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Efficacy of Neem Oil on the Biology and Food Consumption of Epilachna Beetle, *Epilachna dodecastigma* (Wied.)

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ABSTRACT

The efficacy of neem oil on the mortality, growth and feeding responses of epilachna beetle showed that all the larval instars were susceptible to this oil. The LC_{50} values were higher at 3rd instar and it was lowest on 1st instar. The LT_{50} values of oil increases proportionately with increasing larval age and with decreasing oil concentration. Neem oil significantly prolonged larval and pupal periods and some of the treated larvae never reached to the pupae. Pupal recovery and adult emergence were greatly reduced in treated larvae. In addition, neem oil also reduced the food consumption of this beetle by acting as feeding deterrent.

Key words: Neem oil, mortality, growth, feeding response, *Epilachna dodecastigma.*

INTRODUCTION

Epilachna beetle, *Epilachna dodecastigma* (Wied.) is one of the devastating pests of vegetable crops in Bangladesh. It is fairly common throughout the country and causes a considerable damage to a number of solanaceous, cucurbitaceous and leguminous crops (Alam, 1969). Both adults and grubs are injurious and feeding upon the epidermis of the leaves resulting in drying and falling of the infested foliage.

Indiscriminate use of pesticides for the insect pest management results various environmental and ecological problems, which concern human welfare. In this situation, alternation or biodegradable substitutes are now being strongly conceived by all using chemical pesticides. At present researchers paid much emphasis on the biologically active indigenous plant products because they are environmentally safe, biodegradable and cost effective (Saxena *et al.,* 1980, 1981a). During the last few years, a number of investigators isolated and identified several chemical compounds from leaves and seeds of many plant species and screened out as insect feeding deterrents and growth inhibitors (Jacobson *et al*., 1975). Among them, use of neem as an insecticide is the most widespread and widely researched. Its insecticidal effect is known to work in various ways, like antifeedant, repellent, growth inhibitor, egg laying deterrent, impaired reproductive ability etc. (Schmutterer, 1995). Its biological efficacy has been proved against a wide range of agricultural pests since more than 20 years (Ostermann, 1993). However, researches on the use of neem products against vegetable pests are scanty in Bangladesh. The present study was therefore, undertaken to evaluate the efficacy of neem oil on the biology and food consumption of Epilachna beetle, *Epilachna dodecastigma* (Wied.).

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MATERIALS AND METHODS

The experiments were conducted in the laboratory of the Department of Entomology, Bangladesh Agricultural University, Mymensingh. The neem oil was purchased from various grocery shops of Mymensingh town. From the stock solution, a series of neem oil concentrations (0.25, 0.5, 1.0, 2.0 and 4.0%) were prepared mixing with distilled water containing 0.01% nonidet.

Food treatments

Fresh and healthy bean leaves were treated in different neem oil concentrations by leaf-dipping method. In the control, leaves were treated with distilled water containing 0.01% nonidet. The treated leaves were air-dried before offering to the insects. The cut end of the leaf petiole was covered with a water soaked cotton pad to prevent water loss from the leaves.

Mass culture of the test insect

In order to meet the supply of the test insect, a stock of large number of larvae and adult beetles of *E. dodecastigma* were collected from bean plants of the BAU campus. They were sexed and confined in the petridishes (9 cm dia) for mating and egg laying. Fresh and healthy bean leaves were supplied each day to provide food. After oviposition, adult beetles were transferred in different petridishes and the eggs were left undisturbed for hatching. Immediately after hatching, the larvae were transferred in several petridishes and reared up to adult emergence. This procedure was continued several times for obtaining large number of larvae and adult beetles.

Dose-mortality responses

The efficacy of neem oil on the mortality responses was evaluated by exposing larvae of each instar (1^{st}, 2^{nd}, 3^{rd} and final) separately in petridishes having neem oil treated bean leaves. The different concentrations were 0.25, 0.5, 1.0, 2.0 and 4.0% of neem oil. Twenty larvae were maintained in each petridish for each concentration. Control treatments were done simultaneously for each larval instar separately. Mortality data were recorded in every 24h.

