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BIOACTIVITY OF SELECTED ECO-FRIENDLY PESTICIDES AGAINST *CYLAS FORMICARIUS* (COLEOPTERA: BRENTIDAE)

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ABSTRACT

Seven low risk pesticides including 1.2% azadirachtin (*Azadirachta indica*), extracts from *Morinda citrifolia*, petroleum oil 97%, *Beauveria bassiana* strain GHA, mixed essential oils (rosemary oil: 0.25%, peppermint oil: 0.25%, thyme oil: 0.25%, clove oil: 0.25% and other ingredients: 99.00%), spinosad and malathion, were evaluated against adults of the sweet-potato weevil, *Cylas formicarius* (Fabricius) (Coleoptera: Brentidae) to determine potential insecticidal, repellent and feeding deterrence effects. Among the pesticides tested, *A. indica* and spinosad showed high insecticidal, repellent and feeding deterrence activity against *C. formicarius*. Spinosad, *A. indica* and malathion showed significantly higher insecticidal activity against *C. formicarius*. Similarly, these pesticides showed high repellency activity against adults, particularly 3-4 h after the treatment. The lowest food consumption was observed with the *A. indica* (0.8 g/adult/192 h), and the highest (9.9 g/adult/192 h) was with the petroleum oil spray. The other tested pesticides showed comparable activities. The chemicals we tested—particularly neem and spinosad—are therefore promising candidates as ecofriendly chemicals that could potentially replace broad-spectrum synthetic neurotoxins for control of *C. formicarius*.

Key Words: *Azadirachta indica*, *Morinda citrifolia*, *Beauveria bassiana*, spinosad, essential oils, petroleum oil

RESUMEN

Se evaluaron siete plaguicidas de bajo riesgo incluyendo 1.2% de azadiractina (*Azadirachta indica*), extractos de *Morinda citrifolia*, 97% de aceite de petróleo, una cepa GHA de *Beauveria bassiana*, aceites esenciales mezclados (aceite de romero: 0.25%, el aceite de menta: 0.25%, aceite de tomillo: 0.25%, aceite de clavo: 0.25% y otros ingredientes: 99.00%), spinosad y malatión contra adultos del gorgojo del camote, *Cylas formicarius* (Fabricius) (Coleoptera: Brentidae) para determinar los efectos potenciales como insecticidas, repelentes y inhibidores de alimentación. Entre los pesticidas probados, *A. indica* y spinosad mostró una alta actividad de insecticida, repelente y disuador de alimentación contra *C. formicarius*. El spinosad, *A. indica* y malatión mostraron una actividad insecticida significativamente mayor contra *C. formicarius*. Del mismo modo, estos plaguicidas mostraron una alta actividad repelente contra los adultos, sobre todo después de 3-4 horas del tratamiento. Se observó el menor consumo de alimento con la *A. indica* (0.8 g/adulto/192 h) y la más alta (9.9 g/adulto/192 h) fue con la aspersión de aceite de petróleo. Los otros plaguicidas probados mostraron actividades comparables. Los químicos que probamos en particular neem y spinosad, son por lo tanto, candidatos prometedoros que no perjudican el medio ambiente y que potencialmente podrían reemplazar las neurotoxinas sintéticas de amplio espectro para el control de *C. formicarius*.

Palabras clave: *Cylas formicarius*, *Azadirachta indica*, *Morinda citrifolia*, *Beauveria bassiana*, spinosad, aceites esenciales, aceites de petróleo

