UNIVERSITI PUTRA MALAYSIA

POTENTIAL OF AZADIRACHTA EXCELSA VINEGAR TO CONTROL PLUTELLA XYLOSTELLA

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POTENTIAL OF *Azadirachta excelsa* VINEGAR TO CONTROL *Plutella xylostella*

By

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ABSTRACT

Azadirachta excelsa is a plant species that grows naturally in Southern Asia including Borneo that contains marrangin which is believed to be able to control insects. In this study, the potential of this species in controlling a major pest in Crucifers, *Plutella xylostella* is being assessed and effective concentration to control the pest identified. *Azadirachta excelsa* vinegar is used in this study at different dilution rates and third instars *P. xylostella* larvae was used as test subject. The result indicated that vinegar treatments have a significant effect ($p<0.05$) on leaf consumed, adult size, and larval, pupae, adult and final mortality rate when compared with the control. A few adults were also experiencing defects. The effects of *A. excelsa* vinegar to insect are primary antifeedancy and regulating growth of *P. xylostella*. The *A. excelsa* has shown highly potential on controlling *P. xylostella* and dilution of vinegar for 400 times is good enough to control *P. xylostella*.

ABSTRAK

Azadirachta excelsa adalah spesis tumbuhan yang tumbuh secara semulajadi di Asia Selatan termasuk Borneo yang mengandungi marrangin yang dipercayai dapat mengawal serangga. Dalam kajian ini, potensi spesies ini kepada pengawalan perosak utama Crucifers, *Plutella xylostella* dinilai dan rawatan yang berkesan untuk mengawal perosak dikenalpasti. Cuka *A. excelsa* digunakan dalam kajian ini pada kadar pencairan yang berbeza dan larva *P. xylostella* pada peringkat instar ketiga digunakan sebagai bahan ujian. Hasilnya menunjukkan bahawa rawatan cuka mempunyai kesan yang signifikan ($p<0.05$) pada daun dimakan, saiz dewasa, dan kadar kematian larva, pupa, dewasa dan akhir apabila dibandingkan dengan kawalan. Beberapa ekor serangga dewasa juga mengalami kecacatan. Kesan cuka *A. excelsa* kepada serangga *P. xylostella* adalah anti-pemakanan primer dan pengawalan pertumbuhan. *Azadirachta excelsa* menunjukkan potensi yang tinggi dalam mengawal *P. xylostella* dan pencairan cuka 400 kali adalah mencukupi untuk mengawal *P. xylostella*.

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I certify that this research project report entitled **"Potential of *Azadirachta excelsa* vinegar to control *Plutella xylostella*"** has been examined and approved as a partial fulfilment of the requirement for the degree of Bachelor Science Bioindustry in the Faculty of Agriculture and Food Sciences, Universiti Putra Malaysia Bintulu Sarawak Campus.

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CHAPTER 1

INTRODUCTION

Azadirachta excelsa (Jack) Jacobs is a tree species from Meliaceae (Mahogany Family). It has several common names depend on place such as Tiem in Thailand, Sentang in Peninsular Malaysia, Limpaga or Ranggau in Sabah, Ranggu in Sarawak, and Marrango or Philippine neem tree in Philippine (Apannah & Weinland, 1993).

There are only three species from the genus *Azadirachta*: *A. indica*; *A. siamensis*; and *A. excelsa* (Schmutterer & Doll, 1993). Biopesticide of *A. indica* which is extracted from seed has been tested against more than 200 insect species from several orders and other microorganisms such as bacteria, nematode and fungi (Kelly, 1994). Morgan (2008) also claimed that azadirachtin from *A. indica* tree have been tested on 600 or more insect species.

Azadirachta excelsa also contains a type of azadirachtin which is known as marrangin that is valuable as growth-regulating pesticides (Schmutterer & Doll, 1993; Isman, 2005; Morgan, 2008; Hummel *et al.*, 2011). Even they are in different species, *A. excelsa* may have the potential to become a biopesticide similar to *A. indica*.

The wood vinegar or pyroligneous acid from *A. excelsa* can be obtained from pyrolysis process. Liquids obtained from pyrolyisis of wood or wood-based wastes

have several names such as pyrolysis oil, bio-oil, pyrolysis liquid, crude bio-oil, bio-fuel oil or vinegar (Nakai *et al.*, 2005). It usually viscous and has dark brown colour, where the compositions and yields depend on the process condition and compositions of starting material. The most important factor affecting product yields and chemical compositions are heating rate and temperature (Ratanapisit *et al.*, 2009). Previous studies have concluded that slow pyrolysis takes a long period of time and favours char yield while fast pyrolysis takes a short reaction time and favours high liquid yields.

