Effect of botanical insecticides on survival and virulence of *Steinernema carpocapsae* (Weiser)

Achariya Nitjarunkul¹, Sirirut Mangtab¹ and Atirach Noosidum¹*

ABSTRACT

The survival rates of 100 IJs of *Steinernema carpocapsae* (Weiser) soaked in each recommended rate of five different botanical insecticides (neem 15,000 ppm, tobacco 16,000 ppm, tuba root 50,000 ppm, sweet flag 7,500 ppm and wood vinegar 5,000 ppm) for 24 and 96 hours were evaluated. The result showed that survival rates of *S. carpocapsae* in all botanical insecticides ranged from 98.5 to 99.9% at 24 hours and 94.5 to 99.6% at 96 hours after application. Results of tuba root and tobacco treatments did not show a significant difference in nematode survival rate with control (100%) at both 24 and 96 hours (p<0.05). In both periods all nematode survival rates were higher than 94.5%. In the second experiment, nematode virulence of *S. carpocapsae* to kill a larva of *Galleria mellonella* (L.) after soaking in botanical insecticides for 24 hours and 96 hours were evaluated by using a sand column bioassay. For 24 hours after soaking, 100% larval mortalities were observed in control and wood vinegar treatment and they also significantly differed from other treatments (70 to 90%) at 120 hours after application. For 96 hours after soaking, mortality rates of control and wood vinegar treatment were 60% and 50%, respectively while other botanical insecticides were only 12.5 to 37.5%. Larval mortality of a wood vinegar treatment (92.5%) did not differ from control (97.5%) at 120 hours after application while other treatments were observed only 47.5 to 62.5%.

Key words: botanical insecticide, *Steinernema carpocapsae*, entomopathogenic nematode

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INTRODUCTION

Entomopathogenic nematodes (EPNs) in the genera Steinernema or Heterorhabditis and their symbiotic bacteria (Xenorhabdus sp. or Photorhabdus sp., respectively) are obligate pathogens of soil born insects (Koppenhöfer et al., 2002; Grewal et al., 2005). The EPNs can infect and kill a wide range of insect pests developing or long-term survival in the soil such as grubs, cicadas and lepidopterous larvae (Grewal et al., 2005; Noosidum et al., 2010). The infective juveniles (IJs) of EPNs are the third juvenile stage that enter insect host through natural openings (i.e., mouth, anus, spiracle) and the cuticle (Koppenhöfer et al., 2002; Grewal et al., 2005). After infection, nematodes release their symbiotic bacteria into insect hemocoel and the bacteria kill insect host with their toxin productions in 48 hours (Kaya and Gaugler, 1993). Steinernema carpocapsae (Weiser) is a well-known nematode for controlling insect pests in around the world (Grewal et al., 2005), including Thailand (Noosidum et al., 2010). In addition, numerous of scientific research showed that most of a combination between entomopathogenic nematodes and other pesticides are synergistic effect, resulting in a better efficacy (Koppenhöfer et al., 2002; Alumai and Grewal, 2004; Koppenhöfer and Grewal, 2005).

Botanical insecticides can be used as a manner for a crop protection in a part of insect pest management (Dadang et al., 2009). Botanical insecticides affect to insect in various mechanisms such as contact poison, insect attractant and repellent or antifeedant (Isman, 2008). Several researches confirmed that most of botanical insecticides are environment-friendly because they have less toxicity to human and other non-target organisms (Isman, 2008; Dadang et al., 2009). In Thailand, botanical insecticides from several plants are being used for insect control such as Neem (Azadirachta indica A. Juss), tobacco (Nicotiana tabacum L.), tuba root (Derris elliptica Benth) and sweet flag (Acorus calamus L.). These extracts are also effective against insect pests (Areekul et al., 1987; Motely, 1994; Wongtong and Nawanich, 2001). Recently, wood vinegar is becoming an alternative product for pest control and belonging to botanical insecticide as well (Wititsiri, 2011). Thus, we hypothesized that S. carpocapsae might be applied together with the botanical insecticides which presented in this study as same as a successfully using of a combination of S. carpocapsae with various pesticides.

MATERIALS AND METHODS

Nematode and Insect culture

Adult females and males of greater wax moths, Galleria mellonella (L.) (Lepidoptera: Pyralidae) were put in a plastic box (17×13×7 cm) and fed them with 10% honey syrup. Eggs were laid on the paper in 3 days, cut and placed onto a new rearing box containing artificial diet (Noosidum et al., 2010). The larvae were maintained inside the rearing box and fresh diets were
added every three days. The fifth instar larvae were stored at 15°C to use for subsequent studies within 2 weeks.