The practice of using natural compounds (e.g., essential oils) as insect control agents is receiving increased consideration as an alternative to traditional insecticides, because they are practicable and non-toxic to humans and the environment (Lee et al. 2001; Ngamo et al. 2007a). Essential

oils may be more rapidly degraded in the environment than synthetic compounds, and some have increased specificity that favors beneficial insects (Müller & Buchbauer 2011). Plant-derived essential oils are typically steam distillates of fruit, bark, stems, or leaves, and provide a concentrated

source of secondary metabolites, predominantly volatile terpenoids (Koul et al. 2008). Also, these oils are known ingredients of plants, providing them with a characteristic and pleasant odor (Müller & Buchbauer 2011). The practical use of these oils is to mix grains or seeds with the oil or similar substances to provide physical contact of the oil with the insect cuticle, which then evokes behavioral responses (Ngamo et al. 2007b). Their potential for bioaccumulation are not significant and their persistence is exceptionally short (Ngamo et al. 2004). Consequently, essential oil products are good alternatives to industrial pesticides, which have adverse effects on consumers and on the environment because of their toxicity to non-target organisms and potential for bioaccumulation, and thus, when feasible, should be replaced with environmentally friendly pesticides (Müller & Buchbauer 2011).

The sweetpotato weevil, *Cylas formicarius* (Fabricius) (Coleoptera: Brentidae), is considered the single most destructive insect pest of sweetpotato, *Ipomoea batatas* (L.) Lam. (Convolvulaceae) (Chalfant et al. 1990). Weevil infestation ranges from 20 to 50% on many farms and can even reach 100%, depending on the season and variety (Sutherland 1986). The infestation of *C. formicarius* occurs more frequently during dry seasons (Reddy et al. 2012). Adult *C. formicarius* feed on the tender buds, leaves, vines and storage roots, while the larvae, the most destructive stage, feed and tunnel into the mature stems and storage roots (Chalfant et al. 1990). Injuries to the plant include small feeding and oviposition holes on the external and larval tunnels filled with frass in the tissues (Strong 1936). The injury leads to the thickening, drying and cracking of the stems and to secondary infection by microbes (Talekar 1983). Infestation of storage roots makes them unfit for human or animal consumption, even if only a small proportion of the flesh is damaged, as the damaged tissue produces terpenes that give the flesh an unpleasant odor and bitter taste (Loebenstein & Thottappilly 2009). Weevil damage continues to increase during storage (Chalfant et al. 1990).

A previous sampling study revealed that *C. formicarius* is the most destructive pest on sweetpotato in the Mariana Islands (Reddy et al. 2012). This pest was followed by the West Indian sweetpotato weevil, *Eusepes postfasciatus* (Fairmaire), and *Daealus tuberosus* Zimmerman (Curculionidae), which cause damage to sweetpotatoes in the Commonwealth of the Mariana Islands. Therefore, the management and eradication of these weevils are vital for successful production of sweetpotato in the Mariana Islands and also in other parts of the world. Control using insecticides in the field has been shown to reduce *C. formicarius* numbers (Talekar 1983). However, the insecticidal applications are toxic and particularly undesirable on tuber crops. Chalfant et

al. (1990) detailed a range of hormones and insect growth regulators, which have been shown to have varying effects on *C. formicarius*; however, further research is needed in this area. Cultural controls, such as the use of *C. formicarius* resistant cultivars of sweetpotato, non-infested planting material and crop rotation, along with various management regimes, have also been shown to reduce pest numbers (Jansson et al. 1987). Mass-trapping using pheromones has been shown to reduce populations of *C. formicarius* males in several countries (Yasuda 1995), but this has not always resulted in significant reductions in infestation or increases in yield (Braun & Van De Fliert 1999). Recently, an efficient pheromone-based trapping technique has been developed for *C. formicarius*, but it is yet to be commercially available to the growers (Reddy et al. 2012). The derivative of neem (Aza-Direct®) and the insecticide spinosad (Conserve SC®) appear to be promising control agents for various insects pests (Cisneros et al. 2002; Reddy & Bamba 2011). Therefore, research was initiated to find appropriate ecologically friendly pesticides to control *C. formicarius*.

We explored the application of various plant-derived compounds and low-risk insecticides for the purpose of preventing sweetpotato damage caused by *C. formicarius*. Low risk pesticides including azadirachtin, extracts from *M. citrifolia*, petroleum oil, *B. bassiana* strain GHA, mixed essential oils, spinosad and malathion, were evaluated against adults of *C. formicarius*. Their biological activities against adult *C. formicarius*, such as contact toxicity, repellent activity and feeding deterrence, were determined.