Growth and feeding responses

For the treatment 2^{nd} instar larvae were used exposing them to treated foods at 0.25% neem oil concentration. The larvae were exposed either for 24h (24h treatment) or continued throughout the larval period (continuous treatment). Newly emerged 2^{nd} instar larvae were placed individually in petridishes containing treated food and 20 petridishes were maintained for each concentration. Larval, pupal period, percent pupation, adult emergence and food consumption were recorded.

In another experiment, the effect of neem oil on the food consumption of the adult beetles was evaluated. The leaves were changed at regular intervals of 24h. Ten newly emerged adults were individually exposed to treated (24h and continuous treatment) and also in untreated control. Food consumption was recorded upto 30 days. Square millimeter graph papers were used to measure food consumption for both larvae and adults.

Statistical analysis

The mortality data were analyzed by probit analysis originally designed by Finney (1971) using MSTAT Statistical package programme. The data on the growth and feeding response were analyzed by single factor following CRD. LSD test was done taking the probability level 1% as the maximum unit of significance.

RESULTS AND DISCUSSION

Mortality response

The LC_{50} values of neem oil for the 1^{st}, 2^{nd} and 3^{rd} instar larvae at 1, 2 and 4 days after treatment (DAT) were found 3.538, 2.799, 0.621%, 3.377, 2.830, 0.992% and 3.646, 3.225, 1.566% respectively. However, for the final instar larvae the LC_{50} values were found 1.684, 1.076 and 0.653% at 6, 7 and 8 DAT respectively (Table 1).

Table 1. Toxicity of neem oil on different larval instars of *E. dodecastigma* at different days after treatment

Days after treatment	LC_{50}(%) (95% fiducial limit)			
	1st	2nd	3rd	Final
1 DAT	3.538 (2.37-5.28)	3.377 (2.38-4.79)	3.646 (3.04-4.37)	-
2 DAT	2.799 (1.76-4.46)	2.830 (1.77-4.54)	3.225 (2.59-4.02)	-
4 DAT	0.621 (0.45-0.93)	0.992 (0.70-1.40)	1.566 (1.01-2.43)	-
6 DAT	-	-	-	1.684 (1.03-2.75)
7 DAT	-	-	-	1.076 (0.76-1.53)
8 DAT	-	-	-	0.653 (0.44-0.97)

Median Lethal Time (LT_{50})

The values for median lethal time (LT_{50}) of neem oil on *E. dodecastigma* larvae are presented in Table 2. The results of probit analysis showed that with the increase of concentration, the LT_{50} decreased proportionately. The LT_{50} values were 4.7, 4.7, 3.1, 2.5 and 0.6 days at 0.25, 0.50, 1.0, 2.0 and 4.0% concentrations, respectively for the first instar larvae (Table 2). These were 5.5, 3.7, 3.4, 3.0 and 0.6 days for the 2nd instar. The same were 5.5, 4.6, 4.0 and 1.0 days for the 3rd instar larvae, whereas those were 7.0, 5.5, 5.2 and 4.4 days in case of final instar larvae at concentrations of 0.5, 1.0, 2.0 and 4.0%, respectively (Table 2). The results revealed that the LT_{50} values gradually increased with the decrease of concentrations of neem oil.

Table 2. LT_{50} Values of neem oil for 1st, 2nd, 3rd and final instar larvae of *E. dodecastigma*

Concentration (%)	LT_{50} (in days)			
	1st instar	2nd instar	3rd instar	Final instar
0.25	4.7	5.5	-	-
0.5	4.7	3.7	5.5	7.0
1.0	3.1	3.4	4.6	5.5
2.0	2.5	3.0	4.0	5.2
4.0	0.6	0.6	1.0	4.4

Neem oil caused significant larval and pupal mortality of *E. dodecastigma*. Maximum mortality was observed in the continuous treatment, where cent percent larvae died before reaching to pupal stage. The recorded larval mortality were 10% for 3rd instar and 90% for the 4th instar larvae. In 24th treatment, 40% larvae were found dead at 4th instar. In the control, the total larval mortality recorded only 5% (Table 3). None of the larvae reached to the pupal stage in continuous treatment. The recorded pupal mortality was 10% in 24h treatment.