*Steinernema carpocapsae* were received from Department of Agriculture, Ministry of Agriculture, Thailand. Nematodes were maintained in a laboratory by rearing with *G. mellonella* larvae (Kaya and Stock, 1997). A drop containing 500 IJs of *S. carpocapsae* was applied into 5 cm diameter Petri dish lined with Whatman® #1 filter paper. Then, six fifth instar larvae of *G. mellonella* were placed into a Petri dish. Three days later, the cadavers were transferred to a modified white trap (Lewis and Gaugler, 1994). The emerging IJs were collected in a flask and stored at 15°C for subsequent studies within 4 weeks.

**Botanical insecticide preparation**

Following the recommended rates (Hutapeat, 2007), commercial neem and wood vinegar were diluted with distilled water into 15,000 and 5,000 ppm. Grinded tuba root, tobacco and sweet flag were soaked with distilled water in the concentrations of 50,000, 16,000 and 7,500 ppm.

**Survival rates of *S. carpocapsae* combined with botanical insecticide**

The concentration of 100 IJs/2.5 ml of water was prepared in 5 cm diameter Petri dish and filled individually with 2.5 ml of each botanical insecticide and distilled water (control). The experiments were repeated 30 times and the dishes were kept at room temperature (25±2°C). Number of alive IJs was counted at 24 and 96 hours.

**The virulence of *S. carpocapsae* combined with botanical insecticide against *G. mellonella* larvae in sand column assay**

The concentration of 100 IJs/2.5 ml of water was prepared in 5 cm diameter Petri dish, filled individually with 2.5 ml of each botanical insecticide, and kept for 24 and 96 hours. A set of 230 g of sterile coarse sand (~0.5 mm diameter) and 10% moisture (w/w) was filled into a PVC column (4 cm diameter and 10 cm length). Two fifth instar larvae of *G. mellonella* in a cage (made with polyethylene net) were placed on a top of the column and covered with a 5 cm Petri dish. Turned up side the column, then 100 IJs/2 ml of each botanical insecticide was dropped into the column individually and covered with another Petri dish. Distillated water and pure botanical insecticides were used as controls. The experiments were repeated 30 times and the dishes were kept at room temperature (25±2°C). Numbers of dead insect were counted every 24 hours after application.

**Data analysis**

Numbers of alive IJs and dead insect were analyzed using One-Way Analysis of Variance and significant differences were grouped by Tukey’s test (Multiple Comparisons of Means: Tukey Contrasts). A significant difference between two groups of alive IJs from two periods was evaluated...
with Paired simple T-test method by using R program version 3.0.1. (R Development Core Team, 2013).

**RESULTS AND DISCUSSION**

**Survival rates of *S. carpocapsae* combined with botanical insecticide**

The survival rates of *S. carpocapsae* in all botanical insecticides were ranged from 98.5 to 99.9% for 24 hours and 94.5 to 99.6% for 96 hours after application. Treatments of tuba root and tobacco did not show a significant difference of nematode survival rate with control (100%) while nematode survival rates of neem, wood vinegar and sweet flag treatments were significant difference to control at both 24 and 96 hours (df=179, F=11.231, p<0.05 and df=179, F=25.373, p<0.05, respectively). Nematode survival rates of all botanical insecticides at 96 hours were decreasing when compared to 24 hours, especially neem, wood vinegar and sweet flag were significant decreasing from 24 hours (p<0.05). However, all nematode survival rates in both times were higher than 94.5% (Table 1). Nematode survivals in this study were similar to Alumai and Grewal (2004) who reported that a recommended rates of pesticides directly applied with *S. carpocapsae* had no effect on *S. carpocapsae* virulence (more than 96% survival rate) at room temperature for 3 hours. Moreover, another research confirmed that the relationships between nematodes and botanical insecticides or chemical insecticides at recommended doses also indicated the compatibility and allowed higher nematode survival at 72 hours after combination. The results showed that the survival rates of nematode after combining with 0.01% Confidor® (imidacloprid), 0.025% Actara® (thiomethoxan), 0.05% Agropest BT®, 0.05% Conserve® (spinosad) and 2.00% Neemglod® (neem) were 97.2%, 83.2%, 93.6%, 87.2% and 92.4%, respectively (Kulkarni et al., 2013).

**Table 1** The survival rates of *Steinernema carpocapsae* which were soaked in different botanical insecticides for 24 and 96 hours.

