INTRODUCTION

Plant extracts have drawn attention in recent years as potential control agents for a number of plant pests, and hold promise to replace environmentally damaging synthetic agricultural chemicals. For instance, root exudates of the neem tree (Azadirachta indica A. Juss., also known as the neem tree) inhibit hatching and are lethal to second stage juveniles (J2s) of Meloidogyne incognita (Kofoid and White) Chitwood in vitro (Adegbite and Adesiyan, 2005). Moreover, it is clear that many plant extracts also have significant nematicidal activity (Adegbite and Adesiyan, 2005; Martin and Magunacelaya, 2005; Qamar et al., 2005; Usman and Siddiqui, 2011). Okinawa has a subtropical climate that supports a large number of indigenous plants, including more than 1,000 species of medicinal herbs that have traditionally been used to treat human diseases or to maintain and improve health (Yoshikawa, 2002). Tabo et al. (2008a) examined plant extracts from approximately 30 Okinawan wild plant species for nematicidal action on the J2s of M. incognita. From these studies, an aqueous extract from Bidens pilosa L. var. radiata Scherff. was identified as having high nematicidal activity, including immobilization, hatching inhibition, repellence and lethality. Moreover, the plant extracts had high immobilization activities against several plant parasitic nematodes, such as M. arenaria (Neal) Chitwood, M. javanica (Treub) Chitwood, Pratylenchus coffeae (Zimmermann) Filipjev and Schuurmans Stekhoven and Heterodera glycines Ichinohe (Taba et al., 2012). Taba et al. (2008b, 2010) also reported that drenching different types of soils with an aqueous extract of the plant controlled the nematode, whereas the effect of the extract on host plants and other soil microorganisms is negligible. In addition, high immobilization activity was observed in the plants collected at different seasons, locations, growth stage and growing on several soil types (Taba et al., 2012). In this research, several treatment methods were evaluated for control of M. incognita using extracts and tissues of B. pilosa var. radiata.

MATERIALS AND METHODS

Nematodes:

The single egg sac of M. incognita was isolated from eggplant plants (Solanum melongena L., cv. Chojya)
grown in Nishihara, Uruma, Okinawa, Japan, and
cultured on tomato plants (Lycopersicon esculentum L.,
cv. Chibikko) (Marutane Co., Ltd.) in a greenhouse
without temperature control. J2s hatched from egg sacs
in a Petri dish were used in the experiments.

Bidens pilosa L. var. radiata aqueous extracts:

Above-ground tissues from B. pilosa var. radiata
collected from subtropical field science center, University
of the Ryukyus were dried by heat treatment (100°C, 24 hrs)
and, finely chopped. They were then extracted with
50 ml of boiling sterile distilled water containing 10 g
dried plant tissue for 30 minutes. Extracts were filtered
through filter paper (No. 2, Advantec) and used as a stock
solution, with sterile distilled water as diluent to make a
0.1 × diluted solution.

Selection of carriers suitable for B. pilosa var. radiata
aqueous extracts:

The four substrates to be tested were commercially
available perlite (Togawa-Heiwa Plantation Co., Ltd.),
vermiculite (Santeck Co., Ltd.), diatomaceous earth
(Souken Green Co., Ltd.), and hydro ball (ball of clay,
about 5 mm in diameter, Toshi-Engei Research Institute).
Each substrate was sterilized by dry heat (150°C, 60 min),
put into a 50 ml graduated cylinder to 15 ml, and
immersed in either 30 ml of the extract stock solution or a
0.1 × dilution for one day at 25°C. As a control, each
substrate was immersed in sterile distilled water. These
substrates were dried by the clean bench. About fifty
M. incognita J2s in a 0.2 ml suspension were added to a
small test tube (100 × 15 mm in diameter) containing an
approximately 5 mm diameter particle of substrate
loaded with the extract and then incubated without
shaking at 25°C. Immobile nematodes were counted 1, 4
and 7 days after inoculation. Each experiment was
repeated 5 times.