The major pest of Crucifers crop in Malaysia is *Plutella xylostella*. It becomes the worst pest because cultivation of Crucifers is year around and short life cycle makes this pest a constant threat to those crops. The use of synthetic pesticide to control the pests is common trend nowadays. However, it can harm the environment and also may toxic to humans (Smith & Reynolds, 1972; DeBach & Rosen, 1991). Other than that, pest can develop resistance to most frequently used insecticides. Due to care to the environment, biological pesticides are increasingly emphasized nowadays. According to Liu *et al.* (2000) biopesticides are another alternative because they generally have low environmental pollution and have low toxicity to humans.

So, *A. excelsa* is one of the species that can be used as biopesticide because it has insecticidal properties and available naturally in Southern Asia including Borneo. In this study, the potential of this species in controlling *P. xylostella*, the major pest of Crucifers in Malaysia was being assessed and the effective concentration to control the pest was identified.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

These days, the concern on environment in term of pest control is increasingly with the rise in usage of biopesticides while the uses of synthetic insecticides begin to decrease. Many research showed that certain tropical trees produce phytochemicals which have potentials to control pests (Isman, 2005). One of the important species is *A. indica*, family Meliaceae, which was discovered to have insecticidal properties (azadirachtin) and have been commercialized worldwide (Copping & Menn, 2000).

Another species of genus *Azadirachta*, *A. excelsa* have also been identified having marrangin, a compound that is more effective than azadirachtin. This bioactive compound that is not found in *A. indica* products has been observed to be better growth inhibitors (Ng *et al.*, 2003).

2.2 Biopesticides

The use of synthetic insecticides in controlling crop pest around the world has resulted in significant damage to the earth in term of environment, pest resurrection, pest

resistance to insecticides, and mortality effects on non-target organisms (Abudulai *et al.*, 2001). Meanwhile, biopesticides are derived from materials such as plants, microbes and minerals (O'brien *et al.*, 2009). Plant-derived pesticides must be ingested by pests to be effective to cause poisoning in stomach or have contact toxicity against soft bodied of insects and larvae. However, these actions also depend on concentration or dose of the products, behaviour and physiology of insects (Gahukar, 2012).

In developing countries, inadequate product knowledge, uncertainty of supply and high price caused the use of pesticides inefficient, and created additional socio-economy problem among the rich and poor people (FAO, 1992). So a new search, preferably insecticide which is effective and/or low cost is needed. Due to this matter, interest in plant-based pesticides has grown rapidly during recent years (Leng *et al.*, 2011).

2.2.1 Advantages of biopesticides

O'brien *et al.* (2009) stated that the common benefits of biopesticide active ingredients in comparison with synthetic pesticides among other were less toxic, biodegrade faster, more specifically target and action mode, and manage rather than eliminate (maintaining ecological balance). In contrast, synthetic pesticides may affect other organisms rather than the targeted pest, such as plants, birds, insects, and mammals. Besides, biopesticides are often designed to control pest populations until they reached a manageable level rather than completely eradicate them (Lewis *et al.*, 1997).

US Environmental Protection Agency (2010) stated that there is no restricted interval entry or 'grace period' to enter the field when using biopesticide. The restricted interval entry is requirements where one cannot enter for certain period of time to the field when it has been treated. The restricted interval entry period can delay field practices such as irrigation and pruning. It is also same with harvest restriction, where many biopesticides do not have harvest restriction period.

2.2.2 Disadvantages of biopesticides

The common challenges of using biopesticides are they tend to be slower to act or have slower kill rate. Therefore biopesticides are considered as less effective compared to synthetic pesticides (Gahukar, 2012). Beside, biopesticides that are degrading very fast in environment may also have a short shelf life or limited field persistence. This in turn may require multiple applications, which wastes a lot of farmers' times.

The other disadvantages are in terms of a narrow target range and very specific mode of action (Clemson Extension, Home, Garden Information Center, 2007). It gives benefits in term of specificity where it has lower impact on non-target species, but the challenge is the control of dominant pests on a given crop may need more than one product and application, and this increase the management cost (O'brien *et al.*, 2009).

Not all biopesticides can work efficiently like synthetic pesticides. Biopesticides can only be very effective when use as a part of systematic Integrated Pest Management (IPM) with high skill and understanding of its usage (O'brien *et al.*, 2009).

Biopesticides are among the least toxic to control pests, however this is not always true. Some are more toxic than others such as pyrethroids can affect human health in unexpected ways (O'brien *et al.*, 2009).

2.3 *Azadirachta excelsa*

2.3.1 Distribution and habitat

Azadirachta excelsa is a lowland humid forest species native to Borneo, but now grows naturally in Southern Thailand, Peninsular Malaysia and Palawan Island of Philippines (Joker, 2000). It has also been introduced into other tropical countries such as Taiwan, Guatemala, and the State of Hawaii (Appanah & Weinland, 1993; Kijkar & Boontawee, 1995).

This species can be found in up to about 350 m elevation with the best growth usually available in areas where annual rainfall is greater than 2000 mm, mean annual temperature of 22 - 27° C and the dry season which is not more than 2 - 3 months. It does not tolerate cold or frost (Joker, 2000).

