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Efficacy of *Azadirachta excelsa* vinegar against *Plutella xylostella*

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ABSTRACT

The diamondback moth, *Plutella xylostella* L. (Lepidoptera: Plutellidae), is considered a major pest for crucifers in Malaysia. New control materials are constantly required as *P. xylostella* population develops resistance to most of the insecticides used against them. In this study, vinegar derived from *Azadirachta excelsa* (Jack) Jacobs was evaluated against the third-instar larvae of *P. xylostella* and an appropriate application dose required to control the pest was determined in the laboratory trial. Results indicated *A. excelsa* vinegar to be a useful control measure, significantly reducing *P. xylostella* leaf consumption and adult size of *P. xylostella*. Interestingly, mortality rate of larval, pupae and adult was significantly increased by the application of *A. excelsa* vinegar. An in-diet EC_{50} of 80× against larvae was achieved, whilst obvious defects in adult moth were also recorded. The results indicate that *A. excelsa* vinegar exhibits potential to be used against *P. xylostella*.

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Antifeedancy; *Azadirachta excelsa*; biopesticide; crucifer crops; wood vinegar

Introduction

The major pest of cruciferous crops in Malaysia is *Plutella xylostella* L. Because the cultivation of crucifers is a year-round activity, the short life cycle of *P. xylostella* makes this pest a constant threat to these crops. The use of synthetic pesticides to control the pest is common. However, these pesticides can harm the environment and are also toxic to humans (Smith & Reynolds 1973; DeBach & Rosen 1991). Furthermore, this pest has developed resistance to most frequently used insecticides (e.g. Sarfraz & Keddie 2005). As concerns for the environment grow, biological pesticides or botanical insecticides are increasingly emphasized. According to Liu et al. (2000), biopesticides offer an alternative to chemical pesticides because they generally have low environmental pollution rates and have low toxicity to humans. Therefore, with this study our goal is to test the efficiency of a botanical insecticide extracted from *Azadirachta excelsa* (Jack) Jacobs in controlling the severe pest *P. xylostella*.

In the past 40 years, plant secondary compounds have been subjected to systematic study in an attempt to discover new sources of botanical insecticides (biopesticides) and antifeedants. Annonaceae, Asteraceae, Labiatae, Meliaceae, Piperaceae and Rutaceae are among the most promising plant families (Schoonhoven 1982; Jacobson 1989; Isman 1995). Partly due to the existence of triterpenoids called limonoids, two families, Meliaceae and Rutacae, have received much attention (Connolly 1983). Azadirachtin, a complex tetranortriterpenoid limonoid that is extracted from seeds of the neem tree (*Azadirachta indica*, Meliaceae), is the main component responsible for the antifeedant and growth-inhibitory effects against insect pests (Isman 1997).

The biopesticides of *A. indica* have been tested against more than 200 insect species from several orders, as well as other micro-organisms, such as bacteria, nematodes and fungi (Kelly 1994). Morgan (2008) claimed that azadirachtin from the *A. indica* tree has been tested on 600 or more insect species. Schmutterer (1995) reported that insecticides derived from neem seeds have been effective against hundreds of insect pests, including species of Diptera, Hemiptera and Lepidoptera. Neem-derived insecticides also have a minimal effect on natural enemies of insects (Schmutterer 1988; Ascher et al. 2002); thus, the potential for their application in integrated pest management programs is large. Hummel (2006) has described the use of neem-based insecticides in integrated pest management.

In addition to the Indian neem tree (*A. indica*), the genus *Azadirachta* also includes *A. simensis* (Thai neem), which can hybridize with *A. indica* (Sombatsiri et al. 2002) and *A. excelsa* (marrango), a fast-growing tree species that is naturally distributed in Borneo and the southern Philippines (Schmutterer & Doll 1993). *Azadirachta excelsa* also contains a type of azadirachtin known as marrangin that is effective as a growth-regulating pesticide (Schmutterer & Doll 1993; Isman 2005; Morgan 2008; Hummel et al. 2011). Albeit from a different species, *A. excelsa* may have the potential to become a biopesticide similar to *A. indica*.