Control efficacy of the carrier containing B. pilosa var.
radiata aqueous extracts (Pot test 1):

Sixty grams (3.0 tons/10 a) of manure (‘Minori’,
Kitanaka yuki SA, containing cattle dung as a major
ingredient) as a base fertilizer was mixed into sterile
Ryukyu limestone soil ‘Shimajiri mahji’ (pH 7.4) mixed
initially with vermiculite at a volume ratio of 4:1, and
approximately 2.5 kg of this prepared soil was put into
1/5,000 Wagner pots (190 × 159 mm in diameter). A 10
ml suspension containing about 1,000 M. incognita J2s
was added to each pot and mixed in well. In this study, 1,
3, or 5 g of perlite (approximately 5 mm in diameter)
containing a stock solution or a 0.1 × dilution was used.
For the total soil mixing treatment, the perlite was
premixed with the infested soil, and a 3-week-old tomato
seedling (cv. Chibikko; height, 5 cm) was then planted.
On the other hand, in the planting–hole treatment, the
4-week-old tomato seedling was planted with the perlite
in the hole (ca. 5 cm diameter × 5 cm depth). Granular
nematicide (0.3 g; Nematorin-Ace® Ishihara Industries,
Ltd.; 1.5% fosthiazate as active ingredient) (15 kg/10 a)
and untreated samples were used as controls. The
nematicide was mixed with the soil before the seedling
was planted. For the planting–hole treatment, the controls
were not used. There were five replicates per treatment,
and plants were cultivated in a greenhouse without
temperature control. Total soil treatment was from
October 27, 2006 to December 19, 2006, and planting-
hole treatment was from November 4, 2006 to December
formation, and M. incognita population density were
measured at the end of the cultivation period. Root-knot
formation in the tomato plants was rated according to the
following scale: 0: no root-knots, 1: a few (1-2) root-knots,
2: a moderate number (3-10 root-knots) of separated root-
knots, 3: 11-30 root-knots, with many continuous root-
knots, and 4: 31 or more root-knots, mostly root-knots
continuous without fine roots. J2s of M. incognita were
counted in extracts from 20 g of soil collected from each
pot for 48 h by means of the Baermann funnel method
(Sano, 2004) with three replicates per soil.

Control efficacy of Bidens pilosa var. radiata dry chip on
Meloidogyne incognita (Pot test 2):

Sterilized soil in a Wagner pot (1/5,000), prepared as
in pot test 1, was left undisturbed for 7 days. Dry chips
(approximately 2 mm) from above-ground parts of B.
pilosa var. radiata plants were prepared using a hot air
sterilizer (100°C, 120 min), and 2-60 g of dried chips were
mixed with soil. A 10 ml suspension containing about
1,000 M. incognita J2s was inoculated on the same day,
and then the soil was liberally watered. A 4 weeks old
tomato seedlings were planted ten days after nematode
inoculation. Three controls were used: granular
nematicide (same as in Pot test 1), control A (without the
plant chips or M. incognita) and control B (without the
plant chips but with M. incognita inoculation). There
were five replicates per treatment. Plants were cultivated
in a greenhouse without temperature control from
October 20, 2008 to December 4, 2008.

Control effect of the leaf application of Bidens pilosa var.
radiata aqueous extracts on Meloidogyne incognita (Pot
Wagner pots were prepared as above, but leaves of a 4 weeks old tomato seedlings were sprayed five times every other day starting 7 days after planting with 10 ml of the stock solution, the 0.1 × dilution or sterile distilled water as a control. The plant extract was kept from contacting soil by covering the pot upper part with plastic vinyl. A 10 ml suspension containing about 1,000 M. incognita J2s was inoculated on the day following the last spray treatment. The nematode was inoculated three holes in soil surrounded the seedling. Granular nematicide (same as in Pot test 1) and a water treatment were used as controls. There were five replicates per treatment. Plants were cultivated in a greenhouse without temperature control from November 2, 2006 to December 19, 2006.