Table 3. Effect of neem oil on the larval and pupal mortality of *E. dodecastigma*

Treatment	Larval mortality (%)			Total larval mortality (%)	Pupal mortality (%)	Total mortality (%)
	II instar	III instar	Final instar			
Control	5	0	0	5	0	5
Continuous treatments	0	10	90	100	-	100
24th treatment	0	0	40	40	10	50

Islam and Islam (1988) showed that methanolic neem seed kernel extracts caused high larval mortality of *E. dodecastigma*. They also reported that mortality was dose dependent. Doll and Schmutterer (1993) showed that neem oil caused mortality of *E. varivesstis* during their metamorphosos. Similar effects were also recorded by Becker *et al.* (1992). Kareem (1981) observed 25% mortality in *Plutella xylostella* larvae when fed on leaves with 3% neem oil and high mortality induced at higher concentrations. Seventy seven to hundred percent mortality of *Sogatella furcifera* caused by neem oil was reported by Saxena *et al.* (1983).

Rajendran *et al.* (1998) reported partial control of *E. vigintioctopunctata* by using 4% neem oil. Karmarker and Bhole (2001) found a significant percent protection of *E. dodecastigma* upto 6 days using 2% neem oil. This oil was most effective at 48 and 72h post treatment.

Growth responses

The larval period was found longer on treated food. The longest larval period was observed in continuous treatment, whereas the lowest larval period was found in the control. In continuous treatment the larvae took an average of 6.3 days at final instar and none of them was able to pupate. The total larval periods prolonged up to 10.3 days. In 24h treatments the larvae took an average of 4.9 days at final instar compared to 2.8 days in control. The total larval periods prolonged up to 8.9 days compared to 6.8 days for the control. Duration of pupal period (4 days) was same both in 24h and in the control treatment (Table 4).

Table 4. Effect of neem oil on the larval and pupal development of *E. dodecastigma*

Treatment	Larval mortality (%)				Pupal period (days)	Pupal recovery (%)	Adult recovery (%)
	II instar	III instar	Final instar	Total			
Control	2	2	2.8 (2-4)	6.8 (6-8)	4	95	95
Continuous treatments	2	2	6.3 (2-12)	10.3 (6-16)	-	-	-
24th treatment	2	2	4.9 (2-12)	8.9 (6.16)	4	60	50

The results on the growth and development revealed that neem oil had marked influence on the mortality of the test insect. In the present study, continuous treatment showed highest mortality followed by 24h and the control treatment. Although larvae were treated at 2nd instar stage, however, highest mortality was occurred in final instar compared to those in 2nd and 3rd instar. The pupal recovery was recorded 60% in 24h treatment compared to 95% in the control. In case of adult recovery, adult emergence rate was less in 24h treatment than the control. The adult emergence was observed 50% in 24h treatment. On the other hand, 95% emergence occurred in control treatment.

The result of the present study indicated that neem oil significantly prolonged larval period and markedly reduced pupal and adult recovery. The results also indicated that usually the treated larvae were unable to moult and if moulted they took longer time to complete the larval instar. It can be assumed that the chemical compound, present in the neem oil might be responsible for the prolongation of the development period of the insects. Islam and Islam (1988) reported that when the larvae of *E. dodecastigma* were fed on methanolic neem seed kernel extracts, treated food resulted prolongation of larval period.

