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Effectiveness of neem seeds extract to control diamondback moth (*Plutella xylostella*)

Efektivitas ekstrak biji nimba untuk pengendalian ulat kobis (Plutella xylostella)

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ABSTRACT

Synthetic insecticides have detrimental effect to environment. Botanical insecticides are considered to be safer to the environment. Need seeds contain compounds with insecticidal properties. The aim of this study was to examine the effectiveness of neem seeds extract to control Diamondback moth (DBM). A series of neem seeds extract concentration (0.04 %, 0.08 %, 0.16 %, 0.32 %) and control (without neem extract) were tested on second instar larvae in oral test and contact test. The results showed that neem seeds extract have significant effect on the mortality of second instar DBM larvae 24-72 h after treatment in oral test and 72 h after treatment in contact test. In the oral test, the neem seeds extract significantly reduced feeding intensity of the larvae 42 h and 72 h after treatment. Subsequently, the extract also significantly reduced the moth emergence. The neem seeds with concentration 0.16 % effectively control DBM second instar larvae 72 h after treatment.

Key Words: neem seeds extract, diamondback moth, effectiveness

ABSTRAK

Insektisida sintetis memiliki efek merugikan bagi lingkungan. Insektisida botani dianggap aman untuk lingkungan. Biji nimba mengandung senyawa dengan sifat insektisida. Tujuan dari penelitian ini adalah untuk mengetahui efektivitas ekstrak biji nimba untuk mengendalikan ulat kobis Plutella xylostella. Serangkaian konsentrasi ekstrak biji nimba (0,04%, 0,08%, 0,16%, 0,32%) dan kontrol (tanpa ekstrak nimba) diuji pada larva instar kedua dengan uji pakan dan uji kontak. Hasil penelitian menunjukkan bahwa ekstrak biji nimba berpengaruh nyata terhadap mortalitas larva instar II 24-72 jam setelah perlakuan pada uji pakan dan 72 jam setelah perlakuan pada uji kontak. Pada uji pakan, ekstrak biji nimba secara nyata mengurangi intensitas makan larva 42 jam dan 72 jam setelah perlakuan. Selanjutnya, ekstrak juga secara nyata mengurangi munculnya ngengat. Ekstrak biji nimba dengan konsentrasi 0,16% efektif mengontrol ulat kobis instar kedua 72 jam setelah perlakuan.

INTRODUCTION

Diamondback moth (DBM) Plutella xylostella L. is a major pest of Cruciferous crops, including cabbage, cauliflower, broccoli, radish, turnip, brussel sprout, Chinese cabbage, kohlrabi, mustard, rapeseed, collard, pakchoi, saishin, watercress and kale (Rueda & Shelton, 1997). The infestation of DBM will reduce the quantities and qualities of crop production. Cabbage that heavily infested by this pest failed to produce head and yield loses could reach 100% during dry season (Sudarwohadi, 1975). Therefore, failure in controlling this pest will reduce farmer's income.

The importance of DBM as a pest has increased over time. Control of this pest has depended primarily and heavily on insecticides. Thus, the intensive use of insecticides in Cruciferous crops over many years has led to the development of chemical resistance in DBM. Insecticide resistance in DBM was first reported in Indonesia in 1953 (Kao et al., 1989). In addition, resistance of DBM to major classes of insecticides, including organochlorines, organophosphates, carbamates, pyrethroids

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synthetic and insect growth regulators has been reported in many countries, such as China, Japan, North America, Malaysia and Australia (Fauziah et al., 1990; Georghiou, 1983; Kao et al., 1989; Kobayashi et al., 1990; Ridland & Endersby, 1994; Shelton et al., 1993). Due to the conventional chemical control failures farmers are turning to Bacillus thuringiensis Berliner to control DBM. However, resistance to the microbial insecticides B. thuringiensis has evolved in field population. Resistance of DBM to B. thuringiensis has been found in Hawaii (Tabashnik et al., 1990).

With the ever increasing awareness of the safety of the environment, the farmers became interested in using botanical insecticides for pest control. Botanical insecticides are environmentally friendly because rapidly degraded in nature and relatively safe to non-target organisms. One of botanical insecticide that has been widely used and developed is derived from neem seeds. Active ingredients found in neem seeds is azadirachtin which can serve as feeding deterrent, insect growth regulator and inhibitor, larvicidal, ovicidal, and insect reproduction degradation (Schmutterer, 1988; Grainge & Ahmed, 1988; Blaney & Simmonds, 1995; Mordue & Nisbet, 2000; Irigaray et al., 2010). Application of insecticide containing azadirachtin on Grape berry moth (Lobesia botrana Denis and Schiffermüller) resulted in reduced fecundity and fertility, decreased eggs eclosion and larvae unable to molt properly and deformities (Irigaray et al., 2010). Other study showed that application of neem seeds extract and synthetic azadirachtin orally on Plutella xylostella delayed the development of a proportion of surviving larvae but no morphogenetic abnormalities were observed in larvae which reached pupation (Verkerk & Wright, 1993). Insecticidal testing may have different result although it is tested in the same species but different strain. This could happen because some strain (populations) are exposed to different insecticides that may lead to resistance to certain chemicals. Therefore, it is important to test the effectiveness of neem seeds extract against local DBM larvae, so it can be used as an alternative control of this pest.