Statistical analysis:

All experimental data were subjected to an analysis of variance (ANOVA) using Statistica Pro (StatSoft Japan). Treatment means were tested with Tukey’s HSD multiple comparison test at a 5% level of probability.

RESULTS

Selection of carriers:

In the substrate comparison test, perlite gave the highest immobilization of J2s in stock solution for up to 7 days after nematode inoculation in the substrates tested, and with 0.1 × dilution, immobilization was higher than with the other substrates (Table 1).

Control efficacy of the carrier containing B. pilosa var. radiata aqueous extracts (Pot test 1):

No significant increase was observed in plant growth in both total soil mixing and plant–hole treated soil (Table 2). In the total soil mixing treatment, the not-treated soil (control) showed the highest root-knot formation index i.e. 3.8. Other treatments decreased root-knot formation while the lowest formation (0.5) was seen in soil treated with fosthiazate. Perlite (5 g) loaded with a stock solution significantly reduced root-knot nematodes (2.3). Moreover, all treatments significantly reduced the J2 population density in the soil (Table 2). In planting–hole treatments, the root-knot index was reduced to 1.8 in soils treated with 3 and 5 g of perlite loaded with a stock solution. However, no significant decrease was observed in the index of 3 and 5 g of perlite with stock solution and other soil treatments, except soil with 1 g of perlite containing a 0.1 × dilution (3.5) (Table 2). The population density of J2 was not reduced significantly by any treatment (Table 2).

Control efficacy of Bidens pilosa var. radiata dry chip on Meloidogyne incognita (Pot test 2):

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### Table 1. Effect of substrates containing Bidens pilosa var. radiata aqueous extracts on the mobility of second stage juveniles of Meloidogyne incognita

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Substrate</th>
<th>Days after treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stock solution</td>
<td>Diatomaceous earth</td>
<td>82.9 ± 5.8 b²</td>
</tr>
<tr>
<td></td>
<td>Hydro ball⁴</td>
<td>90.4 ± 2.0 ab</td>
</tr>
<tr>
<td></td>
<td>Perlite</td>
<td>96.6 ± 4.6 a</td>
</tr>
<tr>
<td></td>
<td>Vermiculite</td>
<td>89.0 ± 6.0 b</td>
</tr>
<tr>
<td>0.1 × dilution</td>
<td>Diatomaceous earth</td>
<td>0.7 ± 1.5 d</td>
</tr>
<tr>
<td></td>
<td>Hydro ball</td>
<td>2.4 ± 2.4 d</td>
</tr>
<tr>
<td></td>
<td>Perlite</td>
<td>73.2 ± 6.0 c</td>
</tr>
<tr>
<td></td>
<td>Vermiculite</td>
<td>5.1 ± 3.0 d</td>
</tr>
<tr>
<td>Control (Sterile distilled water)</td>
<td>Diatomaceous earth</td>
<td>0.0 ± 0.0 d</td>
</tr>
<tr>
<td></td>
<td>Hydro ball</td>
<td>0.0 ± 0.0 d</td>
</tr>
<tr>
<td></td>
<td>Perlite</td>
<td>0.0 ± 0.0 d</td>
</tr>
<tr>
<td></td>
<td>Vermiculite</td>
<td>0.0 ± 0.0 d</td>
</tr>
</tbody>
</table>

¹ Percent immobilization of 50 M. incognita J2s.
² Mean ± SD. Different letters in the same vertical column indicate significant difference (Tukey’s HSD multiple comparison test, P < 0.05).
³ Ball of clay.
Table 2. Effects of the perlite containing Bidens pilosa var. radiata aqueous extracts on the growth of tomato and the root-knot formation caused by Meloidogyne incognita

