Toxicological Effect of Lauric Acid Based Insecticide on the Reproduction System, Growth Development and Feeding Activity of Aphids, *Aphis gossypii* Glover

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Abstract: The toxicological effect of lauric acid based insecticide was investigated in terms of mode of action on aphids target system. Treated leaves were sprayed with different insecticides, which were lauric acid, cinnamaldehyde and malathion at 50μg/ml concentration to compare the effect of the pesticides on reproduction, growth development and feeding activity of aphids. The total number of new born nymphs produced and the relative development stage of nymphs were significantly reduced in all treatments compared to untreated leaves. Number of new born nymphs treated using lauric acid based pesticide was 6.0 ± 1.41 nymphs/day and the growth development rate at second day post treatment was rDS=1.07 ± 0.10. This data showed no significant difference with the data obtained when cinnamaldehyde and malathion were used as positive controls but the results were significantly different from the results obtained using untreated leaves (22.5 ± 3.54 nymphs/day, rDS=1.82 ± 0.02). Lauric acid was also shown to reduce the feeding activity of aphids. The study demonstrated that lauric acid was toxic to aphids. It has the ability to slow down the reproduction system, reduce growth development rate and decrease feeding activity of aphids (*Aphis gosyypii* Glover).

Keywords: *Aphis gossypii*, Lauric acid, Insecticides, Toxicological effect.

1. INTRODUCTION

Aphids, from the group *Aphis gossypii* Glover, is a species of plant pest that can pose serious problems to the agriculture industry. Aphids suck phloem sap from the underside of leaves. This behaviour causes leaf discoloration, result in direct damage to plants and may even stunt plant growth. Aphids also act as a major vector of plant viruses by excreting honeydew thus allowing fungi formation [1] and the transmission of virus borne diseases [2]. Currently, the most highly applied pesticide for the control of crop pests is chlorpyrifos phosphorothioate, a chlorinated organophosphorus pesticide, which is a broad spectrum pesticide with a 10-120 days half-life in the environment [3]. This pesticide is moderately toxic and can cause health issues [4]. Due to repeated treatments, aphids have become more resistant to this product. One way to reduce insecticidal resistant of aphids against chemical treatment and to control their rapid reproduction rate is by using natural treatment to delay or reduce pest population [5]. The natural compound needs to be environmentally friendly, safe to handle, biodegradable and potent for effective control [1]. Fatty acid is a natural insecticide. It has been reported to have low toxicity against vertebrates, to decompose easily in soil and to result in no resistance by target pest [6]. Lauric acid is a saturated fatty acid with a 12-carbon atom chain (medium chain fatty acid) that acts as an insecticide through both physical and chemical mode of action [7]. Physically, aphids will suffocate from topical application of the highly wettable lauric acid solution. In addition, Mohamad *et al.* [7] also suggested that ingestion of lauric acid might also result in the change in cuticle and cell permeability. Cell contents will leak out causing the insect to dehydrate and die. However, to date, there is no comprehensive study conducted on the chemical mode of action of lauric acid on aphids. This study was carried out as an attempt to determine the effectiveness of lauric acid against aphid productivity, growth development and feeding activity in comparison to conventional pesticides.

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2. MATERIALS AND METHODS

2.1. Chemicals

Lauric acid was purchased from Molekula (formerly Fluka, UK, England). Sodium lauryl ether sulphate was purchased from Ichem solution Sdn Bhd (Johor Bahru, Malaysia). Cinnamaldehyde and malathion were purchased from local pest shop at Johor, Malaysia.

2.2. Insects

The stock of adult Aphids (*Aphis gossypii*) used in this research was collected from a chili host plant (*Capsium annuum*) and maintained on young chili leaves in the area of Institute of Bioproduct Development (IBD), Universiti Teknologi Malaysia.

ISSN: 1927-3037/16

2.3. Lauric Acid Preparations

50µg/ml of lauric acid was mixed with sodium lauryl ether sulphate (SLES) and deionized water to formulate the pesticide formulation. 50µg/ml Malathion (organophosphate insecticide) and 50µg/ml Cinnamaldehyde (botanical insecticide) were used as positive controls.

2.4. Aphid Feeding Apparatus

The feeding apparatus was constructed using a petri-dish with a plastic cover, a piece of filter paper, cotton wool, rubber bands, a magnifying glass and a paint brush. The filter paper and paint brush were used to transfer the aphids and the magnifying glass was used to observe aphid activities. The apparatus and subsequent feeding methods were modified from Ahmed *et al.* [5].

