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Effect of aqueous extracts from vermicomposts on attacks by cucumber beetles (*Acalymna vittatum*) (Fabr.) on cucumbers and tobacco hornworm (*Manduca sexta*) (L.) on tomatoes

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ABSTRACT

Vermicomposts are produced through interactions between earthworms and microorganisms in the breakdown of organic wastes. Aqueous extracts were prepared in commercial brewing equipment from vermicomposts produced from super-market food wastes. The ratio of vermicompost to water was one to five v:v, to produce a 20% aqueous solution which could be diluted to 5% and 10% concentrations. The effects of soil drenches applied at dilutions of 20%, 10%, and 5% vermicompost aqueous extracts, were compared with those of deionized water, in the suppression of cucumber beetles (*Acalymna vittatum*) attacking cucumbers and tobacco hornworms (*Manduca sexta*) attacking tomatoes, in greenhouse cage experiments. Tomatoes and cucumber seedlings were germinated and grown for 4 weeks in 25 cm diameter pots containing a soil-less growth medium – Metro-Mix 360 – and thinned to four plants per pot. They were placed under 0.2 mm mesh cages (40 cm × 40 cm × 40 cm), with one pot containing four plants in each treatment cage. At germination, plants were treated with soil drenches of 5%, 10%, or 20% vermicompost extract or a deionized water control to field capacity and thereafter at weekly intervals. A complete nutrient solution was applied weekly to all plants. In each experiment, eight cucumber beetles or eight tobacco hornworms were released onto the leaves of the appropriate plant species in each cage (four pests per test plant). All treatments were replicated four times per pest experiment, in a randomized complete block design. Numbers of pests were counted and damage rated (0-none to 5-total) on days 1, 3, 5, 7, 9, 11, 13 and 14 after the release of pests into the cages.

All of the concentrations of vermicompost extracts significantly suppressed the establishment of and the damage caused by the two pests on the plants. The higher the rate of aqueous extract application the greater was the suppression of the pests. We concluded that the most likely cause for the unpalatability of the plants to pests was the uptake of soluble phenolic compounds from the vermicompost aqueous extracts into the plant tissues. These compounds are known to make plants unattractive to pests and to affect pest reproduction and survival rates.

Introduction

Vermicomposts are produced from organic wastes through interactions between earthworms and microorganisms, and can be utilized as plant growth media or soil amendments (Edwards and Arancon 2004). They are produced by an aerobic and mesophilic microbial process involving complex interactions between earthworms and microorganisms which stabilizes the organic matter, reduces the C:N ratio, and makes the nutrients that it contains readily available to plants. Vermicompost aqueous extracts are much easier to handle and apply than solid vermicomposts, which are bulky and heavy (Yardim et al. 2006).

It has been shown that various organic amendments to soils, including manures and composts, can help to suppress plant pest populations and damage to plants growing in the amended soils (Chellemi 2002; Altieri and Nicholls 2003; Atkinson et al. 2004). The first reports of vermicomposts suppressing arthropod pests were by Arancon and Edwards (2004) and Arancon et al. (2005) who showed that solid vermicomposts produced significant suppression of mealy bug attacks (*Pseudococcus* sp.) on cucumbers and tomatoes, two-spotted spider mite attacks (*Tetranychus urticae*) on bush beans and eggplants and attacks by aphids (*Myzus persicae*) on cabbages by low application rates of solid food waste vermicomposts (Arancon et al. 2007). Yardim et al. (2006) reported the suppression of tomato hornworms and cucumber beetles by solid vermicomposts in the field and in greenhouse experiments.

More recent research at the Soil Ecology laboratory at O.S.U. has demonstrated significant suppression of plant parasitic

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nematodes, above-ground foliar arthropod pests and both foliar and root plant diseases by soil drenches of aqueous extracts, produced from vermicomposts, (Edwards et al. 2007, 2009).

The greenhouse experiments that are reported in this paper describe the effects of aqueous extracts produced from food waste-based vermicomposts, on populations and damage by cucumber beetles (*Acalymna vittatum*) to cucumber plants (*Cucumis sativa*) and the caterpillars of tobacco hornworm (*Manduca sexta*) to tomato plants (*Lycopersicon esculentum*). These vermicompost liquid extracts were applied to the plants at weekly intervals as soil drenches.

Materials and methods

Design of greenhouse experiments and production of test plants

The greenhouse experiments were organized in the Biological Sciences Greenhouse at The Ohio State University, Columbus, Ohio. Cucumbers and tomatoes were sown and grown in a commercial greenhouse soil-less bedding plant medium – Metro-Mix 360 (MM360). MM360 is a preparation of vermiculite, Canadian sphagnum peat moss, bark, ash, sand, and has a starter nutrient fertilizer in its formulation (Scotts, Marysville, OH). We used a commercial vermicompost produced from food wastes in automated continuous-flow vermicomposting reactor systems designed by Edwards and his colleagues in the UK (Edwards and Arancon 2004). The food wastes were obtained from a range of supermarkets in the Portland, Oregon area by the Oregon Soil Corporation. We have used these vermicomposts with considerable success to assess their effects on plant germination, growth, flowering and yields in a range of other projects funded by the USDA. (Edwards and Arancon 2004; Arancon et al. 2005; Edwards et al. 2007). The earthworm species used in processing these food wastes was *Eisenia fetida* (Savigny). The food wastes contained 1.3% N, 2.7% P, and 9.2% K and trace elements.