MATERIALS AND METHODS

Rearing of Insects

Pheromone lures consisting of rubber septa loaded with *Z3*-Dodecenyl-*E2*-butenoate, sealed in an impermeable bag for shipping and storage, were obtained from Chem Tica Internacional S.A. (San José, Costa Rica). Pherocon unitraps (Trécé Incorporated, Adair, Oklahoma, USA) baited with lures were used to trap adult *C. formicarius* in sweetpotato fields in Latte Heights (Guam, USA). The trapped adults were taken to the laboratory, placed in batches in collapsible cages (12 × 10 × 10 cm), fed leaves and pieces of the sweetpotato, and maintained at 22 ± 2 °C, 70-80% relative humidity and 16:8 h L:D.

Pesticides

Six of the chemicals used in the present study—neem (Aza-Direct), Volck oil spray, BotaniGard, EcoSmart spray, spinosad, and malathion—were obtained from local markets (Table 1),

TABLE 1. LOW RISK PESTICIDES EVALUATED FOR EFFICACY AGAINST ADULT *CYLAS FORMICARIUS*.

| Treatment | Active Ingredient | Dose | Source |
|--------------------------------------|------------------------------------------------------------------------------------------------------|----------------------|----------------------------------------------------|
| Control (no treatment) | No treatment | — | — |
| Control (water spray) | Tap water | — | — |
| <i>A. indica</i> (Aza-Direct®) spray | Azadirachtin 1.2%, other ingredients 98.8% | 10 mL/L of water | Azadirachtin, Gowan Company, Yuma, Arizona |
| <i>Morinda citrifolia</i> | — | whole spray | Locally grown plants |
| Volk oil spray® | Petroleum oil 97%, other ingredients 3% | 20 mL/L of water | The Ortho Group, Marysville, Ohio |
| <i>B. bassiana</i> ® 22WP | <i>Beauveria bassiana</i> Strain GHA 22%, inert ingredients 78% | 2.4 grams/L of water | Laverlam International Corporation, Butte, Montana |
| EcoSmart® (mixed essential oils) | rosemary oil 0.25%, peppermint oil 0.25%, thyme oil 0.25%, clove oil 0.25%, other ingredients 99.00% | whole spray | EcoSmart Technologies, Alpharetta, Georgia |
| Spinosad (Conserve SC®) | spinosad including Spinosyn A and Spinosyn D 11.6%, other ingredients 88.4% | 0.5 mL/L of water | Dow AgroSciences, Indianapolis, Indiana |
| Malathion (Prentox®) Spray | malathion 0,0-dimethyl phosphorodithioate of diethyl mercaptosuccinate 57%, other ingredients 43% | 5 mL/L of water | Prentiss Incorporated, Floral Park, New York |

and the dosages used were those recommended by the manufacturer. The seventh, extracts of *M. citrifolia* (noni, Rubiaceae), were prepared in the laboratory by the freeze-juicing method.

Preparation of *M. citrifolia* Juice Using the Freeze-Juicing Method

Twenty white, hard *M. citrifolia* fruits were harvested from a tree at the University of Guam and were then washed, dried, and stored at room temperature (22.0 ± 2.0 °C, 70-80% relative humidity) for one to three days. Once these fruits were soft-ripened, they were stored at -18 °C for 6-12 h. They were then thawed for 6-12 h at room temperature. The juice from the thawed fruit was collected and stored at 5 °C (Yang et al. 2011).

Adulticidal Assays

The adulticidal assays tested the hypothesis that the chemicals we tested, when topically applied, would exhibit contact toxicity to adults of *C. formicarius* (Table 1). For each replicate, 10 adults were transferred to a disk of Whatman No. 1 filter paper (9 cm diam, Whatman® quantitative filter paper, ashless, Sigma-Aldrich, St. Louis, Missouri, USA) in a 9 cm disposable Petri dish.