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In this study, the potential of *A. excelsa* for controlling *P. xylostella*, a major pest of crucifers in Malaysia, was assessed, and the effective concentration required to control the pest was determined.

Materials and methods

Preparation of Azadirachta excelsa *vinegar*

Branches of *A. excelsa* were taken from an *A. excelsa* tree located near the Botanical Garden on the Universiti Putra Malaysia Bintulu Sarawak Campus (UPMBC). The samples were air-dried for one week before being dried in a furnace. The furnace Carbolite® ELF 11/6B (Carbolite Limited, Hope, UK) was modified to collect and condense smoke into liquid form. The temperature increased at a rate of 1.4 °C min^{-1} from ambient temperature to a final temperature of 550 °C to obtain the maximum yield as described by Ratanapisit et al. (2009).

The smoke released from the furnace was channeled into a pipe via a small hole made at the end of the pipe. The smoke was cooled and condensed with a wet towel. The yield was collected via a small hole into a beaker. Smoke collection began at the point of carbonization and continued until the smoke was clear. Then, the yield was left for 24 hours to obtain the vinegar.

Plutella xylostella *larvae*

Brassica campestris var. *parachinensis* seeds were sown in 20 pots with mixed media (5:3:2 of topsoil: coco peat: sand). The vegetables were propagated in Share Farm II at the UPMBC to attract *P. xylostella*. Third-instar larvae were collected for use in the experiments.

Antifeedant tests

The larvae were randomly selected and divided into groups for the different treatments. The experimental design was a completely randomized design with four treatments, including the control. Three treatments involving vinegar were diluted at different ratios of vinegar and distilled water (1:400 – 400×, 1:200 – 200× and 1:100 – 100×). The control treatment used only distilled water. Each treatment was replicated four times, and each replicate consisted of 10 larvae; thus, 160 larvae were used in this study.

The larvae were weighed and their body weights were recorded. The larvae were starved for three hours before testing. Leaves were dipped in the different solutions for 5 min and were air-dried until the solution on the leaves was completely dry. All the leaves were then placed individually in Petri dishes containing 10 larvae each. The experiments were carried out for 24 hours.

At the end of the experiments, the leaf area consumed by the larvae was measured using a LiCor 3100C Area Meter (LI-COR Biosciences, Lincoln, USA) and recorded. The number of survivors and the larval body weights were also recorded. The larvae then were transferred to Petri dishes containing untreated leaves, where they were kept until they pupated. Physical observations, including the number of adults that subsequently emerged, were recorded.

Statistical analyses

All of the analyses were conducted using SAS software v. 9.2 (SAS Institute Inc., Cary, NC, USA). To examine the effects of different *A. excelsa* vinegar treatments (Control, 100×, 200× and 400× diluted *A. excelsa* vinegar) on the performance of *P. xylostella*, an analysis of variance (ANOVA) was conducted for the different variables (days to pupation, larval weight gain (%), leaf area consumed (%), mortality rate and size of the adult). Assumptions of normality and homogeneity of variance were visually checked, and all of the percentage variables indicated the need for log-transformation before analyses. Other variables did not require transformation. The mean differences were separated using Duncan's New Multiple Range tests. The EC_{50} (effective concentration causing 50% inhibition of larval growth compared with the control) and DC_{50} (concentration causing 50% feeding deterrence compared with the control) values were also calculated.

Research limitation

Field trials were not conducted due to the difficulty in locating the larvae after implementing the treatments. This was due to the high mobility of the larvae, uneven distribution and non-uniform population of growth stages of the insect. The inability to control insect movement in the field would consequently lead to large variation in insect abundance, and thus not allowing for a detailed assessment of treatment effects. In laboratory assays, detailed assessment of the effect of *A. excelsa* vinegar towards insect response can be done but would not be possible in the field. Results from this laboratory trial can be used to predict the effectiveness of vinegar treatment on the insect in field situations and thus enable the design of a valid field experiment.