For all experiments, eight tomato or cucumber seeds were sown into each 25 cm diameter plant pot, containing Metro-Mix 360, and placed in a greenhouse to germinate. After plant emergence was complete, all pots were thinned to four seedlings per pot with one pot per cage. Plants were watered to approximately field capacity moisture content (200 ml) with Peter's Nutrient Solution, three times weekly throughout the experiments, to supply all needed nutrients. Peter's Nutrient

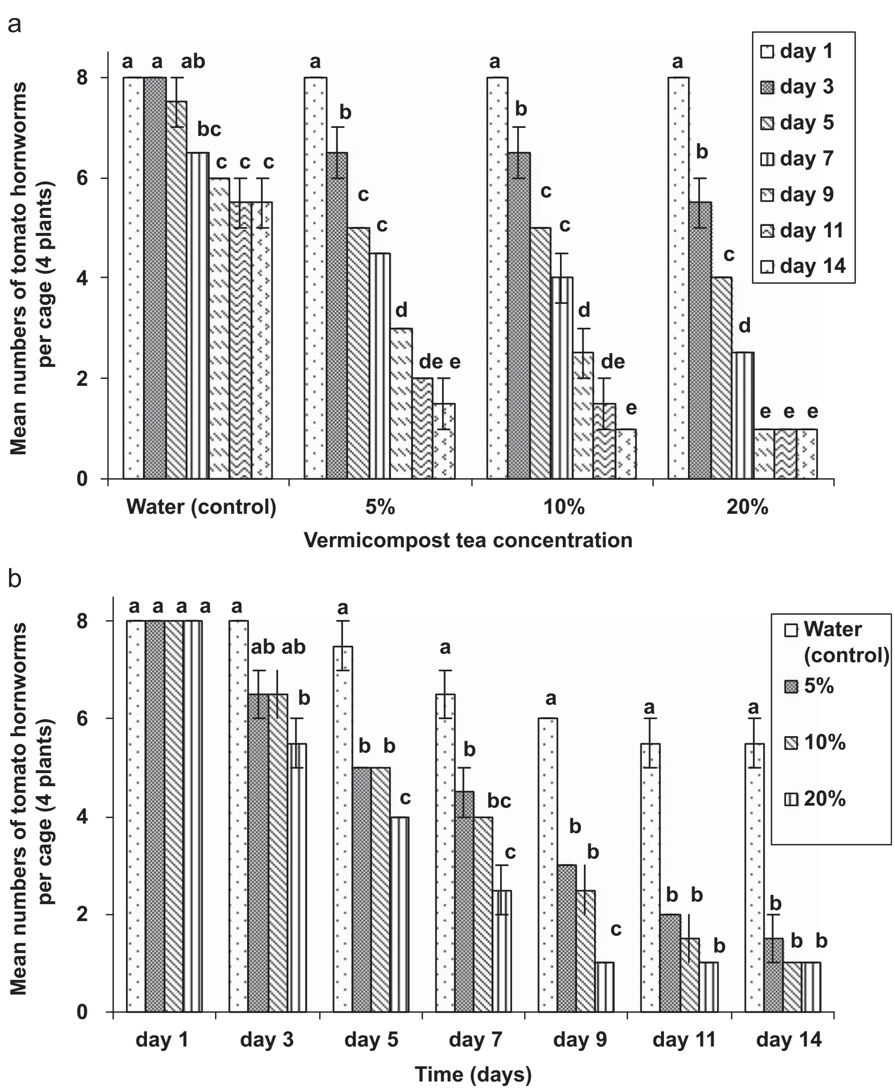

Fig. 1. (a) Changes in numbers of tobacco hornworms (*Manduca sexta*) between treatments on tomatoes treated with a range of concentrations of food waste vermicompost aqueous extracts ('teas'). (b) Changes in time in numbers of tobacco hornworms (*Manduca sexta*) on tomatoes treated with a range of concentrations of food waste vermicompost aqueous extracts ('teas') (Means ± S.E.). Plants grown in MM360 with all needed nutrients supplied. Means followed by the same letters are not significantly different for each treatment concentration ($P \leq 0.05$). LSD was calculated between days of sampling within each treatment.

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Solution is a water-soluble fertilizer, recommended for continuous liquid feed programs of bedding plants, and contains 7.77% NH_4–N, 12.23% NO_3–N, 10% P_2O_5, 20% K_2O, 0.15% Mg, 0.02% B, 0.01% Cu, 0.1% Fe, 0.056% Mn, 0.01% Mo, and 0.0162% Zn.