2.5. Aphid Reproduction, Mortality and Growth Development

To study the effect of lauric acid and two conventional pesticides on the reproduction and growth development of aphids, chilli leaves were sprayed with the respective pesticide from all sides with a manual mist sprayer to the point of run off, then left to dry. Then, five adult aphids from the stock culture were caged under a plastic Petri-dish with one treated leaf. Numbers of new born nymphs were counted at 24 hours and 48 hours. The experiments were conducted in triplicate.

After 48 hours of treatment, adults were removed from the petri dish and the born nymph were caged again. The numbers of living and dead born nymphs were counted every 24 hours for 3 days post treatment. Then, percentages of mortality were calculated.

The length of the different immature stages and the relative developmental stage (rDS) were calculated according to equation 1 [8]. The effects of different formulations of pesticides on the fecundity of aphid adults, from nymphs reared till maturation, per cage were evaluated. For each treatment, 15 adult females were used, which were caged in 3 cages with 5 aphids per cage. The treatments were allowed to proceed for 24 hours.

$$rDS = \sum \frac{(nt\ Sp \times Fp)}{Nt\ S} \qquad \text{Equation 1}$$

nt Sp= number of individuals per development stage at time, t

Nt S = total number of individuals per cage

Fp= multiplication factor of relevant development stage (1st instar nymph) or 2 (2nd instar nymph) or 3 (3rd instar nymph) and 4 (4th instar nymph)

2.6. Effect on Feeding Activity

Five aphids from the stock culture were housed under a plastic petri-dish (35mm in a diameter) with one leaf that was treated with the insecticide. The bottom of the dish was covered with Whatman No 3 filter paper. The amounts of honeydew droplets produced were calculated by spraying with 0.2% ninhydrin reagent after 24 hours observation and compared with the positive and negative controls [1, 9].

2.7. Statistical Analysis

All experiments were carried out in triplicate and reported as mean ± SD (standard deviation). Data were analysed with SPSS 16.0 (SPSS Inc., Chicago, IL, USA) using one-way ANOVA for the result of aphids' growth development (rDS), reproduction and number of honeydew droplets. The mean of each measurement was analysed statistically using Tukey's and Duncan multiple range test with the negative control leaf treated with distilled water and positive control treated with conventional pesticides (Cinnmaldehyde and Malathion). The data are deemed significant if $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1. Effect of Lauric Acid on Aphids Reproduction, Mortality, Growth Development and Feeding Activity

To evaluate the efficacy of lauric acid pesticide towards aphids reproduction until adult stages, two commercial pesticides were used as positive controls and distilled water was used as a negative control. For each treatment, 15 female aphids (*Aphis gosyypii)* were used. There were 3 cages with 5 aphids per cage. *Aphis gosyypii* species may reproduce parthenogenetically throughout the summer and take a week to complete its development. Adult aphids can produced egg by sexual activity or directly producing nymphs by parthenogenesis technique without the presence of male [10]. The treatments were allowed to proceed for 24 hours. The numbers of nymphs produced were counted at 24 hours and 48 hours post

Table 1: Effect of Three Different Treatments on New Born Nymph Reproduction, 24 h and 48 h Post-Treatment (Result Shown as Mean ± SD)

Treatment	Aphid reproductions (number of new born nymph)	
	24 hours	48 hours
Lauric acid	6.0 ± 1.41[a]	7.5 ± 0.71[a]
Cinnamaldehyde	6.5 ± 0.71[a]	8.0 ± 0.00[a]
Malathion	5.5 ± 0.71[a]	7.5 ± 0.71[a]
Negative Control	22.5 ± 3.54[b]	33.5 ± 4.95[b]
F- value	35.215	52.359

Mean in the column followed with different letter(s) are significantly different at P ≤ 0.05 level of probability by Tukey's test.

treatment. Table **1** shows, the comparison of the treatment on aphids reproduction at 24 hour of treatment using Tukey's HSD test for multiple comparison (p=0.00067 @ p ≤ 0.5). The results showed that the treatment with distilled water (negative control) was the only treatment that resulted in a significantly higher reproduction of aphids compared to lauric acid, F (3, 4) = 35.21. 24 hours post treatment with lauric acid, only 6.0 ± 1.41 nymphs/day were produced. 6.5 ± 0.71 nymphs/day were produced following cinnamaldehyde treatment and 5.5 ± 0.71 nymphs/day were produced after malathion treatment. These values were significantly lower than negative control value of 22.5± 3.54 nymphs/day. These outcomes were similar to the ones reported in another study using neem formulation and Margosan-O [11].