<table>
<thead>
<tr>
<th>treatments</th>
<th>Survival rate (Mean±SE)¹</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24 hours</td>
<td>96 hours</td>
<td></td>
</tr>
<tr>
<td>control</td>
<td>100.0±0.0⁴⁰ᵃᵃ</td>
<td>100.0±0.0⁴⁰ᵃᵃ</td>
<td></td>
</tr>
<tr>
<td>tuba root</td>
<td>99.9±0.1⁴⁰ᵃᵃ</td>
<td>99.6±0.1⁴⁰ᵃᵃ</td>
<td></td>
</tr>
<tr>
<td>tobacco</td>
<td>99.6±0.2⁴⁰ᵃᵃ</td>
<td>99.0±0.3⁴⁰ᵃᵃ</td>
<td></td>
</tr>
<tr>
<td>neem</td>
<td>99.2±0.2⁴⁰ᵇᵇ</td>
<td>96.4±0.4⁴⁰ᵇᵇ</td>
<td></td>
</tr>
<tr>
<td>wood vinegar</td>
<td>98.5±0.3⁴⁰ᶜᶜ</td>
<td>95.6±0.5⁴⁰ᶜᶜᵇᵇ</td>
<td></td>
</tr>
<tr>
<td>sweet flag</td>
<td>99.0±0.3⁴⁰ᶜᶜᵃᵃ</td>
<td>94.5±2.9⁴⁰ᶜᶜᵇᵇ</td>
<td></td>
</tr>
</tbody>
</table>

¹ Means±SE followed by the same letters were not significantly different as determined by Tukey’s test (a=0.05). Lowercase letters compared means in row, uppercase letters compared means in column.
The virulence of *S. carpocapsae* combined with botanical insecticide against *G. mellonella* larvae in sand column assay

The nematode virulence for *G. mellonella* larvae searching of *S. carpocapsae* soaked in all botanical insecticides for 24 hours clearly showed the difference with control from 72 hours after application (df=23, F=9.228, p<0.05). The highest larval mortality was observed in wood vinegar treatment (80%), following by tuba root and tobacco treatments (70%). At 120 hours after application, most mortality rates from botanical insecticides (100% larval mortality) were significant difference from the control and wood vinegar treatments (100% larval mortality) (df=23, F=7.885, p<0.05).

Nevertheless, other botanical insecticides still had higher larval mortalities, which ranged from 70 to 90% (Table 2). At 96 hours soaked, the differences of larval mortality between control and botanical insecticides were also found, except the wood vinegar treatment at 72 hours after application (df=23, F=4.132, p<0.05). Mortality rates of control and wood vinegar were 60.0% and 50.0%, respectively while other botanical insecticides were only 12.5 to 37.5%. Only the wood vinegar treatment did not differ from the control treatment at 120 hours after application and showed 92.50% and 97.5% larval mortality, respectively. Mortalities of other botanical insecticides were observed only 47.5 to 62.5% (Table 3). Overall, nematode virulence in all botanical insecticides mostly decreased in longer soaking period comparing between 24 hours and 96 hours, except wood vinegar (Figure 1). The mortality rates of *G. mellonella* larvae were not observed in all treatments which were applied with botanical insecticide alone.

The result from this study is similar to Kulkarni *et al.* (2013) who revealed that nematode in 0.01% Confidor®, 0.025% Actara®, 0.05% Agropest BT®, 0.05% Conserve® and 2.00% Neemglod® infected the *G. mellonella* larvae at 72 hours after application were up to 98.7%, 90.1%, 91.3%, 96.3% and 100%, respectively. In addition, *S. carpocapsae* combined with cypermethrin (0.025 L/ha), chlorpyrifos (0.300 L/ha) and lufenuron (0.150 L/ha) had high efficiency to *Spodoptera frugiperda* (Smith) for 82.0±3.7, 90.0±3.2% and 90.0±4.5%, respectively (Negrisoli *et al*., 2010). Furthermore, Fetoh *et al.* (2009) indicated that an interaction between *S. carpocapsae* with proclaim (0.125mg/L) in 24 hours exposed an additive effect and the mortality rates of forth-instar larvae of *Agrotis ipsilon* (Hufn.) was up to 100%. Whereas, nematode alone (50 IJs) and proclaim (0.125mg/L) killed *A. ipsilon* larvae were 85% and 89%, respectively. Nevertheless, this result also agreed with various researchers who reported that entomopathogenic nematode and pesticide have been compatible (Koppenhöfer *et al*., 2002; Koppenhöfer and Grewal, 2005).
Table 2 The virulence of *Steinernema carpocapsae* soaked in different botanical insecticides for 24 hours against *Galleria mellonella* larvae in sand column assay.

