EFFECT OF NEEM OIL AND NEEM LEAF EXTRACT ON MORTALITY, GROWTH AND FEEDING RESPONSES OF JUTE HAIRY CATERPILLAR, *Spilarctia obliqua* (Walker)

By

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This is to certify that the thesis entitled **"EFFECT OF NEEM OIL AND NEEM LEAF EXTRACT ON MORTALITY, GROWH AND FEEDING RESPONSES OF JUTE HAIRY CATERPILLAR, *Spilarctia obliqua* (Walker)"** submitted to the Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE IN ENTOMOLOGY,** embodies the result of a piece of *bona fide* research work carried out by Mohammad Didarul Islam, Registration No. 25241/00357 under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

I further certify that such help or source of information, as has been availed during the course of this investigation has duly been acknowledged.

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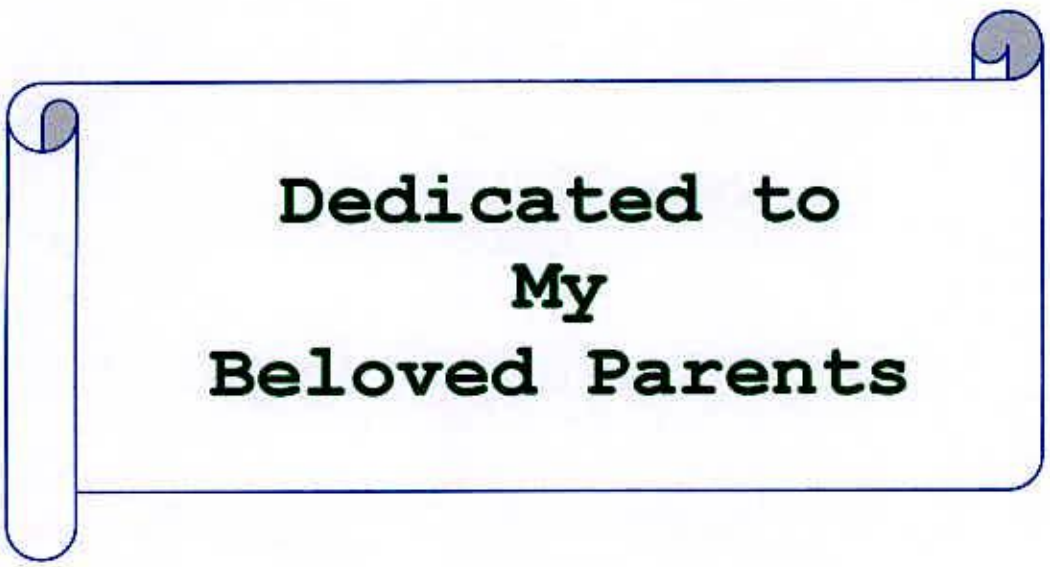
Dedicated to
My
Beloved Parents

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By

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ABSTRACT

Experiments were conducted in the Division of Entomology, Bangladesh Jute Research Institute, Manik Mia Avenue, Dhaka, to study the effect of Neem oil and Neem leaf extract on mortality, growth and feeding responses of jute hairy caterpillar, *Spilarctia obliqua* (Walker). To evaluate the efficacy of neem products on the larval mortality, third, fourth and fifth instar larvae were allowed to feed on jute leaves treated with neem extract (1:20) and neem oil at 0.5, 1.0, 2.5 and 5.0% emulsified with 0.1% Nikalin and their mortality were recorded at different days after treatment. Maximum percent larval mortality occurred at the higher two concentrations (2.5 and 5.0%) in each instar. Neem leaf extract (1:20) and lower concentrations (0.5 and 1.0%) of neem oil, significantly increased the larval mortality. The median lethal dose (LD_{50}) values of neem oil for different larval instars at above concentration were also determined. The effect of neem oil on growth and feeding responses were evaluated by exposing fourth instar larvae to food treated with neem oil at 0.5% concentration either for 48 hours (48 hrs treatment) or continuously throughout the larval period (continuous treatment).Neem oil exhibited growth inhibiting effects on the developing larvae and reduced the food consumption as compared to control. The effects were more pronounced in continuous treatment. Such effects on development were prolongation of larval period, reduction in the larval size, blackish spots on the larval body, hairless larvae and development of abnormal pupae. Adults developed from treated larvae were malformed or had crippled wings. Neem served as a potential growth inhibitor and feeding deterrent against *S. obliqua* and it can be integrated with appropriate cultural and biological pest control tactics.