Azadirachta excelsa is also considered as a fast-growing plant (Appanah & Weinland, 1993) which can reach 45 m high. It prefers good quality soil, sandy loams to loamy soil with a pH of 5.0 - 6.5. This species usually begin to flowering and fruiting after reaching 6 to 7 years (Joker, 2000).

2.3.2 Insecticidal properties

Ermel *et al.* (1991) was the first to report on inhibition of insect growth regulatory activity by an extracts from the seeds of *A. excelsa*, and as well as the isolation of a novel limonoid called marrangin. That compound has similar biological effect as azadirachtin. Florido & De Mesa (2001) claims that marrangin extracted from seed kernel of *A. excelsa* and found that it was two to three times more active than azadirachtin that was obtained from *A. indica* tree. *Azadirachta indica* tree which is in the same family as *A. excelsa* is believe to be a rich source of botanical insecticides (Ng *et al.*, 2003). Hummel *et al.* (2011) found that *A. excelsa* showed a more pronounced effect than *A. indica* on the hormonal system of insects.

Effects of the species on insect pests is inhibits feeding but not directly kill the insects. The insects will finally die through starvation. Among the effects resulted from application of *A. indica* were reduction of food intake, delay in development of larvae and nymphs, permanently larvae, incomplete ecdysis (moulting process), pupae and adults were malformed, and the eggs were sterile, and reduce in fertility (Morgan, 2008). However, although this species has impacts on insects and mite pests, it has no obvious toxicity on vertebrate.

2.4 Diamondback moth, *Plutella xylostella* (Linnaeus) (Lepidoptera: Plutellidae)

Plutella xylostella is a major pests of Crucifers in many tropical countries include Malaysia (Ho *et al.*, 1983), where it was first recorded in Malaysia on 1925 (Loke *et al.*, 1992) and occurs widely in both hot and cold area. *Plutella xylostella* is the worst problem in tropical and subtropical since the cultivation of Crucifers is year round and short life cycle makes this pest continued to be a great threat to production (Grzywacz *et al.*, 2009).

2.4.1 Life cycle

The development stages of *P. xylostella* from egg to pupal are 25 to 30 days depending on the weather and temperature where when temperature increases, the life cycle was shorten (Talekar & Shelton, 1993). Larvae are pale yellow-green to green colour covered with good hair scattered and tense. It bore into the leaves and start mining leaf tissue after hatching from eggs. The larvae moult three times depends on temperature and food availability. At maturity, the larvae are cigar-shaped with approximately of 12 mm long. These larvae are easily identified through active response when disturbed with moves backward and when fall from the plant it will be suspended by a silk thread and very active at dusk and night (Talekar & Shelton, 1993). The larva pupates in a fine, white, open-mesh cocoon attached to the leaves, stems or fruit of the host plant. At first, the pupae are green but as they mature, they become brown as adult butterflies will look through the cocoon (Capinera, 2012).

The natural enemies of this pest can be found during the larval, prepupal and pupal stages. At these stages, they can be killed by parasitiods such as *Microplitis plutellae* (Muesbeck) (Hymenoptera: Braconidae), *Diadegma insulare* (Cresson) (Hymenoptera: Ichneumonidae), and *Diadromus subtilicornis* (Gravenhorst) (Hymenoptera: Ichneumonidae) (Capinera, 2012). In Malaysia, the introduction of parasitoids to kill the pest is not successful due to farmers more prefer insecticides (Talekar & Shelton, 1993).

Intercropping between Crucifers crops with other type of crop, use overhead irrigation where caused the pest's larvae drowning or physically evicted from the surface of the crops, crop rotation and clean cultivation are a few of the cultural practices claim to be quite successful in controlling *P. xylostella* (Talekar & Shelton, 1993).

2.5 Wood vinegar

2.5.1 Process of making vinegar

Wood vinegar or pyroligneous acid is a dark liquid obtained from all oil and liquid contents in plants. This is a by-product produced from charcoal production when the organic materials undergo carbonization (Food and Fertilizer Technology Center, 2005).

The process to produce wood vinegar is known as pyrolysis. The overall process of wood pyrolysis, according to Roberts (1971) is as follows: at around 160°C, all removal of moisture (dehydration) was complete; at around 200 - 280°C, all the hemicelluloses decomposes, producing the volatile products (carbon dioxide and monoxide, and condensable vapours); from 280 - 500°C was decomposition of cellulose and reaching a peak around 320°C; the products were again predominantly volatiles. The decomposition of lignin increases rapidly at temperatures beyond 320°C. Meanwhile, according to Sinha *et al.* (2000), pyrolysis of wood was usually started at 200°C and hold up to 400 - 500°C, depending on the species of the wood species. The end products that obtained from this process are vinegar which condensed from the smokes produced.