These finding demonstrated that lauric acid negatively affected the reproduction of *Aphis gosyypii* similar to malathion and cinnamaldehyde. Other insecticides that resulted in the reduction of aphids population include thiamine [12] and neem [2]. This study supported the work by Mohamad *et al.,* [7] that lauric acid did not only kill aphids by physical mode of action but also through the chemical properties of lauric acid.

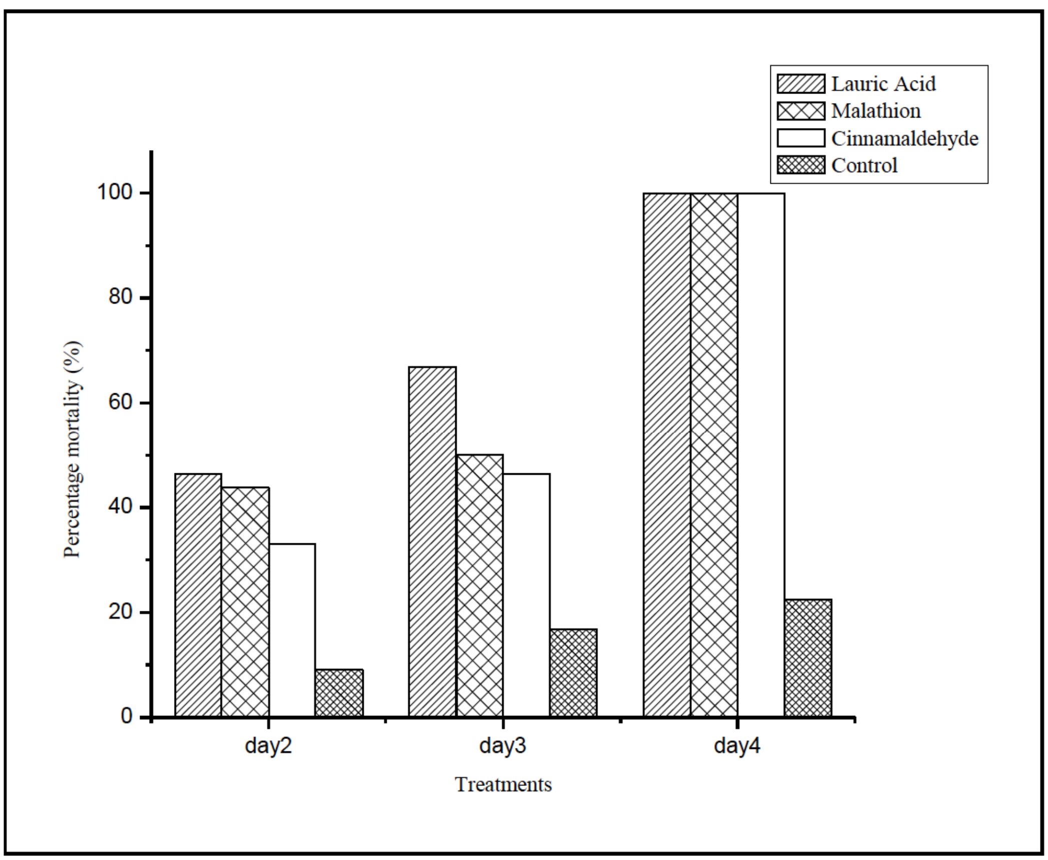

Figure 1: Effect of different pesticide at concentration of 50µg/ml on mortality of new born nymph.

To determine the sub-lethal effect of pesticides on nymph stages, the survivability of new born nymphs was assessed at day 2, day 3 and day 4 post treatment. The effect of lauric acid, malathion and cinnamaldehyde at 0.05% w/v on the mortality of nymphs is shown in Figure **1**. Leaves treated using lauric acid showed sub-lethal effect on nymph, at day 2 post treatment with the mortality percentages of 46%. The mortality rate was 67% at day 3 and reached 100% mortality at day 4 post treatment, respectively. Similar effects were observed with cinnamaldehyde and malathion treatment. The mortality rates of aphids fed with non-treated leaves were very low compared to when the aphids were fed with treated leaves. Toxicity of pesticide on nymphs increased with the increase in amount of feeding time. At day 4 all aphids fed with treated leave died due to time dependent exposure to the pesticides.

The mortality rate of insect was directly correlated to insecticide concentration and feeding time [11,14]. Some insecticides resulted in the inhibition of growth and molting process at the nymphal stage [1]. Aphids at nymphal stages normally take 7 days in average to complete their cycles before entering adult stage [13]. The results in this study showed that after 72 hours of exposure to the treated leaf, 100% mortality of aphids was reached. Lauric acid treatment seemed to shorten aphids' life span to 4 days.