Mosaddeque (1995) observed that neem oil markedly affected the larval growth and development of *Spilarctia obliqua.* A high percentage of larvae died at almost all concentrations of the meem oil tested. He also reported that larvae survived after being fed on neem oil treated leaves, developed poorly and required longer developmental period than those fed on untreated control leaves. Consequently, total larval period was much higher when the larvae were fed on treated leaves than that of control. Some larvae failed to complete their larval period and died before pupation. Loke *et al.* (1990) reported that neem oil was responsible for the retardation of growth (prolonged larval stadia) of *Plutella xylostella.* The juvenomimetic properties of neem leaf extract were also reported by Ambika *et al.* (1981). Rani (1984) stated that neem seed oil delayed development causing larval pupal intermediates, deformed adults and poor emergence of *Corcyra cephalonica.* The findings of the present study were in agreement with findings of these researchers. Kumar and Babu (1998) revealed that Azal-T/S (1% azadirachtin) and Neem Azal-F (5% Azadirechtin) have adverse effects on the fecundity and moderate growth regulatory effects of *E. vigintioctopunctata.*

Feeding responses

Neem oil significantly reduced the rate of food consumption of the test insect when 2nd instar larvae were allowed to feed on treated food. The total food consumption was recorded 10,260 mm^2 and 28,880 mm^2 leaf area in continuous and 24h treatment respectively compared to 40,101 mm^2 leaf area in control (Table 5a). The lowest food consumption was observed in continuous treatment,

whereas the highest was in control. The food consumption gradually increased with the increase of larval age (Table 5a). In all the treatments neem oil was statistically identical regarding its effect on food consumption.

Table 5a. Effect of neem oil on the food consumption of different larval instars of *E. dodecastigma*

Treatment	Food consumption (in sq. mm) of each instar			Total food consumption (in sq. mm)	Mean ± S.E
	II	III	Final		
Control	2664	6248	31189	40101	2005.50±151.43
Continuous treatments	1721	2473	6066	10260	513.00±86.90
24th treatment	1781	3082	24017	28880	1444.00±159.11
LSD at 5%				386.12	
LSD at 1%				514.00**	

**Significant at 1% level

Neem oil significantly reduced the food consumption by the adult beetles in continuous treatment tested as compared to control. The total food consumption was 45356 mm^2 and 73603 mm^2 leaf area in continuous and the control treatments respectively (Table 5b).

Table 5b. Effect of neem oil on the food consumption of adult beetles of *E. dodecastigma*

Treatment	Total food consumption (in sq. mm)	Mean ± S.E
Control	73603.00	7360.30±460.77
Continuous treatments	45356	4535.60±405.51
LSD at 5%	1288.66	
LSD at 1%	1765.24**	

**Significant at 1% level

Haque *et al.* (1996) reported that neem oil significantly reduced the food consumption of third insar larvae and adults of epilachna beetle. In the present study, experiments on the effect of neem oil on the feeding responses also indicated that neem oil significantly reduced the food consumption by the larvae and adult beetles. Continuous treatment showed lower food consumption followed by 24h and control treatment. The lower food consumption of neem oil treated leaves might be due to the feeding deterrent action of neem oil. The feeding deterrent properties of neem oil has been reported by several researchers (Heyde *et al.,* 1984 and Islam, 1984). Saxena *et al.* (1981b) reported that the food consumption by the leaves of rice leaf roller was significantly reduced when they were fed on neem oil treated rice leaves. Shin Foon and Zhang (1984) reported that neem oil protected leaf discs by more than 97.5% from the larvae of fall armyworm. They concluded that feeding deterrent properties of neem oil protected the rice leaves from the insect pest. The reduction in feeding due to the presence of neem oil in the food was also reported by Ladd (1981) on Japanese beetle, *Popilla japonica* Newman. From this finding it can be concluded that neem oil has insecticidal, antifeedant and growth disrupting properties, which could play an important role in the management of this pest. However, the effectiveness of this oil needs to be improved for the benefit of agricultural sector.

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