Treatments

The treatments consisted of a range of three concentrations of aqueous vermicompost extract, namely 5%, 10% and 20%, and their effects were compared with those of a deionized water control. The vermicompost aqueous extracts were produced in commercial vermicompost extract brewing equipment called the Growing Solutions System 10^{TM} Compost Tea Brewing Equipment, with a maximum capacity of 37.5 L. A 20% aqueous vermicompost solution was prepared by placing 7.5 L of food waste vermicompost in a mesh container in the brewing equipment containing 30 L of water. This was extracted for 24 h whilst aerating continually. The 20% aqueous extract was diluted to 10% v:v and 5% v:v for use in the experiments. The vermicompost was placed in a mesh container suspended in water and aerated for 24 h. Vermicompost aqueous extracts were used within 24 h of preparation to minimize loss of microbial activity.

The 5%, 10% and 20% aqueous vermicompost extracts and a deionized water control were applied as drenches to field capacity (200 ml) of the plant growing medium at sowing, and at weekly intervals thereafter. The tomatoes or cucumbers were exposed to test infestations by two species of arthropod pests – tobacco

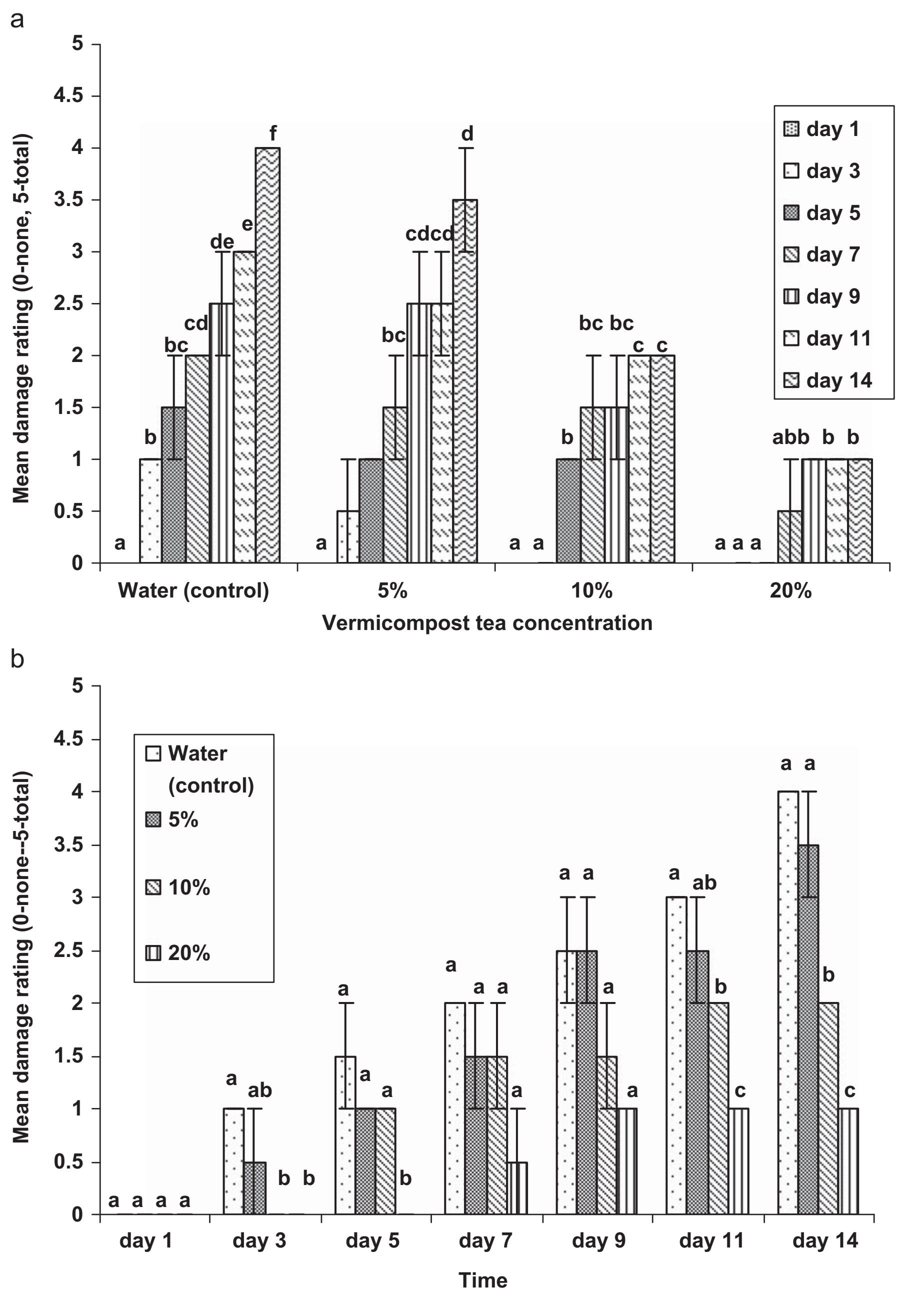

Fig. 2. (a) Changes in tobacco hornworm (*Manduca sexta*) damage ratings between treatments on tomato plants treated with a range of concentrations of food waste vermicompost aqueous extracts ('teas') (Means ± S.E.). (b) Changes in time in tobacco hornworm (*Manduca sexta*) damage ratings on tomato plants treated with a range of concentrations of food waste vermicompost aqueous extracts ('teas') (Means ± S.E.). Plants grown in MM360 with all needed nutrients supplied. Means followed by the same letters are not significantly different for each treatment concentration ($P \leq 0.05$). LSD was calculated between days of sampling within each treatment.

hornworms and cucumber beetles – for 2 weeks in cages. At the end of each experiment plants were harvested for mean shoot dry weight and leaf area measurements. Shoots were oven-dried at 60 °C for 24 h before weighing to measure shoot dry weights. On the same date mean leaf areas were measured using a Licor Model 3100 leaf area meter.