2.5.2 Components

The wood vinegar has approximately 10 – 20% organic compounds, and 300 chemicals compounds such as furan compounds, various phenol compounds and organic acid. The carboxylic acids give pungent and rancid smell, and syringol (phenol compound) gives pungent smell palliative (Yokomori, 2011). The acetic acid is the largest component of acid portion and also for wood vinegar (Yatagai *et al.*, 2002). Due to the large portion of acid, the pH of wood vinegar is around 2 – 3 (Yokomori, 2011).

2.5.3 Usage of vinegar

According to Apai & Tongdeethare (2001), and Pangnakorn (2008), vinegar can help to improve the soil quality, kill pests, accelerates plant growth (in roots, stems, tubers, leaves, flowers and fruit) and acting as plant growth regulator. In term of killing pests, Yatagai *et al.* (2002) shows that acetic acid in vinegar can kill termite. Meanwhile, Pangnakorn *et al.* (2012) found that the *Musca domestica* larvae are susceptible to vinegar treatments compared to adults.

2.5.4 Insect control

Since the marrangin and azadirachtin have similar biological effect, the *A. excelsa* vinegar may have potential in controlling *P. xylostella* larvae as neem products do. The effect of *A. indica* extracts to the larvae include disrupting or inhibiting the development of larvae, blocking the moulting of larvae or nymphs, repelling larvae and adults, poisoning larvae and adults, deterring feeding and caused metamorphosis incomplete at various stages (Anon., 1992) .

CHAPTER 3

MATERIALS AND METHODS

3.1 Preparation of *Azadirachta excelsa* vinegar

The branches of *A. excelsa* were taken from an *A. excelsa* tree located near the Botanical Garden in Universiti Putra Malaysia Bintulu Sarawak Campus. The branches were cut to 3 cm length and 1.5 – 2.5 cm in diameter. The samples then were air-dried for one week before dried into a furnace (a pyrolysis process). The furnace (Carbolite®, ELF model) used, was modified to collect and condense smoke to liquid form (Figure 1). The temperature was increased at a rate of 1.4°C min^{-1} from ambient temperature to final temperature at 550°C, to get maximum yield as described by Ratanapisit *et al.* (2009).

The smoke released from the furnace was channelled into a pipe. A small hole was made at the end of the pipe. The smoke was cooled with wet towel to condense it. The yield produced was collected from the small hole into a beaker. The smoke collection was started at the beginning of carbonization and continued until the smoke was clear. Then, the yield collected was left for 24 hrs to get the vinegar.

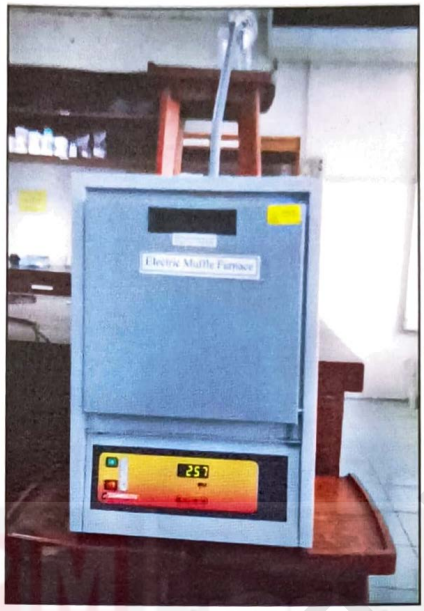

Figure 1: Modified method to collect vinegar from furnace

3.2 *Plutella xylostella* larvae

Brassica campestris var. parachinensis (or Sawi bunga) seeds were sown in 20 pots of mixed media (5:3:2 of Top soil: Cocopeat: Sand). The pots were put in roofed area until the vegetables were grown (Figure 2). After second week, the vegetables were placed in Share Farm II of Universiti Putra Malaysia Bintulu Sarawak Campus area to attract the *P. xylostella* (Figure 3). Third instars of larvae were collected to be used in the experiments.

Figure 2: Pots under roofed area

Figure 3: Pots placed in open area on the farm

3.3 Antifeedant tests

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

The larvae body weight were weighed and recorded, then starved for three hours before the testing. Leaves were dipped in different solution for 5 minutes and air dried until the solution on leaves is completely dry. All the leaves were then place individually in Petri dishes containing 10 larvae each. The experiments were carried out for 24 hours.

Table 1: The treatments used and its description

Treatment	Description
Control	Distilled water
400X	The vinegar dilute with water at ratio 1:400 (2.5 ml vinegar + 997.5 mL distilled water)
200X	The vinegar dilute with water at ratio 1:200 (5.0 ml vinegar + 995 mL distilled water)
100X	The vinegar dilute with water at ratio 1:100 (10.0 ml vinegar + 990 mL distilled water)

At the end of the experiments, the amounts of leaves consumed by the larvae were measured using LiCor 3100 Area Meter and recorded. The number of survivors and the larval body weight also were recorded. The larvae then were transferred to other

Petri dishes containing untreated leaves where they were kept until pupated. The numbers of adults' subsequently emerging and physical observations were recorded.