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Plant height (cm)</th>
<th>Shoot weight (g)</th>
<th>Root weight (g)</th>
<th>Root-knot index</th>
<th>Number of M. incognita /20 g soil</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total soil mixing</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stock solution (1 g)</td>
<td>11.3 ± 7.5 a2</td>
<td>0.9 ± 1.0 a</td>
<td>0.6 ± 0.5 b</td>
<td>3.5 ± 0.5 ab</td>
<td>9.3 ± 0.6 b</td>
</tr>
<tr>
<td>Stock solution (3 g)</td>
<td>15.1 ± 4.3 a</td>
<td>1.8 ± 1.0 a</td>
<td>1.2 ± 0.6 ab</td>
<td>3.2 ± 0.8 ab</td>
<td>1.7 ± 2.1 b</td>
</tr>
<tr>
<td>Stock solution (5 g)</td>
<td>17.4 ± 7.3 a</td>
<td>1.9 ± 1.8 a</td>
<td>0.7 ± 0.6 ab</td>
<td>2.3 ± 0.5 b</td>
<td>17.3 ± 8.1 b</td>
</tr>
<tr>
<td>0.1× dilution (1 g)</td>
<td>15.8 ± 10.5 a</td>
<td>2.6 ± 3.8 a</td>
<td>1.0 ± 0.7 ab</td>
<td>3.5 ± 1.0 ab</td>
<td>6.3 ± 4.2 b</td>
</tr>
<tr>
<td>0.1× dilution (3 g)</td>
<td>18.3 ± 7.3 a</td>
<td>2.2 ± 1.7 a</td>
<td>0.7 ± 0.3 ab</td>
<td>3.3 ± 0.5 ab</td>
<td>8.7 ± 7.5 b</td>
</tr>
<tr>
<td>0.1× dilution (5 g)</td>
<td>22.1 ± 12.1 a</td>
<td>3.6 ± 2.7 a</td>
<td>1.7 ± 1.0 a</td>
<td>3.2 ± 0.5 ab</td>
<td>4.7 ± 4.0 b</td>
</tr>
<tr>
<td>Granular nematicide (0.3 g)</td>
<td>20.0 ± 5.6 a</td>
<td>2.9 ± 1.5 a</td>
<td>1.0 ± 0.8 ab</td>
<td>0.5 ± 0.5 d</td>
<td>0.0 ± 0.0 b</td>
</tr>
<tr>
<td>Control (no treatment)</td>
<td>18.1 ± 4.8 a</td>
<td>2.7 ± 1.7 a</td>
<td>1.2 ± 0.2 ab</td>
<td>3.8 ± 0.4 a</td>
<td>36.7 ± 3.1 a</td>
</tr>
<tr>
<td><strong>Planting hole</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stock solution (1 g)</td>
<td>21.4 ± 7.0 a</td>
<td>4.1 ± 2.2 a</td>
<td>0.5 ± 0.5 b</td>
<td>2.7 ± 0.5 ab</td>
<td>1.0 ± 0.0 b</td>
</tr>
<tr>
<td>Stock solution (3 g)</td>
<td>19.3 ± 6.5 a</td>
<td>3.8 ± 2.1 a</td>
<td>0.8 ± 0.3 ab</td>
<td>1.8 ± 0.8 b</td>
<td>0.3 ± 0.6 b</td>
</tr>
<tr>
<td>Stock solution (5 g)</td>
<td>17.9 ± 6.4 a</td>
<td>3.6 ± 2.5 a</td>
<td>0.9 ± 0.4 ab</td>
<td>1.8 ± 0.9 b</td>
<td>0.0 ± 0.0 b</td>
</tr>
<tr>
<td>0.1× dilution (1 g)</td>
<td>20.0 ± 10.7 a</td>
<td>3.4 ± 3.0 a</td>
<td>0.8 ± 0.4 ab</td>
<td>3.5 ± 0.8 a</td>
<td>2.0 ± 2.6 b</td>
</tr>
<tr>
<td>0.1× dilution (3 g)</td>
<td>19.8 ± 8.8 a</td>
<td>3.2 ± 3.1 a</td>
<td>0.6 ± 0.3 ab</td>
<td>3.2 ± 0.8 ab</td>
<td>14.0 ± 6.1 b</td>
</tr>
<tr>
<td>0.1× dilution (5 g)</td>
<td>21.9 ± 9.2 a</td>
<td>3.6 ± 2.4 a</td>
<td>0.9 ± 0.1 ab</td>
<td>3.0 ± 0.8 ab</td>
<td>1.0 ± 1.0 b</td>
</tr>
</tbody>
</table>