In addition, the evaluation of the toxicity of insecticides on growth development of the aphids was also conducted. Mortality of born nymphs was correlated to the growth development at each cycle. Aphids have four nymphal stages [13]. Relative development stages (rDS) of aphids at day 2, 3 and 4 post treatment were calculated and presented in Table **2**. Lauric acid treatment showed significant reduction on growth development of nymphs at day 2 and day 3 with the lowest value of rDS of 1.07±0.10 and 0.99±0.19, respectively. At day 4 post treatments no growth of aphid was detected due to total mortality of aphids. The result was comparable among the three pesticides investigated. This work is the first report that indicated lauric acid inhibited growth development of aphids when ingested and the results were comparable with commercial pesticides from the organophosphate class and botanical class [15]. Therefore, lauric acid has the potential to be used as an insecticide to reduce aphids' population by inhibiting nymphal development at day 3 instead of the normal period of 7 days required to enter adult stage.

Honeydew is a term for sugar-rich secretions by aphids as a result of their feeding on plant's phloem sap [16]. Honeydew droplet can be correlated to Aphid's feeding activity [17]. The result on the effect of pesticides on honeydew excretion of aphid is tabulated in Table **3**. Untreated leaves as a control showed the highest number of honeydew spots during 24 hours of treatment with 12.5±3.53 spots/day. Cinnamaldehyde treatment resulted in the lowest spots production in this study with 0.5±0.71 spots/day. A study reported by Brown and Emy [15] and also by Nauen *et al*., [9] showed that cinnamaldehyde was identified as a botanical pesticide and it inhibited energy production, which caused the pest to stop eating, moving and then finally dying. Similar result were obtained by Daniels *et al.* [18] who reported that the use of thiamethoxam resulted in low honeydew droplets excretion, indirectly affecting aphids performance.

Malathion and lauric acid treatment reduced honeydew spots excretion to 5.0±1.41 spots/day and 6.0 ± 1.41 spots/day, respectively. Cocker *et al.,* [19] reported that malathion was categorized in the organophosphate class of pesticides and the reaction was specific to the disruption of the nervous system which led to aphids mortality. This will not directly affect the feeding activity of aphids as was shown here.

This study shows the potential of lauric acid treatment in reducing the fecundity of aphids. Ahmed *et al.* [5] reported that neem treatment also showed the

Table 2: Relative Development Stage (rDS) of Aphids at 2, 3 and 4 Days Post Treatment

Treatment	Relative Development Stages (rDS)		
	day 2	day 3	day 4
Lauric acid	1.07±0.10[a]	0.99±0.19[a]	0[a]
Malathion	1.34±0.13[a,b]	1.61±0.15[a,b]	0[a]
Cinnamaldehyde	1.13±0.18[a]	1.5±0.53[a,b]	0[a]
Negative Control	1.82±0.03[b]	2.49±0.14[b]	3.10±0.05[b]

Mean in the column followed with different letter(s) are significantly different at (p ≤0.05) level of probability by Tukey HSD test.

Table 3: The Amount of Honeydew Spots Excreted by *Aphis gosypii* During 24h of Treatments

Treatment (0.05% w/v)	Honeydew droplet, spots/day (mean ± SD)
Lauric Acid	6.0±1.41[a,b]
Cinnamaldehyde	0.5±0.71[a]
Malathion	5.0±1.41[a,b]
Control (Untreated leaves)	12.5±3.53[b]

Mean followed by different letters within column are significantly different by Tukey test at P ≤0.05.

same outcome. Interruption of the molting process at nymphal stages can lead to growth development inhibition and finally increasing the mortality rate of aphids at a younger stage [20].

In general, fatty acid based insecticides are known to be less toxic to vertebrates and can degrade easily in soil [6,21]. This study shows that apart from physically suffocating insects, lauric acid can also affect aphids' reproduction system, interrupt aphid growth development and reduce aphids feeding activity, thus leading to aphid mortality.

4. CONCLUSION

Lauric acid has been shown to act on insect physically ie by suffocating the insect and quite possibly change the insect's cuticle and cell permeability [7]. The findings in this study demonstrated that lauric acid can also affect aphid reproduction system, interfere with aphid growth development rate and reduce aphids feeding activity. This shows that lauric acid has the potential to be utilized as an effective natural insecticide.

ACKNOWLEDGEMENT

We would like to express our gratitude to Fundamental Research Grant (FRGS) Scheme by Ministry of Higher Education Malaysia for funding this research.

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Received on 15-03-2016 Accepted on 20-05-2016 Published on 04-10-2016

DOI: http://dx.doi.org/10.6000/1927-3037.2016.05.03.2