Results and discussion

Plutella xylostella *infestation*

The crops were attacked by the pest after 26 days in the field. The slow attack by *P. xylostella* may have been due to weather conditions, as it was the rainy season. The low *P. xylostella* population in the area may also have contributed to slow infestation, as Share Farm II was not practicing the continuous cultivation of crucifers and cabbage. According to Ooi (1986), the

Table 1. Effect of treatments on pupation period, leaf consumption, weight gain and adult size of *Plutella xylostella*.

Treatment	Larvae (day)	Pupae (day)	Leaf consumed (%)	Larvae weight gain (%)	Adult size (mm)
Control	2.0 ± 0.0^{a}	4.8 ± 0.5^{a}	29.60 ± 0.60^{a}	15.24 ± 2.98^{a}	6.2 ± 0.2^{a}
400×	1.8 ± 0.5^{a}	4.5 ± 0.6^{a}	20.42 ± 3.84^{b}	12.71 ± 3.71^{a}	5.2 ± 0.8^{b}
200×	2.3 ± 0.5^{a}	4.5 ± 0.6^{a}	17.93 ± 3.10^{b}	13.13 ± 4.78^{a}	5.0 ± 0.7^{b}
100×	2.3 ± 1.3^{a}	4.5 ± 0.6^{a}	15.56 ± 0.85^{c}	8.69 ± 0.08^{b}	5.3 ± 0.3^{b}

Note: Values in columns followed by the same letter are not significantly different by Duncan's New Multiple Range test ($p < 0.05$).

presence of *P. xylostella* on crops depends on weather conditions (high presence was at 25.2–35.0 °C according to Ahmad & Ansari 2010) and the type of *Brassica* vegetables. No *P. xylostella* larvae were observed in the afternoons; they could only be observed at dusk and in the early morning.

Morphological changes

Third-instar larvae take approximately 4–5 days to become adults. Larvae take 1–4 days to become pupa and pupa take approximately 5–8 days to become adults (Table 1). The duration of larva morphology was not influenced by the treatments (Table 1). The current study found that *A. excelsa* vinegar does not affect the morphological development period of larvae to become pupa or pupa to become adults. This finding, however, is in contrast to the results of Schmutterer (1988), Mordue (Luntz) and Nisbet (2000) and Hummel et al. (2012), who reported that azadirachtin from *A. indica* and *A. excelsa* delayed growth and developmental processes, such as molting, and that the larvae took a longer time to become adults.

Adult mortality occurred within 1–2 days after the pupae become adults. The dead were usually adults that suffered from defects, such as wings that did not develop properly and a too-small body size (Figure 1). Normal *P. xylostella* adults can survive up to five days, even without food.

Leaf consumed and larval weights

Significant effects were observed in leaf consumption. The control recorded the highest percentage of leaves consumed. As the concentration of vinegar increased, the consumption of leaves by larvae was reduced (Table 1). This result may be due to the primary antifeedant effect of the vinegar on the larvae. The primary antifeedant activity is due to sensitivity to secondary compounds in their diet, as insects are incapable of digesting food that eventually inhibits feeding appetite (Schmutterer 1985). Azadirachtin from *A. indica* can inhibit the feeding appetite of many insect species. Marrangin, an analog of azadirachtin in *A. excelsa*, has a higher efficacy than azadirachtin as a feeding deterrent in insects (Hummel et al. 2011).

The appetite of insect larvae relies on the taste receptor or the chemoreceptor response on their mouthparts, tarsi and oral cavity (Mordue (Luntz) & Nisbet 2000). In the current study, *A. excelsa* vinegar stimulated specific deterrent effects as a chemoreceptor of larvae and disrupted and blocked receptor cells that usually stimulate feeding of larvae (Simmonds et al. 1990; Mordue (Luntz) et al. 1999). Furthermore, Lepidopterous pests are very sensitive to azadirachtin compared with other insect orders, such as Coleoptera, Hemiptera and Homoptera (Mordue (Luntz) & Nisbet 2000). The DC_{50} value for the current study was 81×. The results indicate that a higher concentration of vinegar is needed than that used in the current study (only up to 100×).