Each dish received a 10-g piece of sweetpotato and two 7-cm sweetpotato branches with leaves (4-8) as food for the insects. Five replicate Petri dishes of 10 adults were sprayed (Household Sprayer, Do It Best Corp., Ft. Wayne, Indiana, USA) with 0.5 mL of each of the chemical. Two control treatments were maintained; in one, the dishes were sprayed with 0.5 mL of tap water, and in the other, no treatment was applied. Following this application, dishes were maintained under laboratory conditions (previously described) and adult mortality was assessed at 24, 48, 72-96, 120-144 and 168-192 h after the treatment.

Repellent Assays

Adult repellency was evaluated using bioassay methods similar to that of Ferrero et al. (2007). Circular white filter paper No. 40 (9 cm diam Whatman® quantitative filter paper, ashless, Sigma-Aldrich Inc., St. Louis, Missouri, USA), was divided into two halves. One half was either left untreated or treated with 0.5 mL water; the other half was treated with 0.5 mL of the solutions of the extract/compound at the dosages shown in Table 1. Each essential oil (Table 1) was sprayed at the dosages shown in Table 1. After spraying, each treated half-disc was then attached lengthwise--edge-to-edge--to a control half-disc with adhesive tape, forming a full disc in a 9 cm disposable Petri dish. Safeguards were taken so that the attachment did not prevent the free movement of the insects from one half to another; but an insignifi-

cant distance between the filter-paper halves was left to prevent outflow of the test samples from one half to the other. The Petri dish had a seam oriented in 1 of 4 randomly selected directions to avoid any incidental stimuli affecting the distribution of insects. The positioning of the seam was altered in replicates. Ten adults of *C. formicarius* were released in the middle of each filter-paper circle and a plastic cover with several slight holes was positioned on the Petri dish. Each treatment was replicated 5 times. Counts of the insects existing on each filter paper disc half were made after 1 h and subsequently hourly, up to the fourth hour. The average of the counts was converted to percentage repellency. Repellency averages were categorized following Ferrero et al. (2007) and the scale used to categorize repellency of the tested pesticides was according to Talukder & Howse (1995); class and percentage repellency rate used was 0 (> 0.01 to < 0.1%), I (0.1 to 20%), II (20.1 to 40%), III (40.1 to 60%), IV (60.1 to 80), V (80.1 to 100).

Feeding Deterrence Assay

To assess feeding deterrence, five replicate petri dishes (9 cm) were set up, each containing 10 adults on a Whatman No. 1 filter paper disc. Each dish was loaded with diet consisting of 5 g of sweetpotato tuber that had been sprayed directly with 0.5 mL of test chemical. Two controls were included, one with untreated diet and one with diet sprayed with 0.5 mL water. The diet was weighed and recorded daily until the eighth day. The dead adults after the treatment are recorded and removed immediately.

Data Analyses

All the data were transformed by log ($x + 1$) to fit the assumption of homogeneity of variance for ANOVA. The data from the mortality and feeding deterrence assays were analyzed using the repeated measures ANOVA in using SAS PROC

Generalized Linear Model Version 9.3 (SAS Institute 2009). The data from repellent tests was analyzed using nonparametric Chi-square tests.

RESULTS

Adulticidal Effect

The results of the toxicity tests are presented in Table 2. The data are expressed as percentage mortality. All the tested pesticides caused significant ($F = 12.8$; $df = 8,15$; $P < 0.05$) adult mortality compared to both the control treatments. In this test, the spinosad caused 100% adult mortality at 48 h after the treatment, followed by *A. indica* and malathion at 72 to 96 h after the treatment. Similarly, 100% adult mortality caused by petroleum oil at 168 to 192 h followed by *B. bassiana* at 93%. The extracts from *M. citrifolia* caused a maximum of 72% mortality at 168 to 192 h after the treatment. The chemical mixed essential oils (EcoSmart®), which caused 46% mortality, was found to be the least effective among the tested pesticides.