Pest infestations

Each experimental treatment involved growing seedlings of either tomatoes or cucumbers, in four pots with four seedlings per pot per treatment, confined in a single mesh cage (40 cm × 40 cm × 40 cm) covered with a 0.2 mm aperture nylon mesh. Each treatment was replicated four times. The seedlings were raised in an insect-free greenhouse environment for 4 weeks. Plants in cages were placed on capillary mats for easy and uniform watering with (1) deionized water (control), or (2) vermicompost extracts and irrigation water without removing the cage, according to the experimental protocol designated. All pots received Peter's Nutrient Solution, which provided all the required nutrients three times weekly. For all experiments, one pot containing four plants was placed in each individual cage for all treatments, which consisted of drenches to the growing medium with 5%, 10%, or 20% vermicompost aqueous extracts or a deionized water control.

The cucumber beetles were collected from the field and the tobacco hornworms were bred from eggs on tomato plants in the greenhouse. Pests were released into the cages after the plants had grown for 4 weeks. Either eight cucumber beetles or eight tobacco hornworm caterpillars were released into each cage (i.e. the equivalent of two pests per plant).

The numbers of cucumber beetles or tobacco hornworms surviving in each cage on the tomatoes and cucumbers were counted on days 1, 3, 5, 7, 9, 11, 13, and 14 after infestation. On the same days, assessments of damage to the plants, on a rating scale of 0 (none) to 5 (total damage) were made. A damage rating of three equates to 60% damage of the total foliage. These ratings are very reliable once technicians making the assessments have been appropriately trained.

The pots for the treatments in each experiment were laid out in the greenhouse in a completely randomized design (CRD). Data were analyzed using the same design in SAS (SAS ver. 9.1) statistical software. Damage ratings and herbivore numbers were analyzed using repeated measurement ANOVA in SAS. Differences between means were separated using LSD (least significant differences) at $P \leq 0.05$ as indicated in figure captions.

Results

Tobacco hornworms

In the experiments on tobacco hornworms attacking tomatoes, numbers of hornworm caterpillars were suppressed by all three concentrations of aqueous vermicompost extracts (F=7.38, df=18, 24, $P \leq 0.0001$) compared with the deionized water control (Figs. 1a, b). Mean numbers decreased from 8.0 caterpillars per cage on day 1 to 5.5 per cage on day 14 in treatments receiving the deionized water (control). For comparison they decreased from 8.0 caterpillars per cage to 1.5 caterpillars in response to 5% vermicompost extract applications, and 1.0 caterpillar per cage in response to 10% and 20% extract applications over 14 days. These decreased numbers were due to pest mortality. Tobacco hornworm pest counts were significantly different between times (F=205.35, df=6, 24, $P \leq 0.0001$).

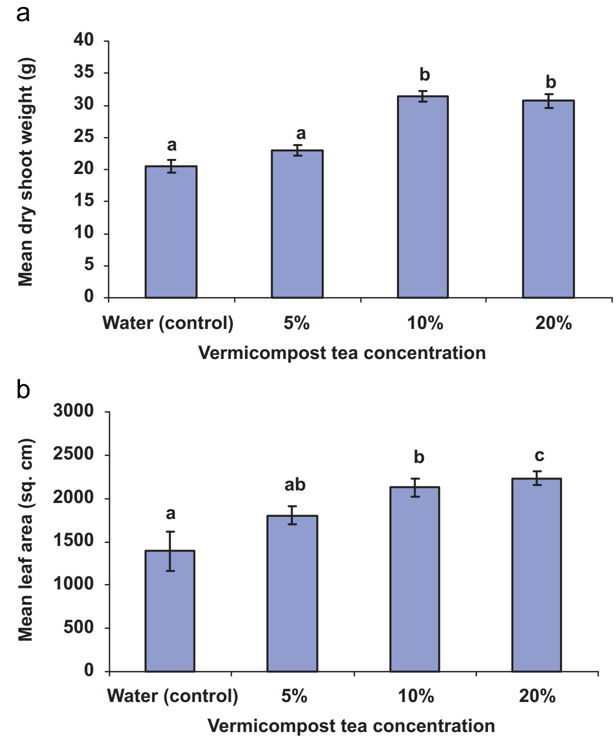

Fig. 3. (a) Effects of tobacco hornworm (*Manduca sexta*) on mean dry shoot weight on tomato plants treated with a range of concentrations of food waste vermicompost aqueous extracts ('teas') (Means ± S.E.). (b) Effects of tobacco hornworm (*Manduca sexta*) on tomato leaf areas on plants treated with a range of concentrations of food waste vermicompost aqueous extracts ('teas') (Means ± S.E.). Plants grown in MM360 with all needed nutrients supplied. Means followed by the same letters are not significantly different for each treatment concentration ($P \leq 0.05$).