<table>
<thead>
<tr>
<th>treatments</th>
<th>24 hours</th>
<th>48 hours</th>
<th>72 hours</th>
<th>96 hours</th>
<th>120 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>10.0±0.0(^b)</td>
<td>57.5±10.3(^bc)</td>
<td>100.0±0.0(^a)</td>
<td>100.0±0.0(^a)</td>
<td>100.0±0.0(^a)</td>
</tr>
<tr>
<td>neem</td>
<td>0.0±0.0(^b)</td>
<td>35.0±6.5(^bc)</td>
<td>57.5±6.3(^bc)</td>
<td>60.0±8.2(^b)</td>
<td>90.0±4.1(^ba)</td>
</tr>
<tr>
<td>wood vinegar</td>
<td>0.0±0.0(^b)</td>
<td>67.5±10.3(^ac)</td>
<td>80.0±10.8(^bc)</td>
<td>92.5±2.5(^ab)</td>
<td>100.0±0.0(^a)</td>
</tr>
<tr>
<td>tuba root</td>
<td>0.0±0.0(^b)</td>
<td>70.0±9.1(^a)</td>
<td>70.0±9.1(^a)</td>
<td>72.5±7.5(^a)</td>
<td>75.0±6.5(^ba)</td>
</tr>
<tr>
<td>sweet flag</td>
<td>0.0±0.0(^b)</td>
<td>2.5±2.5(^c)</td>
<td>40.0±10.8(^cb)</td>
<td>70.0±12.3(^c)</td>
<td>82.5±8.5(^ba)</td>
</tr>
<tr>
<td>tobacco</td>
<td>0.0±0.0(^b)</td>
<td>55.0±6.5(^ab)</td>
<td>70.0±9.1(^a)</td>
<td>77.5±6.3(^b)</td>
<td>80.0±4.1(^ba)</td>
</tr>
</tbody>
</table>

1 Means±SE followed by the same letters were not significantly different as determined by Tukey's test (\(\alpha\)=0.05). Lowercase letters compared means in row, uppercase letters compared means in column.

Table 3 The virulence of *Steinernema carpocapsae* soaked in different botanical insecticides for 96 hours against *Galleria mellonella* larvae in sand column assay.

<table>
<thead>
<tr>
<th>treatments</th>
<th>24 hours</th>
<th>48 hours</th>
<th>72 hours</th>
<th>96 hours</th>
<th>120 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>5.0±2.9(^ac)</td>
<td>15.0±8.7(^ac)</td>
<td>60.0±10.8(^ab)</td>
<td>87.50±4.8(^a)</td>
<td>97.5±2.5(^a)</td>
</tr>
<tr>
<td>neem</td>
<td>0.0±0.0(^ac)</td>
<td>5.0±5.0(^ac)</td>
<td>37.5±8.5(^abc)</td>
<td>55.00±9.6(^ab)</td>
<td>62.5±11.1(^ba)</td>
</tr>
<tr>
<td>wood vinegar</td>
<td>0.0±0.0(^ad)</td>
<td>12.5±7.5(^ad)</td>
<td>50.0±7.1(^abc)</td>
<td>75.00±6.5(^ab)</td>
<td>92.5±4.8(^a)</td>
</tr>
<tr>
<td>tuba root</td>
<td>5.0±5.0(^ac)</td>
<td>10.0±10.0(^ac)</td>
<td>25.0±8.7(^bc)</td>
<td>47.50±11.8(^abc)</td>
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<tr>
<td>sweet flag</td>
<td>0.0±0.0(^ac)</td>
<td>20.0±7.1(^abc)</td>
<td>30.0±10.0(^abc)</td>
<td>45.00±11.9(^abc)</td>
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<td>12.5±4.8(^c)</td>
<td>32.50±4.8(^c)</td>
<td>47.5±7.5(^ba)</td>
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</table>

1 Means±SE followed by the same letters were not significantly different as determined by Tukey's test (\(\alpha\)=0.05). Lowercase letters compared means in row, uppercase letters compared means in column.

Figure 1 The efficiency of *Steinernema carpocapsae* soaked in different botanical insecticides against *Galleria mellonella* larvae in sand column assay at 120 hours after application.
CONCLUSION

The survival rates of *S. carpocapsae* combined with all botanical insecticides had higher survival rates in both 24 and 96 hours after combinations and their survival were over 95.61%. However, the nematodes in wood vinegar showed the efficiency against *G. mellonella* larvae in sand column assay by soaking for 24 hours and 96 hours, comparing to other formulations of 120 hours after experiments. Nematode virulence in botanical insecticides in this study mostly decreased in longer soaking period. Finally, the results from these experiments demonstrated that entomopathogenic nematodes and the above botanical insecticides can be used together and help in controlling agricultural pests.

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