3.4 Statistical analysis

Data were subjected to Analysis of Variance (ANOVA) using SAS version 9.2. The percentage data was subjected to Log transformation before ANOVA. Mean differences were separated using Duncan's New Multiple Range tests. EC (effective concentration causing inhibition of larval growth relative to the control) and DC (concentration causing feeding deterrence compared with the control) values also calculated.

CHAPTER 4

RESULTS

4.1 *Plutella xylostella* infestation

The crops were attacked by the pest after 26 days in the field. The slow attack by *P. xylostella* may be due to weather condition where at the time it was raining season. The low *P. xylostella* population in the area may also be a factor contributing to slow infestation as the Share Farm II is not practising continuous cultivation of crucifers and cabbage. According to Ooi (1986) the presence of *P. xylostella* on the crops depend on weather (high presence is on 25.2° - 35°C of temperature according to Ahmad & Ansari, 2010) and type of *Brassica* vegetable. There was no larvae of *P. xylostella* observed in the afternoon and also can be seen at dusk and early morning.

4.2 Morphological changes of larvae

The time for third instars larvae used in this study to become adult were about 2.3 – 4.8 days, where the time for larvae (Figure 5 and 6) to become pupae takes 1.8 – 2.3 days, and pupae to adult (Figure 7) take about 4 days (Table 2). Adult mortality occurred within 1 – 2 days after the pupae become adult. The dead were usually adults that suffer

adults that suffer from defects such as wings do not develop properly and body size is too small (Figure 4). The normal adult of *P. xylostella* can survive up to five days even without food.

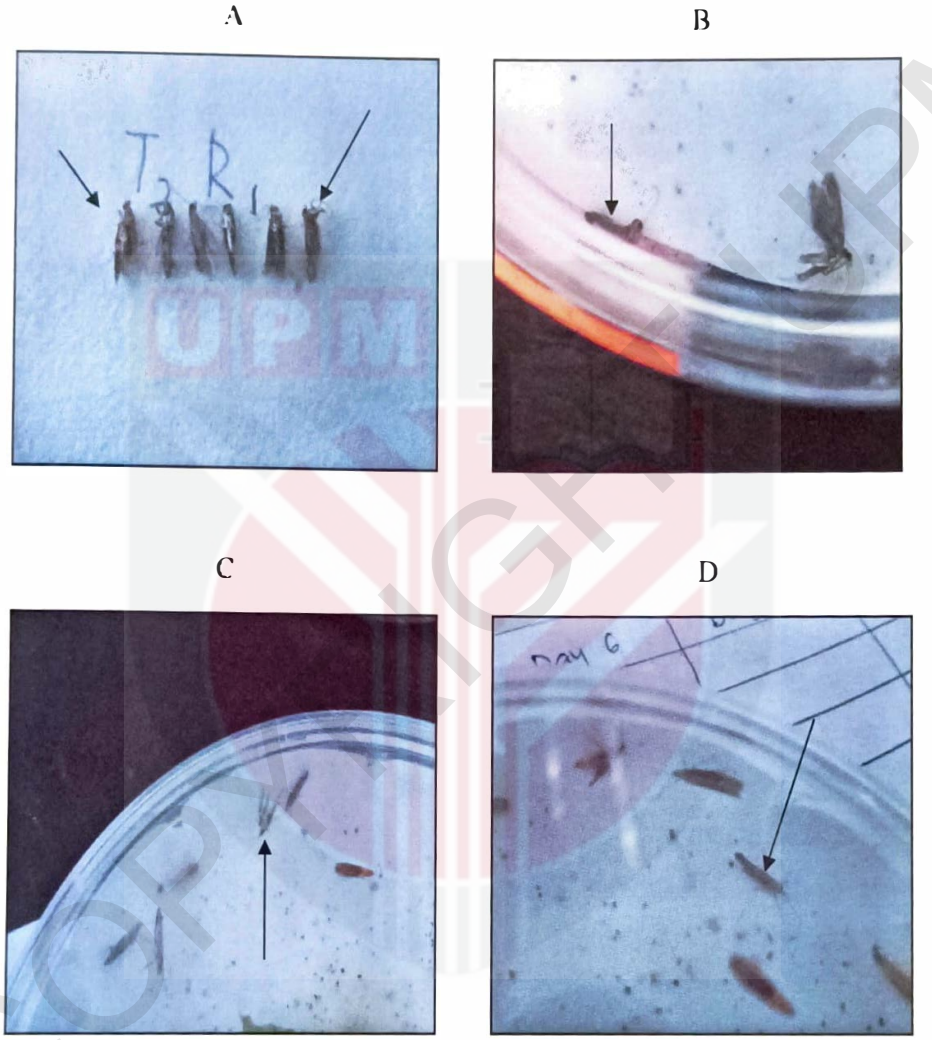

Figure 4: Defects occur on insect after emerged as adult (with arrow). A. Legs and wings do not grow properly; B. No wings developed; C. Small wings and; D. Size of insect is smaller