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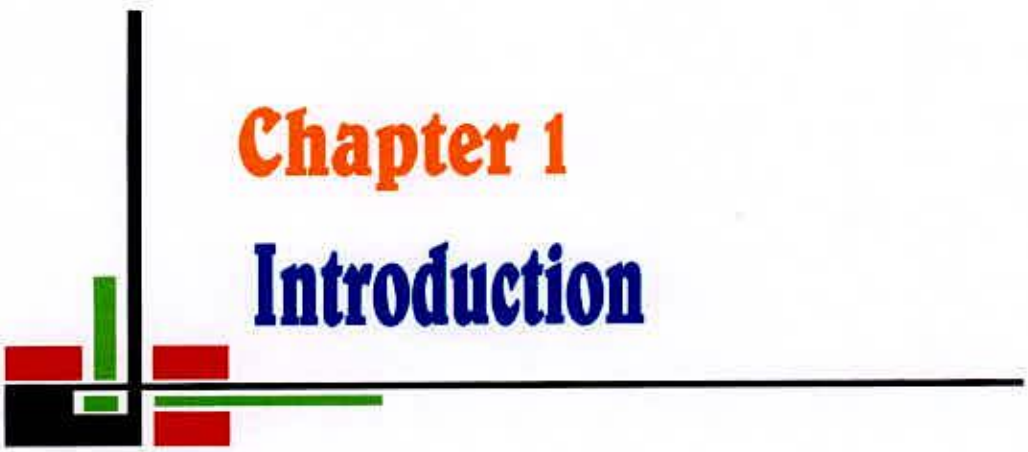
ACRONYMS

BBS	=	Bangladesh Bureau of Statistics
%	=	Percent
^{0}C	=	Degree Centigrade
BARI	=	Bangladesh Agricultural Research Institute
BJRI	=	Bangladesh Jute Research Institute
cm	=	Centimeter
CRD	=	Completely Randomized Design
CV %	=	Percentage of Coefficient of Variance
cv.	=	Cultivar (s)
CVL	=	Capsularies variety late
DAT	=	Days after treatment
DF	=	Degrees of Fredom
DMRT	=	Duncan's Multiple Range Test
Et al.	=	And others
etc	=	Etcetera
G	=	Gram (s)
Hrs	=	Hour(s)
Kg	=	Kilogram (s)
LC	=	Median lethal concentration
LD_{50}	=	Median lethal dose
LSD	=	Least significant difference
M^2	=	Meter squares
mg	=	Milligram
mm	=	Millimeter
No.	=	Number
ppm	=	Parts per million
SAU	=	Sher-e- Bangla Agricultural University
SD	=	Standard deviation
µg	=	Microgram
var.	=	Variety
Ml	=	Millilite



Chapter 1

Introduction

Chapter I

INTRODUCTION

Jute, a fibre crop of international eminence, is the most important cash crop and one of the major foreign exchange earners of Bangladesh. It is cultivated for its blast or phloem fibre, which yields the strongest and most durable fibre of commerce. Jute fibre is extensively used in the world for its versatility, durability and fineness as it is used for the production of newsprint, carpet making, hessians, gunny bags, ropes etc. Jute sticks are now used in making partex. Our agricultural community is dependent to a large extent on jute and jute products.

Jute is mostly grown in the Indo-Bangladesh region and in some countries of the Southeast Asia. It ranks second only to cotton among all the natural fibres in production (Talukdar *et al.* 1989). About 90 percent of world's jute is produced in Bangladesh and India (Atwal, 1976). With respect to production, Bangladesh ranks second among the jute growing countries of the world. The land and climate conditions of Bangladesh are congenial to the production of good quality jute. Deshi jute (*Corchorus capsularies* L.) and Tossa jute (*Corcorus olitorius* L.) belonging to the family Tiliaceae, are cultivated for fibre. In Bangladesh, about .4 m hectares were under jute cultivation for the year of 2004 and total yield was 4.3 m bales (Anon, 2004). Millions of people in Bangladesh earn their livelihood from agricultural and industrial activities based on jute. Moreover, 100 thousand traders and 250 thousand industrial laborers earn their livelihood from the jute business (Khandaker, 1987).

Jute is liable to damage by various insect and mite pests at all stages of its growth from seedling to harvest. All parts of the plants including flowers and seedpods are subjected to attack. About 40 species of insects and mites are considered as pests of jute in Bangladesh (Kabir, 1975). Due to their attack the

yield is greatly reduced and the quality of fibre is deteriorated. Among the jute pests, *Spilarctia obliqua* (Walker) is the worst one (Sharif, 1962; Alam *et al.*, 1964; Kabir and Khan, 1968).

Spilarctia obliqua popularly known as jute hairy caterpillar/Bihar hairy caterpillar, is one of the most serious polyphagous and widely distributed insect pests causing damage to a large number of cultivated as well as non-cultivated plant species (Kabir and Khan, 1969; Viswanath andGowda, 1975; Deshmukh *et al.*, 1976; Bhattacharya and Rathore, 1977). Besides Bangladesh, this pest has also been reported from India, Myanmar, Pakistan, China and many other countries of the world (Kabir and Khan, 1968; Singh and Sehgal, 1922).

