P < 0.05$). (**B**) Effect of compost teas on the stem lesions caused by *Didymella bryoniae* in organically grown greenhouse cucumbers. Water (W), un-amended aerobic compost tea (T1), aerobic compost tea amended with clove (T2), thyme (T3), Japanese knott weed (T4) and un-amended an-aerobic compost tea (T5). Values represent mean ± standard error. Bars with different letters indicate a significant difference ($P < 0.05$).

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