In terms of damage ratings for tobacco hornworm caterpillars on a scale of 0 (none) to 5 (total damage), there were significant differences between time (F=61.55, df=6, 24, $P \leq 0.0001$) and also between time and aqueous extract concentrations (F=3.48, df=18, 24, $P \leq 0.0025$). The damage reached a rating of 4.0, 3.5, 2.0, and 1.0 on plants treated with deionized water (control), 5%, 10% and 20% vermicompost extract applications, respectively, after 14 days (Figs. 2a, b).

After 14 days, dry shoot weights of tomatoes attacked by tobacco hornworms were greater for plants receiving 10% or 20% vermicompost aqueous extracts than for those receiving the deionized water (control) applications but not for those receiving the 5% extract applications (Fig. 3a). For the same time period, the responses in terms of tomato leaf areas were similar for all of the vermicompost aqueous extract applications with only the 10% and 20% extract drenches resulting in increased leaf areas (Fig. 3b). Any increase in dry shoot weight or leaf area could not have been due to any fertilizer effects of the soil vermicompost extract drenches, since the experimental plants were receiving all the nutrients they needed from application of Peter's Nutrient Solution.

Cucumber beetles

In the experiments investigating cucumber beetle attack on cucumbers, there were significant differences over time (F=50.17, df=7, 196, $P \leq 0.0001$) and also between time and vermicompost aqueous extract concentrations (F=2.07, df=21, 196, $P \leq 0.0054$). After 14 days, beetle establishment decreased from 8.0 per cage

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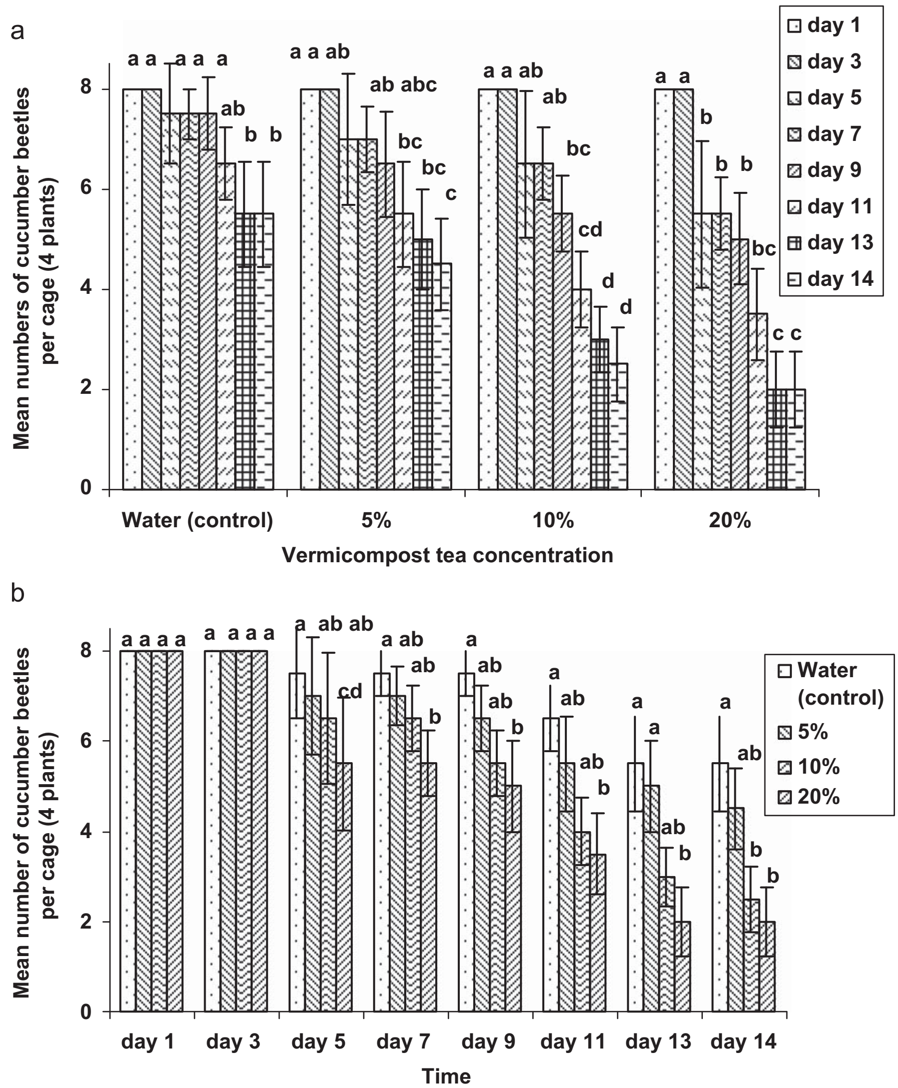