A higher concentration of vinegar also significantly reduced the weight gain of larvae (Table 1). Similar results were reported by Akhtar and Isman (2004), who found that azadirachtin in *Melia volkensii* (family Meliaceae) efficiently inhibited the larval growth of *P. xylostella* at 9.1 ppm. This antifeedancy resulted from a reduction in the food intake and digestion efficiency of larvae (Mordue (Luntz) & Nisbet 2000). The value of EC_{50} for *A. excelsa* vinegar on *P. xylostella* was 80×.

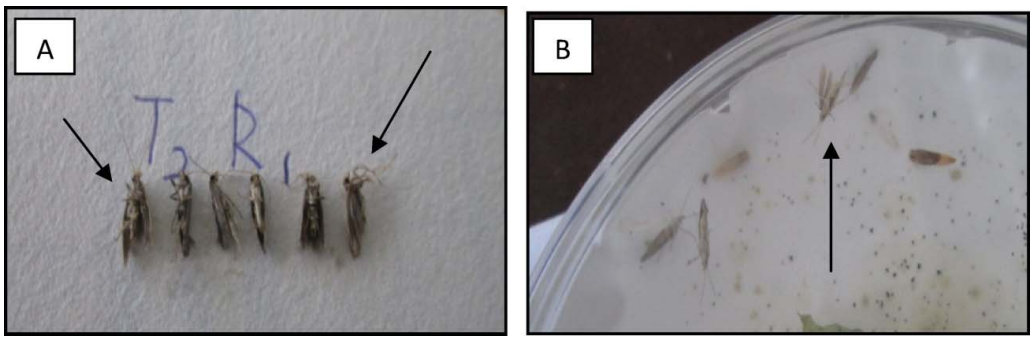

Figure 1. Defects on insects emerging as adults (with arrow): (A) Legs and wings do not grow properly, and (B) small wings.

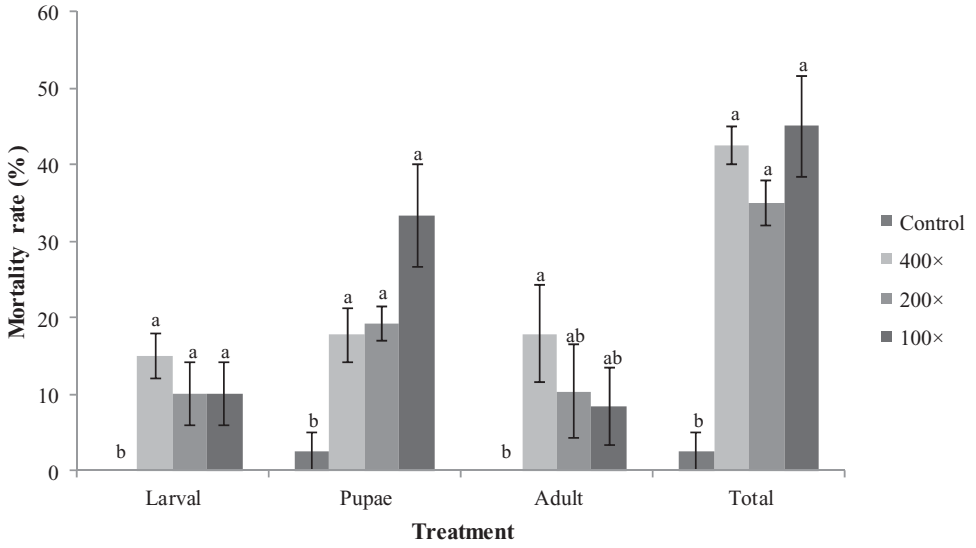

Figure 2. Mortality rate of *Plutella xylostella*.

Mortality

Treatments with vinegar showed significant differences in rates of larval mortality compared with the control (Figure 2). This result may be due to appetite inhibition and the inability to ingest food due to larval sensitivity to the vinegar compound, which caused starvation and eventual larval death. The percentage of larval mortality was low but similar to the results reported by Anon (1992). It was noted that azadirachtin more profoundly affects insect growth than feeding inhibition.