MATERIALS AND METHODS

Insect culture

Diamondback moth larvae were collected from the field and fed with caisim (Chinese cabbage) until pupation. The pupae were collected and placed in a petridish then kept in a cage. Two weeks old Chinese cabbage plant was placed in the cage when the moth emerged, so the moth laid eggs on the plant. The plant was changed daily and kept in separate cage until the eggs hatched. Some larvae were used for experiment and some others were kept to maintain the DBM culture.

Neem extract preparation

Neem seeds extract were obtained from Bandung Institute of Technology Inter University Centre (PAU ITB) laboratory. The neem seeds extract was diluted with water to obtain a series of concentration of 0.04%, 0.08%, 0.16% and 0.32%.

Feeding tests

Chinese cabbage leaf was into 4×4 cm2 and dipped into water (Control) or neem seeds extract (0.04%, 0.08%, 0.16%, 0.32%, depending on treatment), air dried, then placed in a Petri dish lined with filter paper. Ten second instar (2 days old) DMB larvae were fasted for 3 hours before used as tested insects then placed in the Petri dish. Fresh clean leaf cut was added into the Petri dish, when previous leaf cut has consumed by the larvae. Larval survival and leaf area consumed by the larvae was measured at 24 h, 48, 72 h after treatment. Died larvae were removed from the Petri dish and the remaining larvae were kept until pupation. The time taken for pupation was recorded. The test was repeated five times.

Contact tests

Filter paper dipped into water (Control) or neem seeds extract (0.04%, 0.08%, 0.16%, 0.32%, depending on treatment), air dried, then placed in a Petri dish. Ten second instar (2 days old) DMB larvae were fasted for 3 hours before used as tested insects then placed in the Petri dish. Fresh clean leaf cut was added into the Petri dish. Larval survival was examined at 24 h, 48, 72 h after treatment. Died larvae were removed from the Petri dish and the remaining larvae were kept until pupation. The time taken for pupation was recorded. The test was repeated five times.

Statistical analysis

Data were analysed using a One-Way ANOVA followed by Post Hoc Multiple Comparisons with Duncan in SPSS 15.0 version.

RESULTS AND DISCUSSION

Application of neem seeds extract at feeding test significantly reduced diamondback moth (DBM) larvae survival. Survival rate of DBM larvae fed with leaf cut dipped into 0.32% neem seeds extract was significantly lower than those fed with leaf treated with lower concentration 24 h after treatment. Neem seeds concentration did not affect larval survival at 48 h after treatment. Survival rate of DBM larvae fed with leaf cut dipped into 0.32% neem seeds extract was similar to those fed with leaf dipped into 0.16% and was significantly lower than those fed with leaf treated with lower concentration 24 h after treatment. Larval survival decreased over time, after 72 h the survival rate of larvae treated with 0.16% or 0.32% neem seeds extract decreased by 50% than larval survival at 24 h after treatment, but larval survival of those treated with 0.04% or 0.08% neem seed extract only decreased by 25% after 72h (Table 1).

The low mortality of larvae 24 h after treatment showed that the neem seed extract has less potent effect as a stomach poison. Apparently, neem seeds extract acts as an anti feedant. This is in accordance with the opinion of Blaney & Simmonds (1995) and Grainge & Ahmed (1988) which states that azadirachtin contained in the neem seeds extract has anti feedant effect. Larval mortality occurred 72 h after treatment presumably because the larvae starved.

On contact test 24 h after treatment, the survival rate of larvae treated with 0.32% neem seeds extract was significantly lower than those treated with 0.08% or 0.04% neem seeds extract or control. However, after 48 h onwards, the survival rate of larvae treated with various concentration of neem seeds extract was no significantly different from control larvae. This showed that neem seeds extract work as weak contact poison toward DBM larvae, because the mortality rate was very low (Table 2).

Host plant acceptance by insect is determined by the response of gustatory organ and olfactory sensilla. Active ingredient of neem extract Azadirachtin is non-volatile; therefore, response of insect gustatory neurons receptors is critical in determining host. On several lepidopteran larvae, azadirachtin stimulate at least one deterrent neuron at maxillary styloconic sensilla that inhibit larvae to feed (Blaney & Simmonds, 1995). Effect of neem seeds extract as anti feedant can be examined from feeding capacity of DBM larvae after treatment. On feeding test, 24 h after treatment the amount of leaf consumed by the larvae fed with leaf treated with neem seeds extract at various concentration was not different from those eaten by larvae fed with clean leaf (control). However, after 48 h onward the feeding capacity of treated larvae was significantly lower than the control larvae. After 72 h, larvae that previously fed with leaf dipped into 0.32% neem seeds extract, significantly consumed less leaf than those fed with leaf treated with 0.04 % or 0.08% or fed with clean leaf (control), but consumed no significantly differ from leaf treated with 0.16% neem seeds extract (Table 3). On the other hand, anti feedant effect was not significant through contact. On contact test feeding capacity of DBM larvae released on Petri dish lined with filter paper treated with various concentration of neem seeds extract and fed with clean leaf was not significantly different from the control larvae that were not exposed to neem seeds extract (Table 4).