Repellent Effect

There were significant differences in repellency among treatments ($F = 18.2$; $df = 8,12$; $P < 0.05$, Table 3). Among all the pesticides tested, *A. indica* had the strongest repellent effect on *C. formicarius* (class V) with 100% repellency at 3 h after the treatment, followed by the petroleum oil and spinosad (class V). Malathion and *M. citrifolia* had a 32 to 24% repellency effects, respectively. On the other hand, *B. bassiana* had no repellent effect on adults. The mixed essential oils (EcoSmart®), was able to repel a maximum of 12% after 4 h of treatment.

Feeding Deterrence Effect

There were significant differences in adult feeding deterrence among treatments ($F = 14.4$; df

TABLE 2. ADULTICIDAL EFFECTS OF THE SELECTED CHEMICALS AGAINST *CYLAS FORMICARIUS* AT VARIOUS INTERVALS AFTER TREATMENT.

| Chemical | Cumulative mean percentage \pm SEM mortality at different intervals (h) | | | | |
|---------------------------|---------------------------------------------------------------------------|-------------------|-------------------|------------------|-------------------|
| | 24 | 48 | 72-96 | 120-144 | 168-192 |
| Control (no water) | 0.0 \pm 0.0 e | 0.0 \pm 0.0 f | 0.00 \pm 0.0 e | 1.20 \pm 0.6 e | 0.00 \pm 0.0 d |
| Control (water spray) | 0.0 \pm 0.0 e | 0.0 \pm 0.0 f | 0.00 \pm 0.0 e | 0.00 \pm 0.0 e | 0.5 \pm 0.2 d |
| <i>Azadirachta indica</i> | 18.0 \pm 0.6 c | 34.0 \pm 1.3 c | 100.0 \pm 2.1 a | — | — |
| <i>Morinda citrifolia</i> | 8.0 \pm 1.4 d | 22.0 \pm 0.6 d | 42.0 \pm 2.1 c | 63 \pm 1.3 c | 72.0 \pm 0.4 b |
| Petroleum oil | 20.0 \pm 2.3 c | 38.0 \pm 0.6 c | 62.0 \pm 1.4 b | 81.0 \pm 1.6 a | 100.0 \pm 2.1 a |
| <i>Beauveria bassiana</i> | 0.00 \pm 0.0 e | 0.00 \pm 0.0 f | 43.0 \pm 0.0 c | 76.0 \pm 0.0 b | 92.5 \pm 0.6 a |
| Mixed essential oils | 0.0 \pm 0.0 e | 10.0 \pm 1.2 e | 24.0 \pm 2.2 d | 33.0 \pm 1.8 d | 45.5 \pm 1.3 c |
| Spinosad | 80.0 \pm 1.8 a | 100.0 \pm 1.5 a | — | — | — |
| Malathion | 54.0 \pm 0.9 b | 82.0 \pm 2.3 b | 100.0 \pm 0.8 a | — | — |

Each value is the mean of 5 replicates, with 10 adults per replicate. Values within a column followed by the same letter did not differ significantly at the 5% level of probability ($P < 0.05$, repeated measures ANOVA and Tukey's tests).

TABLE 3. REPELLENCY OF SELECTED CHEMICALS FOR *CYLAS FORMICARIUS* AFTER VARIOUS TIMES OF EXPOSURE.