A significantly higher mortality rate was also observed in pupae compared with the control (Figure 2). The pupae mortality may be due to insect growth regulation, which affects them physiologically. Compounds in wood vinegar may interfere with the endocrine system (Mordue (Luntz) & Nisbet 2000) and therefore disrupt larvae homeostasis, behavior, insect growth, development and other physiological activities (Meyer 2006). Thus, wood vinegar controls larval growth and molting. The main target is the insect cuticle, where the compound disturbs the neurosecretory system of the insect brain and blocks morphogenetic peptide hormones and allatostatins from release. These compounds in turn inhibit the prothoracic glands and corpora allata functions. The molting hormone from prothoracic glands is inhibited, and new cuticle formation and ecdysis in turn will also be inhibited (Mordue (Luntz) & Nisbet 2000). The pupae cannot emerge into adults and eventually die. Morgan (2008) also reported similar findings, in which azadirachtin from *A. indica* disrupted and inhibited larvae and nymph growth, resulting in permanent pupae and eventual death.

Only the 400× recorded a significant difference compared with the control in terms of adult mortality (Figure 2). There were no differences observed in the 100× and 200× compared with the control. The results showed that insect growth continued (larvae to adult) at a lower concentration of wood vinegar and that a higher mortality rate only occurred at the adult stage. Death may be due to the effect of secondary antifeedants and insect growth regulation resulting from the incomplete metamorphosis of pupae to adult.

Final mortality also showed similar results compared with the different stages of growth (Figure 2). Mortality resulted from a few different processes. Larval mortality was highly affected by feeding inhibition (primary antifeedancy) due to appetite blockage. Thus, the larvae starved and eventually died. In contrast, for pupae and adults, mortality resulted from secondary antifeedant and growth regulation. The food consumption of the insects was reduced and caused the insects to become weak; growth was inhibited due to interference with the endocrine system, eventually leading to pupae and adult mortality.

Adult size

Even though the adult mortality rate was less than 20% (Table 1), some of the pupae that emerged were smaller (14.5–19.4%) than normal size (the control). The control had a significantly larger body size compared with those that received other treatments (Table 1). This finding was similar to that of Mordue (Luntz) et al. (1995), where azadirachtin caused growth reduction in *Oncopeltus fasciatus* and resulted in smaller insects. Capinera (2012) stated that the normal size of *P. xylostella* was approximately 6 mm; however, in this study, the size of the adults that were treated with wood vinegar was less than 5.3 mm. This result may reflect the influence of the compounds in wood vinegar on insect growth regulation. Even though the

endocrine system of larvae was disrupted, some of the larvae were still able to develop into adults. However, the adults that emerged were smaller because the molting process did not occur properly. Adult defects included incomplete wings and legs, and some adults had no wings at all in the current study. These results are supported by Mordue (Luntz) andNisbet (2000), who found that azadirachtin can cause abnormalities in *Schistocerca gregaria,* leading to death. Because the endocrine system is disrupted by compounds in wood vinegar, wood vinegar also interrupts the prothoracic glands, which serve as ecdysteroids (molting hormones) for the epidermal molting process. Thus, wood vinegar caused the adults that emerged from pupae to have defects.

Conclusion

The active compound contained in *A. excelsa* vinegar inhibited the consumption of leaves and caused mortality in larvae, pupae and adults. The size of larvae, pupae and emerging adults was affected and found to be smaller than normal. Body defects were also observed in the adults. Although higher concentration of *A. excelsa* vinegar was needed to achieve EC_{50} and DC_{50}, the dilution at 400× was sufficient to increase *P. xylostella* mortality. The present study which was conducted in controlled environment represents an important first step to determine the negative effects of *A. excelsa* on *P. xylostella*. A management plan will then be implemented after confirming the effects of vinegar on the pest. This approach is similar to work done by Arutselvi et al. (2012) where they found that a mixture of *Azadiracta indica* kernel and *Vitex negundo* leaf extracts was able to control *Panchaetothrips indicus*, a pest of *Curcuma longa* both in the laboratory and field trials.

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Disclosure statement

Currently there is no financial interest or benefit arising from the direct applications of this research.

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