1 Thousand of M. incognita J2s in 10 ml suspension was inoculated. The cultivation period was 33 days.
2 Concentration of extracts of the substrate. Parentheses indicate treatment quantity.
3 Mean ± SD. Different letters in the same vertical column indicate significant difference (Tukey’s HSD multiple comparison test, P < 0.05).
4 1.5% fosthiazate as active ingredient, equivalent to 15 kg/10 a.
5 Only nematode inoculation.

Table 3. Effect of dry plant chips of Bidens pilosa var. radiata on the growth of tomato and the root-knot formation caused by Meloidogyne incognita

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Plant height (cm)</th>
<th>Shoot weight (g)</th>
<th>Root weight (g)</th>
<th>Root-knot index</th>
<th>Number of M. incognita /20 g soil</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Dried</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 g</td>
<td>28.4 ± 10.2 ab2</td>
<td>8.7 ± 5.3 ab</td>
<td>1.7 ± 0.8 a</td>
<td>2.9 ± 0.4 a</td>
<td>6.3 ± 2.1 a</td>
</tr>
<tr>
<td>10 g</td>
<td>31.6 ± 1.1 ab</td>
<td>11.6 ± 1.5 ab</td>
<td>2.1 ± 0.3 a</td>
<td>0.7 ± 0.5 b</td>
<td>1.3 ± 0.6 b</td>
</tr>
<tr>
<td>20 g</td>
<td>27.8 ± 4.0 ab</td>
<td>10.0 ± 2.1 ab</td>
<td>1.6 ± 0.3 a</td>
<td>0.6 ± 0.0 b</td>
<td>0.0 ± 0.0 b</td>
</tr>
<tr>
<td>30 g</td>
<td>22.4 ± 7.5 b</td>
<td>7.3 ± 3.6 ab</td>
<td>1.4 ± 0.6 a</td>
<td>0.5 ± 0.5 b</td>
<td>0.7 ± 0.6 b</td>
</tr>
<tr>
<td>60 g</td>
<td>19.6 ± 5.1 b</td>
<td>4.9 ± 2.2 b</td>
<td>1.9 ± 0.8 a</td>
<td>0.4 ± 0.0 b</td>
<td>1.0 ± 1.0 b</td>
</tr>
<tr>
<td>Granular nematicide (0.3 g)</td>
<td>30.0 ± 5.3 ab</td>
<td>9.3 ± 3.8 ab</td>
<td>1.2 ± 0.5 a</td>
<td>0.0 ± 0.0 b</td>
<td>0.0 ± 0.0 b</td>
</tr>
<tr>
<td>Control A2</td>
<td>37.5 ± 5.6 a</td>
<td>13.6 ± 1.9 a</td>
<td>2.2 ± 0.4 a</td>
<td>0.0 ± 0.0 b</td>
<td>0.0 ± 0.0 b</td>
</tr>
<tr>
<td>Control B2</td>
<td>23.7 ± 10.8 ab</td>
<td>6.7 ± 5.7 ab</td>
<td>1.4 ± 1.2 a</td>
<td>4.0 ± 0.0 a</td>
<td>9.3 ± 1.5 a</td>
</tr>
</tbody>
</table>