Figure 5: Larva after 12 hours of experiment

Figure 6: Larva pupated in cocoon

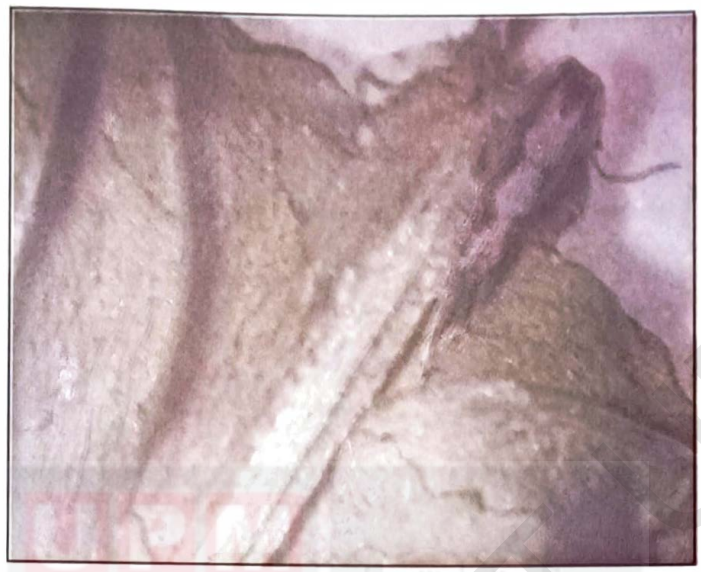

Figure 7: The adult emerged

Table 2: The period of time larvae and pupae before emerged to next life stage

Treatment	Larvae (day)	Pupae (day)
Control	2.0[a]	4.8[a]
400X	1.8[a]	4.5[a]
200X	2.3[a]	4.5[a]
100X	2.3[a]	4.5[a]

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

4.3 Leaf consumed and larvae weight

Result of leaf consumed and larvae weight gain for all treatment are shown in Table 3. Significance effect was observed for leaf consumed. The control recorded highest percentage of leaf consumed. However, the larvae weight gain shows no significant

difference among treatments. The lowest value was observed in 100X treatment for both parameters.

The highest values of EC (effective concentration causing inhibition of larval growth relative to the control) and DC (concentration causing feeding deterrence compared with the control) were recorded by 100X (Table 3). While 200X was recorded as lowest value for EC and 400X for DC.

Table 3: Leaf consumed, gain in larvae weight, effective concentration and deterrence concentration 24 hours after treatment

Treatment	Leaf consumed (%)	Larvae weight gain (%)	EC (%)	DC (%)
Control	29.51[a]	18.34[a]	-	-
400X	20.18[b]	12.45[a]	32.0	30.4
200X	17.78[b]	12.74[a]	30.5	38.5
100X	15.49[b]	8.71[a]	52.5	47.5

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

4.4 Mortality

The mortality rate of larvae, pupae and adult are shown in Table 4. The control has significantly lower mortality as compared to other treatments. There was no different among the treated samples. 400X was recorded as the highest percentage of larval mortality. While for pupae, the highest percentage of mortality occurs by applying

100X treatment. The highest mortality rate for adult was recorded when treated with 400X.

Table 4: The mortality rate of *Plutella xylostella*

Treatment	Larval (%)	Pupae (%)	Adult (%)	Total (%)
Control	0^{b}	2.5^{b}	0^{b}	2.5^{b}
400X	15.0^{a}	17.7^{a}	17.9^{a}	42.5^{a}
200X	10.0^{a}	19.4^{a}	10.3^{ab}	35.0^{a}
100X	10.0^{a}	33.3^{a}	8.3^{ab}	45.0^{a}

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

4.5 Adult size

The lengths of adult of all treatments are shown in Figure 8 and Table 5. The control has significantly larger body size as compared with other treatments. There is no adult size different among treated sample.

Table 5: Adult size of *Plutella xylostella* at the end of experiment

Treatment	**Size (mm)**
Control	6.20^{a}
400X	5.18^{b}
200X	4.95^{b}
100X	5.25^{b}