The application of neem seeds extract did not affect larval developmental time after treatment, either in feeding test or contact test (Table 5). The DBM larval developmental time ranged 5.6-6 days. This larval stage is shorter than those recorded by Kalshoven (1981), i.e. 7-11 days. This could be because the laboratory temperature was 30±1°C, which was higher than average temperature at highland where the cabbages usually were planted. At high temperature, life cycle of DBM will be shorter (Rueda & Shelton, 1997).

Diamondback moth larvae that survive after feeding test were successfully completed their life cycle. The moth emergence of those treated insects was not significantly different from untreated insects. However, not all larvae that survive after contact test could complete their life cycle. The moth emergence of treated insects in contact test was significantly lower than the control insects that were not contaminated by neem seeds extract. The application of 0.16%

and 0.32% neem seeds extract resulted in the lowest moth emergence significantly than those treated with lower concentration (Table 6). Moreover, moths that emerged from treated larvae had abnormal wings, so they cannot fly. This result showed that although Azadirachtin works as poor contact poison that causing high survival rate during contact test (Table 2), it has effect as insect growth regulator as suggested by Mordue & Nisbet (2000) and Irigaray et al. (2010).

CONCLUSION

Based on this study, it can be concluded that neem seeds extract have a potential to be used to control DBM through feeding and contact with the extract. Neem seeds extract can be applied at concentration 0.16% to have a significant effect.

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Table 1. Survival rate of Plutella xylostella larvae on feeding test 24 h, 48 h, 72 h after treatment.

Neem seeds extract concentration	Survival after treatment (%)		
	24 hours	48 hours	72 hours
0.04%	92.0 b	83.6 b	68.9 b
0.08%	92.0 b	79.3 b	67.1 b
0.16%	85.6 bc	68.5 b	33.1 c
0.32%	83.8 c	66.7 b	31.3 c
Control	100.0 a	100.0 a	100 a

Means followed by same letter within a column are not significantly different from one another at P≤ 0.05 (Duncan’s Multiple Rate Test).

Table 2. Survival rate of Plutella xylostella on contact test 24 h, 48 h, 72 h after treatment.

Neem seeds extract concentration	Survival after treatment (%)		
	24 hours	48 hours	72 hours
0.04%	100.0 a	98.0 a	93.0 a
0.08%	100.0 a	96.0 a	92.0 a
0.16%	96.0 ab	92.0 a	90.0 a
0.32%	94.0 b	92.0 a	88.0 a
Control	100 a	100.0 a	100 a

Means followed by same letter within a column are not significantly different from one another at P≤ 0.05 (Duncan’s Multiple Rate Test).

Table 3. Feeding capacity of Plutella xylostella on feeding test 24 h, 48 h, 72 h after treatment.

Neem seeds extract concentration	Leaf area consumed (mm^2/day/larvae) after treatment (%)		
	24 hours	48 hours	72 hours
0.04%	38.8 a	65.2 a	93.0 bc
0.08%	34.5 a	64.9 a	93.6 bc
0.16%	32.4 a	64.1 a	87.0 ab
0.32%	29.9 a	63.2 a	57.0 a
Control	63.8 a	79.0 b	119.3 c

Means followed by same letter within a column are not significantly different from one another at P≤ 0.05 (Duncan’s Multiple Rate Test)

Table 4. Feeding capacity of Plutella xylostella on contact test 24 h, 48 h, 72 h after treatment.

Neem seeds extract concentration	Leaf area consumed (mm^2/day/larvae) after treatment (%)		
	24 hours	48 hours	72 hours
0.04%	33.7 a	94.2 a	124.5 a
0.08%	27.3 a	93.7 a	122.5 a
0.16%	26.8 a	81.4 a	122.4 a
0.32%	26.6 a	81.3 a	122.3 a
Control	53.1 a	96.0 a	126.6 a

Means followed by same letter within a column are not significantly different from one another at P≤ 0.05 (Duncan's Multiple Rate Test)

Table 5. Larval developmental time after treatment

Neem seeds extract concentration	Larval developmental time (days)	
	Feeding test	Contact test
0.04%	5.8 a	5.6 a
0.08%	5.9 a	5.6 a
0.16%	6.0 a	5.7 a
0.32%	6.0 a	5.7 a
Control	5.7 a	5.6 a

Means followed by same letter within a column are not significantly different from one another at P≤ 0.05 (Duncan's Multiple Rate Test).

Table 6. Percentage of moth emergence after treatment

Neem seeds extract concentration	Moth emergence after treatment (%)	
	Feeding test	Contact test
0.04%	80 a	54 b
0.08%	74 a	46 b
0.16%	74 a	12 a
0.32%	70 a	10 a
Control	82 a	84 c

Means followed by same letter within a column are not significantly different from one another at P≤ 0.05 (Duncan's Multiple Rate Test).

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