| Chemical | Cumulative mean percent \pm SEM repellency after various times of exposure | | | | | | | |
|---------------------------|------------------------------------------------------------------------------|-------|------------------|-------|-------------------|-------|-------------------|-------|
| | 1 h | Class | 2 H | Class | 3 h | Class | 4 h | Class |
| <i>Azadirachta indica</i> | 28.0 \pm 0.6 a | II | 83.0 \pm 1.3 a | V | 100.0 \pm 2.1 a | — | — | — |
| <i>Morinda citrifolia</i> | 0.0 \pm 0.0 d | 0 | 12.0 \pm 0.2 d | I | 24.0 \pm 0.9 e | II | 24.0 \pm 0.9 c | II |
| Petroleum oil | 0.0 \pm 0.0 d | 0 | 22.0 \pm 1.4 c | II | 66.0 \pm 2.4 c | IV | 100 \pm 1.7 a | V |
| <i>Beauveria bassiana</i> | 0.0 \pm 0.0 d | 0 | 0.0 \pm 0.0 e | 0 | 0.0 \pm 0.0 g | 0 | 0.0 \pm 0.0 e | 0 |
| Mixed essential oils | 0.0 \pm 0.0 d | 0 | 0.0 \pm 0.0 e | 0 | 12.0 \pm 1.6 f | I | 12.0 \pm 1.6 d | I |
| Spinosad | 22.0 \pm 1.5 b | II | 44.0 \pm 2.8 b | III | 82.0 \pm 1.8 b | V | 100.0 \pm 2.0 a | V |
| Malathion | 8.0 \pm 0.2 c | II | 24.0 \pm 1.4 c | II | 32.0 \pm 1.5 d | II | 32.0 \pm 1.5 b | II |

Each value is the mean of 5 replicates, with 10 adults per replicate. Values within a column followed by the same letter did not differ significantly at the 5% level of probability ($P < 0.05$, ANOVA, Chi-square tests).

= 8,16; $P < 0.05$, Table 4). The pesticides *A. indica*, *B. bassiana*, spinosad and malathion significantly inhibited the feeding activities of *C. formicarius*. Interestingly, *A. indica* and spinosad exhibited high feeding deterrence effects on the adults at 120 h after the treatment. Moreover, all adults were died in the dishes treated with spinosad and *A. indica* after 120 and 144 h, respectively. However, *M. citrifolia*, petroleum oil and mixed essential oil had no significant effect on the feeding and the cumulative mean differences among these treatments were not statistically significant ($P > 0.05$) from the control treatments.

DISCUSSION

Conventional pesticides have not only become less effective as target insect populations have developed resistance, but also kill non-target species and natural enemies of many insect pests (Whalon et al. 2008). Although *C. formicarius* has no record of such pesticide resistance, it may occur in the future. For that reason, we have observed and evaluated low-risk pesticides. Our results show that certain ecofriendly chemicals can cause adult mortality of *C. formicarius* or can repel or deter feeding by adults. Unfortunately, because of the cryptic nature of the larvae, these chemicals may not be effective in bringing down the larval population, but by killing or interfering with adults they can prevent oviposition on sweetpotato crops.

In the current study, neem (Aza-Direct) (a vegetable oil pressed from the fruits and seeds of an evergreen tree, *A. indica*) is a botanical, broad-spectrum insecticide and its active ingredient is azadirachtin, a complex biological compound, which is extracted from seeds of the neem tree (Koul & Wahab 2004). Its primary mode of action is through insect growth regulation. Spinosad (spinosyn A and spinosyn D), on the other hand, is a bacterial waste product produced by fermentation on a nutrient food source used by the bacterium *Saccharopolyspora spinosa* (Hertlein et al. 2011). This product is a white crystalline solid, soluble in water, but breaks down on the