Fig. 4. (a) Changes in numbers of cucumber beetles (*Acalymma vittatum*) between treatments on cucumbers treated with a range of concentrations of food waste vermicompost aqueous extracts ('teas') (Means ± S.E.). (b) Changes in time in numbers of cucumber beetles (*Acalymma vittatum*) on cucumbers treated with a range of concentrations of food waste vermicompost aqueous extracts ('teas') (Means ± S.E.). Plants grown in MM360 with all needed nutrients supplied. Means followed by the same letters are not significantly different for each treatment concentration ($P \leq 0.05$). LSD was calculated between days of sampling within each treatment.

on plants receiving deionized water (control) to 4.6 per plant on plants treated with 5% vermicompost aqueous extract, and further to 2.6 and 2.0 per plant on plants treated with 10% and 20% vermicompost aqueous extract, respectively (Figs. 4a, b). These decreases in numbers were due to pest mortality.

In the cucumber beetle damage ratings, there were significant differences over time (F=179.63, df=7, 196, $P \leq 0.0001$) and also between time and aqueous concentrations (F=20.02, df=21, 196, $P \leq 0.0001$). Records of cucumber plant damage caused by the beetles present a more realistic picture of the suppression of cucumber beetle attacks by the vermicompost extract applications. In cages receiving only deionized water (controls), damage ratings, on a scale of 0 (no damage) to 5 (total damage), increased from less than 1.0 on the first day to 5.0, after 14 days (Figs. 5a, b). These damage ratings were higher compared to plants receiving the 5%, 10% and 20% vermicompost extract applications with ratings of 2.2, 1.8 and 1.35, respectively.

After 14 days, all concentrations of vermicompost aqueous extracts (5%, 10%, and 20%) resulted in increased foliage dry weight (Fig. 6a) and leaf area (Fig. 6b), compared with those of the controls, which received only drenches of deionized water. Any increases in dry shoot weight or leaf area could not have been due to any fertilizer effects of the soil vermicompost aqueous extract drenches, since the experimental plants were receiving sufficient nutrients from the application of Peter's Nutrient Solution.

Discussion

Tobacco hornworms and cucumber beetles cause very serious damage to tomato and cucumber plants, respectively, and economic losses to the pests on these valuable crops can be very high in the US. Horticultural growers of these crops have to resort to extensive use of inorganic pesticides. However, this option is not open to organic growers and gardeners, hence methods of suppression of the pests and damage, of the orders of magnitude reported in these experiments are welcomed who are prohibited from using inorganic pesticides. Vermicomposting is a process that can be carried out at a range of scales and complexities (Edwards and Arancon 2004). Moreover, a range of relatively inexpensive equipment for brewing aqueous vermicompost extracts at different scales is available. This is the first report of

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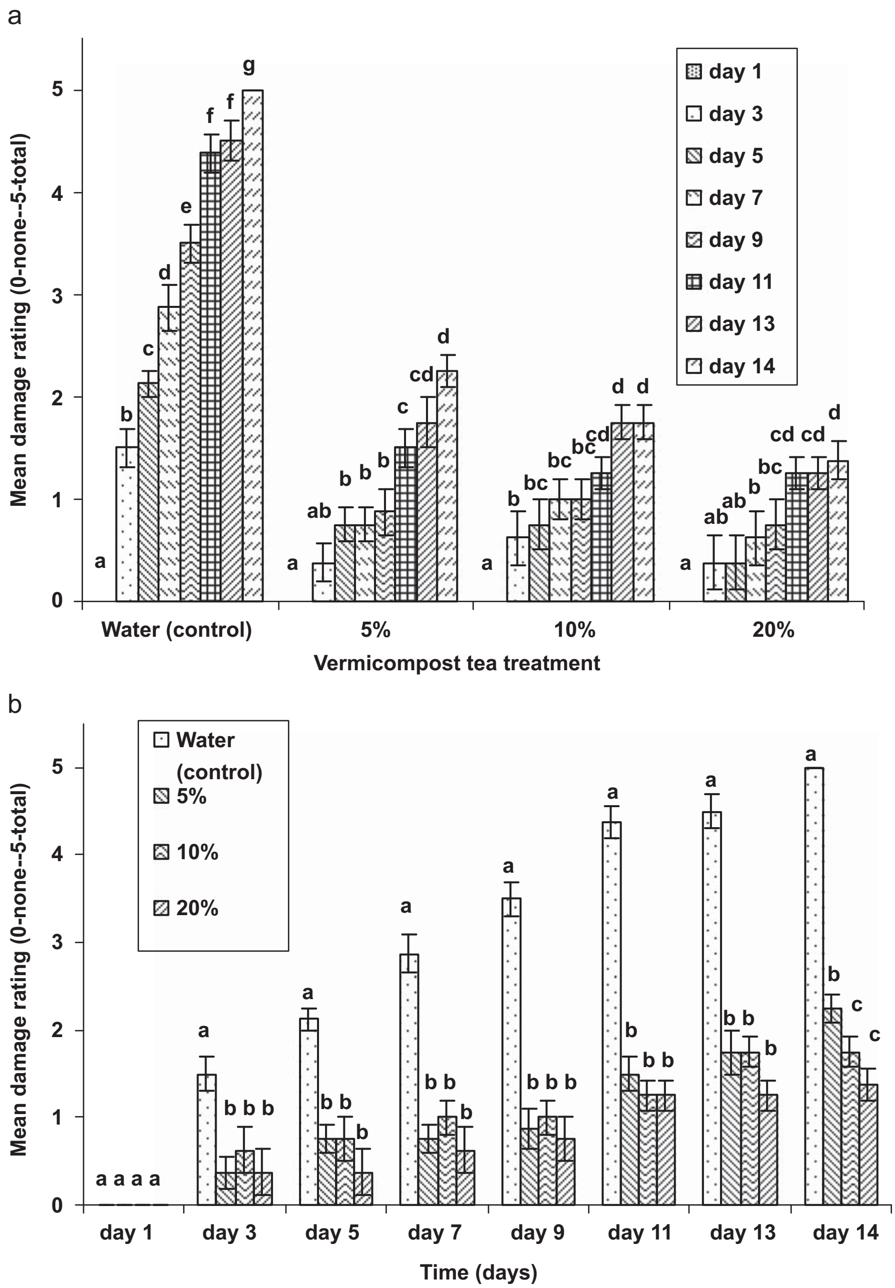