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

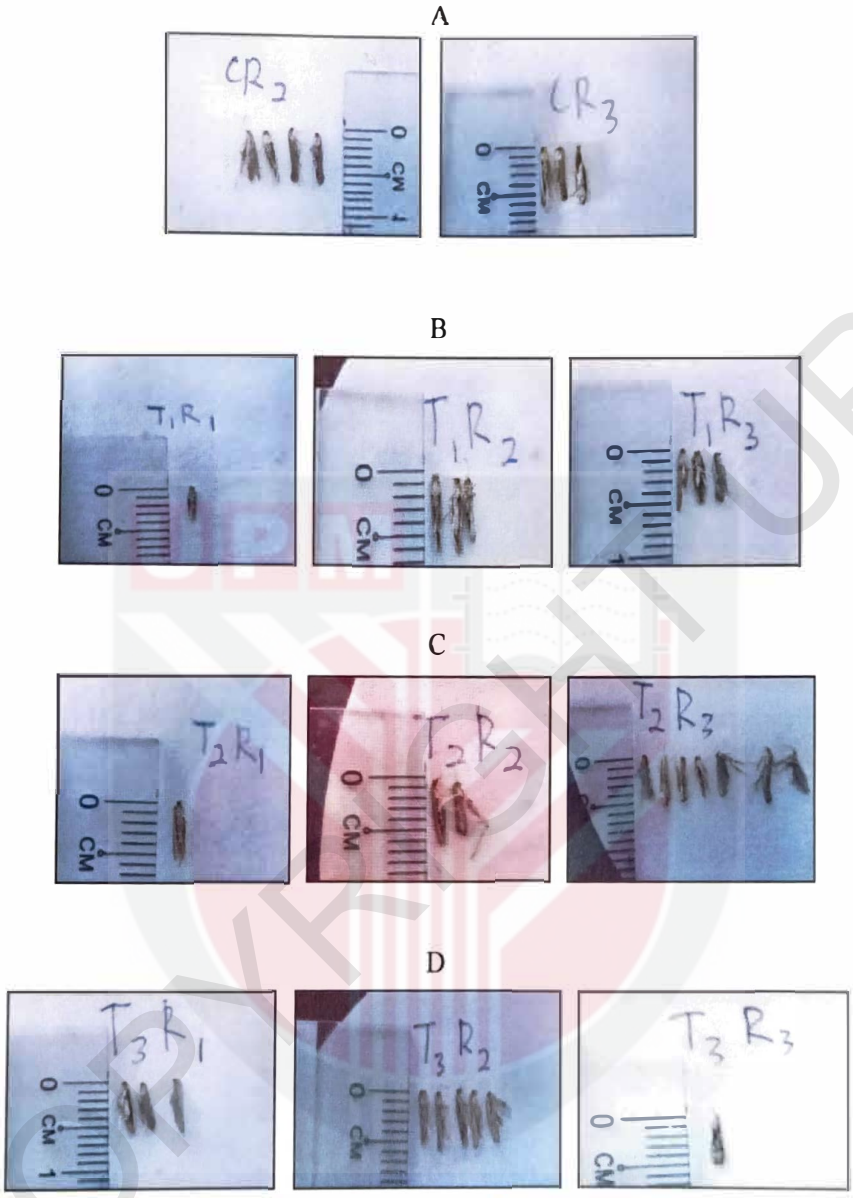

Figure 8: Adults of *Plutella xylostella*, A. Adults subjected to control; B. Adults subjected to 400X; C. Adults subjected to 200X; and D. Adults subjected to 100X

CHAPTER 5

DISCUSSION

5.1 Antifeedant tests

Consumption of leaf by larvae is reduced as the concentration of vinegar increases. It might be due to primary antifeedant effect on larvae. This primary antifeedant is inability of the insect to ingest food on certain level because of sensitive to secondary compound in their diet that eventually caused the feeding appetite inhibited (Schmutterer, 1985). Azadirachtin from *A. indica* have proven to have the ability to inhibit the feeding appetite of many insect species. Marrangin, an analogue of azadirachtin in *A. excelsa*, was also found to have higher efficacy than azadirachtin on feeding deterrent of insect (Hummel *et al.*, 2011).

The feeding appetite of larvae are rely on neural input from insect chemical sense such as taste receptor on tarsi, mouthparts and oral cavity, and central nervous integration of 'sensory code' (Mordue (Luntz) & Nisbet, 2000). Thus, marrangin in vinegar treatments stimulates specific "deterrent cells" in chemoreceptor of larvae, disrupt and block the "sugar" receptor cells that usually stimulate feeding of larvae (Mordue (Luntz) & Nisbet, 2000). Furthermore, study on insect also found that Lepidopterous pests are very sensitive to azadirachtin compared to other insect orders (Anon., 1992). The DC' value has increased in accordance with increasing of vinegar

concentration u ed. This shows that the higher concentration of vinegar used the more active compound of marrangin in treatment to inhibit larval feeding activity.

In term of weight gain there is no significant difference between all treatments. However it is believed that if using high concentration there will be a significant effect on larvae weight gain. This was supported by Akhtar & Isman (2004) where there found that azadirachtin in *Melia volkensii* (family Meliaceae) can inhibit efficiently larval growth of *P. xylostella* relative to the control at 9.1 ppm. The antifeedancy occurred with reduction of food intake and digestion efficiency of larvae (Mordue (Luntz) & Nisbet, 2000).

5.2 Mortality

In term of larval mortality, treatments with vinegar have significant difference as compared to the control. This might due to the feeding appetite inhibition and disability to ingest food due to sensitivity of the vinegar compound caused starvation and larvae eventually die. The percentage of larval mortality was low similar to the reported by Anon (1992). They stated that azadirachtin affect more profoundly on insect growth than feeding inhibition.