leaf surface or in the soil after a few days so that it does not pose a threat to ground water (Mertz & Yao 1990). Spinosad has proved to be toxic to wider variety of insects and recommended to use to control pests in both agricultural and horticultural environments, and also in greenhouses, golf courses, gardens, and around homes (Thompson et al. 1995). The extracts from *M. citrifolia* fruit, are known to contain a number of nutrients and phytochemicals (Wang et al. 2002). Among these, iridoids have recently been found to be the most abundant, especially deacetylasperulosidic acid and asperulosidic acid (Deng et al. 2010; West et al. 2011). Recently, West et al. (2012) demonstrated that deacetylasperulosidic acid and asperulosidic acid, the major phytochemical constituents of noni fruit, possess antibacterial activity. Further, Shapiro et al. (2009) reported *M. citrifolia* extracts as ultraviolet radiation protectants to *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae) nucleopolyhedrovirus. *Beauveria bassiana* strain GHA, applied as *B. bassiana*® Mycoinsecticide is an effective biological insecticide that is used to control whitefly, thrips, aphids, and many other insects (Vey et al. 2001). The applied spores infect directly through the insect's cuticle. Spores adhering to the host will germinate and produce enzymes that attack and dissolve the cuticle, allowing it to penetrate the cuticle and grow into the insect's body (Liu & Bauer 2008). Likewise, horticultural oils used as petroleum oil and mixed essential oil are reported to be effective alternatives to synthetic pesticides. Horticultural oils are lightweight oils, either petroleum or plant based, that can be an effective and low toxicity choice for managing certain insect and mite pests (Johnson 1991). The oils provide control mostly by smothering; i.e., blocking the spiracles through which they breathe (Müller & Buchbauer 2011). Malathion (O,O-Dimethyl S- 1,2-(diethoxycarbonyl) ethyl phosphorodithioate) is an organophosphate insecticide and one of the most commonly used to control a large variety of insect pests. Malathion is a nerve poison and inhibits the action of an enzyme acetylcholinesterase (Flessel et al. 1993). This enzyme works to breakdown another chemi-

TABLE 4. FEEDING DETERRENCE ACTIVITY OF SELECTED CHEMICALS AGAINST *CYLAS FORMICARIUS* AT AFTER VARIOUS TIMES OF EXPOSURE.

| Chemical | Mean percent \pm SEM (g) of food consumed at different intervals (h) | | | | | | | | Cumulative |
|---------------------------|------------------------------------------------------------------------|---------------|---------------|---------------|---------------|---------------|---------------|------------------|------------|
| | 24 | 48 | 96 | 120 | 144 | 168 | 192 | | |
| Control (no water) | 1.6 \pm 0.7 | 1.8 \pm 0.2 | 1.6 \pm 0.2 | 1.7 \pm 0.6 | 1.3 \pm 1.2 | 1.4 \pm 0.4 | 1.2 \pm 0.3 | 10.8 \pm 2.6 a | |
| Control (water spray) | 1.3 \pm 1.2 | 1.4 \pm 0.4 | 1.4 \pm 1.3 | 1.3 \pm 0.2 | 1.3 \pm 0.5 | 1.2 \pm 0.6 | 1.1 \pm 1.2 | 9.1 \pm 1.4 a | |
| <i>Azadirachta indica</i> | 0.2 \pm 0.4 | 0.3 \pm 0.1 | 0.2 \pm 0.1 | 0.1 \pm 0.1 | dead | dead | dead | 0.8 \pm 0.2 d | |
| <i>Morinda citrifolia</i> | 1.1 \pm 1.2 | 1.0 \pm 0.3 | 2.4 \pm 0.6 | 1.6 \pm 1.2 | 1.7 \pm 1.2 | 1.2 \pm 1.3 | 0.5 \pm 0.3 | 9.6 \pm 2.2 a | |
| Petroleum oil | 1.2 \pm 0.3 | 1.1 \pm 0.6 | 2.7 \pm 1.8 | 1.5 \pm 0.4 | 1.3 \pm 0.8 | 1.3 \pm 0.4 | 0.7 \pm 0.2 | 9.9 \pm 1.8 a | |
| <i>Beauveria bassiana</i> | 0.4 \pm 0.6 | 0.7 \pm 0.2 | 0.1 \pm 0.0 | 1.3 \pm 1.2 | 1.4 \pm 0.3 | 1.1 \pm 0.2 | 0.5 \pm 0.1 | 5.5 \pm 1.2 b | |
| Mixed essential oils | 0.8 \pm 1.3 | 1.8 \pm 1.5 | 1.7 \pm 0.4 | 1.6 \pm 0.6 | 1.7 \pm 1.1 | 1.1 \pm 0.2 | 0.9 \pm 0.4 | 9.5 \pm 2.4 a | |
| Spinosad | 1.7 \pm 0.8 | 1.3 \pm 1.3 | 0.1 \pm 0.0 | dead | dead | dead | dead | 3.1 \pm 1.2 c | |
| Malathion | 0.6 \pm 1.1 | 0.7 \pm 0.2 | 0.8 \pm 0.3 | 1.4 \pm 1.0 | dead | dead | dead | 3.5 \pm 1.6 c | |