Fig. 5. (a) Changes in cucumber beetle (*Acalymma vittatum*) damage ratings between treatments on cucumbers treated with a range of concentrations of food waste vermicompost aqueous extracts ('teas') (Means ± S.E.). (b) Changes in time in cucumber beetle (*Acalymma vittatum*) damage ratings on cucumbers treated with a range of concentrations of food waste vermicompost aqueous extracts ('teas') (Means ± S.E.). Plants grown in MM360 with all needed nutrients supplied. Means followed by the same letters are not significantly different for each treatment concentration ($P \leq 0.05$). LSD was calculated between days of sampling within each treatment.

these aqueous vermicompost extracts having major effects on such serious crop pests, although Yardim et al. (2006) reported that solid food waste vermicomposts could suppress both tobacco hornworm attacks on tomatoes and cucumber beetle attacks on cucumbers, in laboratory and field experiments. Other records of solid vermicomposts suppressing chewing insect pests include suppression of *Spodoptera litura* and *Helicoverpa armigera* on ground nuts (Rao et al. 2001; Rao 2002).

Solid vermicomposts are known to provide a slow, balanced nutritional release pattern to plants, particularly in terms of release of plant available N, soluble K, exchangeable Ca, Mg and P (Edwards and Fletcher 1988; Edwards 2004b). Vermicomposts also have much greater microbial diversity and activity than conventional thermophilic composts, because organic wastes fragmented by earthworms have a much greater surface area and can support greater microbial activity. Moreover, microbial activity tends to be suppressed by the high temperatures reached during thermophilic composting. Pest suppression might be due to soluble compounds passing from the solid vermicompost into the vermicompost aqueous extract, which are, in turn, taken up by plants from soil drenches with vermicompost aqueous extracts. The compounds that could pass into plant tissues in this manner include soluble nutrients, free enzymes, soluble phenolic compounds, and a wide range of microorganisms.

In an attempt to identify the pest suppression mechanism further, it is necessary to determine which of these materials could be responsible for the pest suppression via plant uptake from soil drenches. Soluble nutrients can be ruled out since all of

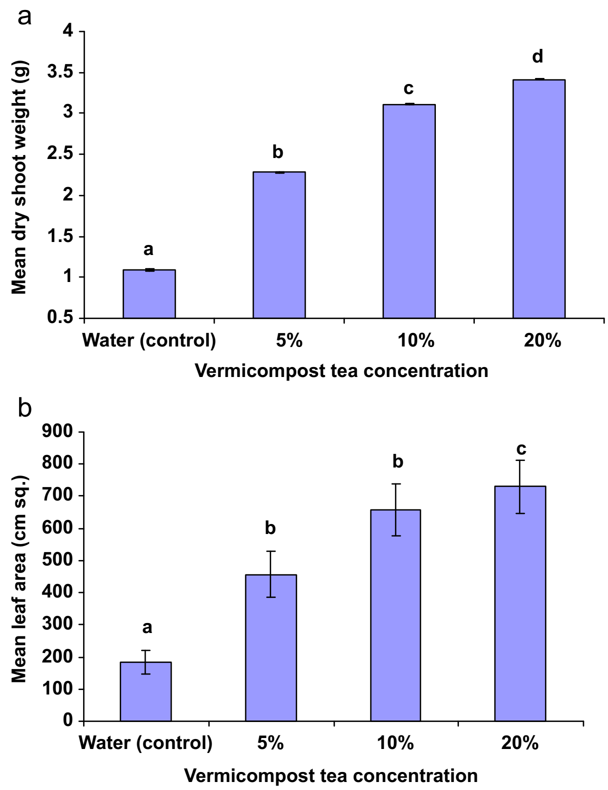

Fig. 6. (a) Mean dry weights of cucumbers in response to attacks by cucumber beetle (*Acalymma vittatum*) on cucumbers treated with a range of concentrations of food waste vermicompost aqueous extracts ('teas') (Means ± S.E.). (b) Cucumber leaf areas in response to attacks by cucumber beetle (*Acalymma vittatum*) on cucumbers treated with a range of concentrations of food waste vermicompost aqueous extracts ('teas') (Means ± S.E.). Plants grown in MM360 with all needed nutrients supplied. Means followed by the same letters are not significantly different for each treatment concentration ($P \leq 0.05$).