Significantly higher mortality rate as compared with the control also have been observed in pupae. The pupae mortality may due to insect growth regulation which affects them physiologically. Compounds in wood vinegar may interfere with endocrine system (Mordue (Luntz) & Nisbet, 2000) such as disrupted larvae homeostasis,

behaviour, insect growth, development and other physiological activities (Meyer, 2006). Thus, it controls larvae growth and moulting. The main target is insect cuticle where the compound disturbed neurosecretory system of insect brain and caused morphogenetic peptide hormones and allatostatins are blocked from released. These in turn inhibit the prothoracic glands and corpora allata functions. Moulting hormone from prothoracic glands is inhibited and new cuticle formation and ecydsis in turn will also inhibit (Mordue (Luntz) & Nisbet, 2000). The pupae cannot emerge to adult, and eventually will die. Morgan (2009) also reported similar finding where azadiracthin from *A. indica* disrupted and inhibited larvae and nymph growth, become permanently pupae and eventually dies.

Only 400X recorded a significant different when compared to the control in term of adult mortality. There was no different observed for 100X and 200X when compared with the control. The result showed that insect growth was allowed to continue (larvae to adult) at lower concentration and higher mortality rate only occurred at the adult stage. The dead may due to effect of secondary antifeedant and insect growth regulation which resulted from incomplete metamorphosis of pupae to adult.

The final mortality also showed similar result as compared to different stages of growth. The mortality is resulted from a few different processes. Larval mortality is highly affected by feeding inhibition (primary antifeedancy) due to feeding appetite blocked. Because of that, larvae suffer starvation and eventually die. While for pupae and adult, the mortality are due to secondary antifeedant and growth regulation. Food consumption of insects reduced caused the insects become weak and growth inhibited

due to interference in endocrine system. This eventually led to pupae and adult mortality.

5.3 Adult size and morphological changes

Even though the adult mortality rate is less than 20%, some of the pupae that emerged to become adults are smaller than their normal size (the control). This finding is similar to Mordue (Luntz) & Nisbet (2000) result where azadirachtin treatment on *Oncopeltus fasciatus* caused growth reduction and insect become smaller. Capinera (2012) stated that normal size of *P. xylostella* was about 6 mm, however the size of adults treated with wood vinegar in this study are less than 5.3 mm. This also may due to influence of compounds in wood vinegar on insect growth regulation. Even though the endocrine systems of larvae have disrupted, some of the larvae still can able to develop to become adult. However, the adult emerged become smaller due to moulting process not occur properly. It is as well as the defects occur in adult. The adult defects are such as incomplete wing and leg, and some has no wing at all. This result is supported by Mordue (Luntz) & Nisbet (2000) where they found that azadirachtin can cause abnormalities on *Schistocerca gregaria* and lead to death. Since endocrine system disrupted by compounds in wood vinegar, it also interrupt the prothoracic glands which is serves as secreting ecydysteroid (moulting hormone) for epidermal moulting process. Thus, it caused the adult emerged from pupae has defect.

The times taken by larvae to change their morphology do not influenced by the treatments. So, this study found that *A. excelsa* vinegar do not affect the morphological

developmebty period of larvae to become pupae to become adult. This finding however in in contrast with results reported by Schmutterer (1988), Mordue (Luntz) & Nisbet (2000) and Hummel et al. (2012) that showed azadirachtin caused delay growth development such as moulting process and larvae take longer time to become adult.

CHAPTER 6

CONCLUSION

Azadirachta excelsa vinegar is found to have potential in controlling *P. xylostella*. The active compound contained in *A. excelsa* vinegar inhibited the consumption of leaf, and caused mortality to larvae, pupae and adult. The reduction of leaf consumed resulted from primary antifeedant effects. The larvae mortality is caused by indirect effect of primary antifeedancy where the larvae starve and eventually die. The pupae and adult mortality are continuous from reduced larvae weight gain effect (secondary antifeedant effect), which eventually caused to death. Adult size also affected by application of treatment where *P. xylostella* adults emerged from cocoon is smaller than normal. In addition body defects also observed on adults. Based on this study, although values of EC and DC have not achieved 50%, dilution of vinegar at 400 times is good enough to control *P. xylostella*.

Further works are needed to fully understand the *A. excelsa* mode of action on insect pests. It is also recommended that further study using higher concentration of vinegar should be carried out to determine their effect on *P. xylostella*. A field experiment testing is recommended to determine the effectiveness of treatment in the field.

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PUBLICATION OF THE PROJECT UNDERTAKING

This is to certify that i have no objection to publish the project entitled **"Potential of *Azadirachta excelsa* vinegar to control *Plutella xylostella*"** by the supervisor in a joint authorship. However, it has to be evaluated by the Faculty of Agriculture and Food Sciencesd, Universiti Putra Malaysia Bintulu Sarawak Campus and published in the form of approved by the Faculty.

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Date: 27/06/13