Each value is the mean of 5 replicates, with 10 adults per replicate. "Dead" signifies that all insects in that treatment were dead at that interval. Values within a column followed by the same letter did not differ significantly at the 5% level of probability ($P < 0.05$, repeated measure ANOVA and Tukey's tests).

cal, acetylcholine, which is essential in transmitting impulses between nerves in the body of the insect (Chamber 1992).

In the current study, *A. indica* and spinosad exhibited high insecticidal, repellent and feeding deterrence activity against *C. formicarius*. There are many reports (Thompson et al. 1995) indicating spinosad as a good candidate for a variety of insects. Spinosad is a reduced-risk insecticide and moreover, recommended field application rates for spinosad are much lower than those for diazinon and other organic pesticides (Stark & Banks 2001). This chemical also effectively controls economically important beetle and moth pests associated with stored grain and is also effective against certain psocid species (Hertlein et al. 2011). Spinosad is also reported to be highly active against Lepidoptera, but is reported to have low toxicity to the natural enemies (Cisneros et al. 2002). Since *C. formicarius* also attacks sweetpotatoes in storage, it is important to keep this chemical in mind when control is necessary. On the other hand, *A. indica* extracts contain many compounds which are useful for pest control (Koul & Wahab 2004). Unlike chemical insecticides, an *A. indica* compound works on the insect's hormonal system, not on the digestive or nervous system, and therefore does not lead to the development of resistance in future generations (National Research Council 1992). These compounds belong to a general class of natural products called "limonoids" (Gilbert & Gill 2010).

In the present study, malathion showed adulticide, repellency and feeding deterrence significantly higher than the control. Although malathion is less toxic to humans than many other organophosphate insecticides, it is highly toxic to aquatic organisms and bees. Several field studies recording direct mortality of nontarget arthropods resulting from malathion treatments demonstrate that a large number and wide variety of species can be affected if malathion is applied in the field. For example, Troetschler (1983) recorded that isopods, earwigs, tree crickets, and carabids were directly killed by malathion bait sprays. In addition, numerous dead predators and parasitoids, as well as herbivores, were collected from beneath treated corn plants 2 h after malathion bait applications (Messing et al. 1995). For these reasons, malathion is not advisable for the control of *C. formicarius* under field conditions. These findings are in accordance with the results of Trdan et al. (2006), who proved the satisfying efficacy of malathion against *Eurydema ventral* and *E. oleracea* (Heteroptera: Pentatomidae) on white cabbage under field conditions, but they recommended the use of more environmentally friendly insecticides, i.e., refined rape oil.

Pesticides such as petroleum oil, *B. bassiana* and mixed essential oil have been demonstrated as moderately effective against *C. formicarius*.

Because of environmental safety and reduced risk, different countries are assaying natural compounds of plant origin as pest control alternatives. A large number of powders and essential oils from natural products have been used as sustainable control measures against different insect pests since they present no known risk to humans and the environment, unlike more conventional pesticides (Jayasekara et al. 2005). Furthermore, Reddy & Bamba (2011) suggested that the current, reduced-risk recommended practice including petroleum oil, *B. bassiana* and mixed essential oil is highly valuable and effective in controlling pests on cabbage.

The research concludes that reduced-risk pesticides such as *A. indica* and spinosad are promising candidates as environmentally friendly pesticides that can be compatible with *C. formicarius* IPM programs hence may potentially substitute for harmful broad spectrum pesticides in the control of this insect pest. However, further studies are needed to make these pesticides more effective and to investigate their efficacy under field conditions. Specifically, it is well-known fact that the results of laboratory experiments cannot be transferred uncritically into conditions which hold for the environment (Jagers op Akkerhuis et al. 1999).

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