the experimental treatments were supplied with all required nutrients from Peter's Nutrient Solution, applied to the plants three times a week. It is conceivable that some free enzymes could influence pest suppression, but not on the scale and consistency demonstrated in these experiments. The enzyme chitinase has been reported to be present in vermicomposts (Hahn 2001) and it is feasible that this could affect arthropod pest molting. However, this enzyme has never been reported in the literature to have any effects on pests, although it does appear to have some influence on plant pathogens. There is unlikely to be a mechanism by which microorganisms might be taken up into the tissues of plants and could thereby influence arthropod pest feeding.

Hence, the most likely way in which vermicomposts and similar organic materials may inhibit attack by arthropod pests on the foliage and fruits of crop plants, is to change the arthropods' feeding responses, due to soluble phenolic substances taken up into plants from vermicomposts. It is well known that phenolic substances are distasteful to secondary invertebrate decomposers in soil systems and inhibit the breakdown of dead and decaying plant materials (Edwards and Heath 1963; Heath and Edwards 1964). Asami et al. (2003) reported that the total phenolic contents were significantly higher in marionberries, strawberries, and corn plants grown organically than in those grown with inorganic fertilizers.

An endogenous phenoloxidase has been obtained from an earthworm, *Lumbricus rubellus*, that is sometimes used to produce vermicomposts. This phenoloxidase can bioactivate compounds to form toxic phenols, such as p-nitrophenol (Park et al. 1996). Polychlorinated phenols and their metabolites have been reported from a range of soils containing earthworms (Knuutinen et al. 1990). Vinken et al. (2005) found that monameric phenols could be absorbed by humic acids in the gut of earthworms. In another study, Koul (2008) identified phenolics as insect anti-feedants in a review on these and other chemicals.

It has also been shown that sprays of phenols and phenolic acids extracted from gingko plants were as effective in controlling attacks by caterpillars, as the use of several pesticides approved for use against these pests (QiTian 2004). Stevenson et al. (1993) reported inhibition of the development of *Spodoptera litura* caterpillars by a phenolic compound in wild ground nuts. Summers and Felton (1994) proposed that lepidoptera larval feeding was decreased by oxidative stress caused by phenolic compounds. Haukioja et al. (2002) reported that phenolics in plant tissues decreased the rates of consumption of tissues by a geometrid caterpillar *Epirrita autumnata*.

Elsewhere, phenols have been reported to deter feeding by southern armyworms, *Spodoptera eridania* (Lindroth and Peterson 1988). Kurowska et al (1990) reviewed the effects of 46 phenols as insect repellents and feeding deterrents and concluded that many can have significant effects on pest attacks. Bhonwong et al. (2009) reported that polyphenol oxidases in tomatoes could produce resistance to cotton bollworm (*Helicoverpa armigera*) and beet armyworm (*Spodoptera exigua*). They demonstrated a clear feeding deterrent effect of these chemicals to the pests. Chrzanowski (2008) reported that phenolic acids in blackcurrant and sour cherry leaves slowed the reproduction and general fecundity of grain aphids. Tomato phenol oxidase in tomato plants decreased feeding and rates of growth of the common cutworm, *Spodoptera litura* F. (Mahanil et al. 2008). Hawida et al (2007) reported that plant phenolics affected the rates of development and survival of the autumn moth, *Epirrita autumnata*. Hoellrigl-Rosta et al. (2003) reported that phenolic xenobiotics could be bound reversibly to dissolved organic matter in soil.

These results all point to the probability that water-soluble phenols, extracted from the vermicompost during the brewing process and taken up by plants from soil receiving drenches of vermicompost aqueous extracts, is the most likely mechanisms by which vermicompost aqueous extracts can suppress pest attacks. A similar mechanism would account for the suppression of arthropod pests by solid vermicomposts (Arancon and Edwards 2004; Arancon et al. 2005).

This conclusion is based on circumstantial evidence but the breadth and weight of the evidence makes it extremely likely that water-soluble phenols, passing from vermicomposts into aqueous extracts and thence into plants, may be the main way in which vermicompost aqueous extracts influence the suppressions of pest feeding, reproduction and mortality reported in this paper.

It is hypothesized that the decrease in insect pest numbers and damage to plants grown with vermicompost extracts in our greenhouse experiments, could be attributed to the presence of water-soluble phenolic compounds in plants grown with vermicomposts and vermicompost aqueous extracts, which make the plants less attractive to pests and interfere with their reproduction.

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