1 Dry B. pilosa var. radiata chips were mixed into a 1/5,000 Wagner pot filled with soil and 1,000 M. incognita J2s in 10 ml were inoculated. Tomato seedlings were planted 10 days after nematode inoculation. The cultivation period was 45 days.
2 Mean ± SD. Different letters in the same vertical column indicate significant difference (Tukey’s HSD multiple comparison test, P < 0.05).
3 1.5% fosthiazate as active ingredient, equivalent to 15 kg/10 a.
4 Without B. pilosa dry chips or M. incognita.
5 Without B. pilosa dry chips but with M. incognita inoculation.
There was a significant difference in plant height between control A treatments and plants treated with *B. pilosa* dry chips at 30 and 60 g, as well as a significant decrease in shoot weight in the dry chip treatment with 60 g (Table 3). Dry chip treatment with 2 g gave the highest root-knot index (2.9), which was not significantly different from the no treatment control with nematode inoculation. Other dry chip treatments gave a high level of control of root-knot formation, 0.4–0.7, which was not significantly different from the index level of 0.0 with granular nematicide treatment. Nematode population density decreased significantly compared with no treatment (with nematode inoculation) in all treatments except the 2 g dry chip treatment (Table 3).

Control effect of the leaf application of *Bidens pilosa* var. *radiata* aqueous extracts on *Meloidogyne incognita* (Pot test 3):

There were significant increases in plant height, shoot and root weight with leaf spray treatments of the 0.1 × dilution extracts (Table 4). There was no significant difference in root-knot formation or the nematode population density in soil between treatments and no treatment, though root-knot formation decreased slightly in each treatment (Table 4).

**DISCUSSION**

In the result of selection carriers test, perlite gave the best performance for nematode immobilization (Table 1), perhaps because of its ability to absorb the extracts most and to release the extract at a reasonable concentration over time. The microbial density and storage period are important in the case of carrier included the natural enemy microorganisms (Nagayama, 2003), however as the carrier of plant extracts, adsorption and leaching behavior as well as storage period are especially considered to be important.

In the pot test, using perlite as the substrate demonstrated the effectiveness of treating the planting-hole with 3 and 5 g of the stock solution and of the total soil mixing treatment at 5 g stock solution (Table 2). Although direct comparison is not possible as a non-treated soil sample (control) was not used in both the experiments, we hypothesize that the planting-hole treatment may be more effective as treatment with 3 and 5 g significantly reduced root-knot formation (1.8) compared with treatment with 1 g containing a 0.1 × dilution where the root-knot index was almost similar to that of no treatment. With total soil mixing, the active ingredient is spread uniformly in the soil. However, the limited distribution of extract in the planting hole may be more effective for immobilizing or killing J2s because of a higher concentration in the rhizosphere. However, before a similar method can be adopted on an agricultural scale, it will be necessary to further concentrate the extract, or deliver it more precisely to be competitive with granular fosthiazate. Because substrate loading was determined solely by immersion time, it may be necessary to control the amount of compound actually loaded into the substrate more precisely to minimize batch-to-batch and particle-to-particle loading differences.

Ahmed et al. (1996) found that the root-knot index of eggplant caused by *M. javanica* decreased with a soil amendment of dried *Calotropis procera* (Aiton) W.T. Aiton, which coincidently increased plant growth. Khan et al. (1974) and Alam et al. (1978, 1979) reported that nematicidal activity toward plant parasitic nematodes could be attributed to substances such as ammonium, phenol and aldehyde, which are released from organic matter undergoing decomposition in the soil. In fact, it was cleared that several phenolic substances are contained in *B. pilosa* var. *radiata* (Deba et al., 2007; Kusano et al., 2003). However, it is necessary to examine since the other constituents may also be participating in the root-knot nematode control. When dry chips of *B. pilosa* var. *radiata* aqueous extracts to the leaf surface on the growth of tomato and the root-knot formation caused by *Meloidogyne incognita*.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Plant height (cm)</th>
<th>Shoot weight (g)</th>
<th>Root weight (g)</th>
<th>Root-knot index</th>
<th>Number of <em>M. incognita</em> /20 g soil</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stock solution</td>
<td>50.3 ± 2.4 a²</td>
<td>14.0 ± 1.3 b</td>
<td>5.0 ± 1.4 ab</td>
<td>2.5 ± 0.5 a</td>
<td>4.3 ± 0.6 a</td>
</tr>
<tr>
<td>0.1% dilution</td>
<td>57.2 ± 5.9 a</td>
<td>18.6 ± 1.5 a</td>
<td>6.5 ± 0.8 a</td>
<td>2.7 ± 0.5 a</td>
<td>28.7 ± 21.9 a</td>
</tr>
<tr>
<td>Granular nematicide (0.3 g)³</td>
<td>51.5 ± 14.6 ab</td>
<td>14.6 ± 1.7 b</td>
<td>4.2 ± 0.4 b</td>
<td>2.2 ± 0.4 a</td>
<td>2.0 ± 2.0 a</td>
</tr>
<tr>
<td>Control (sterile distilled water)</td>
<td>45.5 ± 3.5 b</td>
<td>13.4 ± 2.6 b</td>
<td>4.8 ± 0.8 b</td>
<td>3.0 ± 0.6 a</td>
<td>7.7 ± 2.5 a</td>
</tr>
</tbody>
</table>

¹ One thousand *M. incognita* J2s in 10 ml were inoculated on the day following the last spraying.
² Mean ± SD. Different letters in the same vertical column indicate significant difference (Tukey’s HSD multiple comparison test, *P* < 0.05).
³ 1.5% fosthiazate as active ingredient, equivalent to 15 kg/10 a.
**pilosa** var. *radiata* (pot test 2) were mixed in the soil, only the 2 g treatment showed no significant difference in root-knot formation or J2 population density (Table 3), possibly because there is not enough exudation from dry plant tissue chips. Moreover, premixing chips into moistened soil may be required to induce nematicidal activity. Incorporation of undegraded organic matter into soil can result in the exudation of phenolics, induction of nitrogen starvation, and an increase in fungal plant pathogens, all of which are detrimental to cultivated plants (Nishio, 2001). In the case of *B. pilosa* var. *radiata*, inhibition of tomato growth was observed in the 30 and 60 g (1.5, 3 tons/10 a respectively) treatment (Table 3), presumably due to the leaching a lot of phenols. Although the influence of nematode should also be considered, it is guessed that the influence is small because the root-knot indexes of 10-60 g treatments are low. Additional research will be required to determine the influence of undegraded organic matter on other crops. There was no obvious reduction of root-knot formation or J2 population density when *B. pilosa* var. *radiata* was composted and mixed with soil (unpublished data), suggesting that the nematicidal substances contained in the plant were not released, or were inactivated by composting.

Sitaramaiah and Pathak (1979) found that *M. javanica* populations and root-knot formation could be decreased by spraying catechol, cinnamic acid or salicylic acid on leaves of tomato seedlings. Fujimoto *et al.* (2009) confirmed that infecting of *M. incognita* to tomato root was inhibited by spraying methyl jasmonate. Although these substances are within the plant body and can play an important role in resistance to plant pathogens and plant parasitic nematodes (Giebel, 1974; Sitaramaiah and Pathak, 1979), it is thought that these substances acted as elicitors of phytoalexins and possibly other defense compounds. Taba *et al.* (2008b) reported that a seed-dip treatment of tomato using *B. pilosa* var. *radiata* aqueous extracts showed a high control effect, though the same result was not obtained in spraying to the leaf of this study. Moreover, Mateus *et al.* (2013) reported that the gall number decreased compared with control as a result of *B. pilosa* aqueous extracts applied to the tomato plant. These observations suggest that the extract can act indirectly as an elicitor, and that an examination of the individual constituents of the extract could provide a much better clue as to how these extracts work to reduce root-knot formation.

We evaluated the efficacy of several control methods on the J2 of *Meloidogyne incognita*. It was cleared that perlite loaded with extracts and the dried plant chip mixing treatment had inhibitory effects on root-knot formation and J2 viability. It will be necessary to evaluate control efficacy at the field level in the future.

**LITERATURE CITED**


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