S. obliqua attacks crops throughout the year, the infestation of jute by this pest begins in the month of April, when the plants are about two to three feet high, but heavy infestation occurs in the month of June- July. The adult female lays eggs in clusters on lower surface of the leaves and after hatching of eggs, the young caterpillars begin to feed on the lower epidermis of the leaves in clusters condition. The early damage of leaves assumes a peculiar membranous appearance. *S. obliqua* larvae pass through six instars (Adsule and Kadak, 1969). The first and second instars larvae are gregarious having different color patterns greenish yellow to dark orange (Islam and Sardar, 1971). The third, fourth, fifth and sixth instars larvae are dispersed over the entire field. The fifth instar larvae are the most damaging stage (Gyawali, 1988). The whole leaf tissues are eaten up by the caterpillars, leaving only the ribs and the plants may be completely defoliated. In the caterpillars appear in swarm, nothing but bare stems of jute remains in the field (Anon, 1939-40). Although the caterpillars prefer mature leaves, the top shoots are also eaten up in case of severe attack (Das, 1948). As a result of infestation, the plant growth becomes stunted and the yield is reduced considerably. Hazarika (1952) reported that jute hairy caterpillar reduced the yield by three mounds of fibre per acre. Zaman and Kabir (1990) reported that 7.3 and 13.2% yield loss by hairy caterpillar

assessed of Tossa and Deshi jute respectively. Besides jute, this pest also attacks bean, cotton, groundnut, brinjal, cabbage, cauliflower, radish, linseed, pea, soybean and leguminous crops (Lefroy, 1907; Hazarika, 1952; Kabir, 1966). The host range of this pest is still increasing (Singh and Sehgal, 1992).

Traditionally, the insect pests are controlled by conventional routine use of chemical insecticides. The non-judicious use of synthetic insecticides for the control of insect pests created several problems in agro-ecosystem, such as direct toxicity to beneficial insects, fishes and human (Goodland *et al.*, 1985; Munakata, 1977; Pimentel,1981), pesticides resistance (Brown, 1968; Fukuda, 1966; Goerghiou and Taylor. 1977; Waiss *et al.*, 1981. Schmutterer, 1981), out break of secondary pest (Hager and Franz, 1973), health hazards (Bhaduri *et al.*, 1989), environmental pollution (Kavadia *et al.*, 1984; Desmarchellier, 1985; Devi *et a*l., 1986; Fishwick, 1988), susceptibility of crop plants to insect pests (Pimentel, 1977) and increases environmental and social cost (Pimentel *et al.*, 1980).

To minimize the use of chemical insecticides in pest control programmers, alternative or biodegradable substitutes are now strongly felt in many developed countries. The biologically active natural plant products can play a significant role in this regard. These products may help to keep the drawbacks of conventional methods within bounds. Natural plant products are environmentally safe, less hazardous, less expensive, biodegradable and readily available. The costs of production of indigenous plant products are likely to be less than that of chemical insecticides (Saxena *et al.*, 1980).

Recently search for naturally occurring feeding deterrents and antifeedants have been intensified to suppress pest of field crops and storages. A number of researchers isolated and identified several chemical compounds from leaves

Chapter 2

Review of literature

and seeds of many plants and screened out for insect feeding deterrents and growth inhibitors (Jacobson *et al.*, 1975; Bernays and Chapman, 1977; Warthen, 1979; Carpenter, 1979; Menn, 1980; Jurd and Manners, 1980).Among them, neem seed kernel extracts containing azadirachtin, salanin and meliontriol have extensively been studied and demonstrated the pest control efficacy against several insect pests both in field and storages (Saxena *et al.*, 1981a; Heyde *et al.*, 1983; Mariappan and Saxena, 1983; Haque and Islam, 1988). Its biological efficiency has been proved against a number of agricultural pests since more than 20 years (Ostermann, 1983). The potential antifeedant and growth regulating effects of azadirachtin and crude neem oil have been reported in several rice insect pests (Saxena *et al.*, 1981b; Heyde *et al.*, 1983). However, reports on the use of neem oil in jute pest management are scanty (Schmutterer, 1984).

The present study was, therefore, designed to evaluate the effect of neem oil and neem leaf extract on the development and food consumption of jute hairy caterpillar, *Spilarctia obliqua* (Walker) with the following objectives:

- To investigate the efficacy of insecticidal properties of neem leaf and neem seeds oil against different larval stages of *S. obliqua*
- To fix up the economic doses of neem oil against *S. obliqua*
- To study the growth inhibition and antifeedant effect of neem against *S. obliqua.*

Chapter II

REVIEW OF LITERATURE

The jute hairy caterpillar is a sporadic pest and is widely distributed in the World. In India, Bangladesh and South-East Asia it is a serious pest. The pest breeds on jute from April to August. The pupal stage lasts 9 to 10 days in the active period and the larval period varies from 18 to 20 days in jute season and 28 to 35 days in winter. The jute hairy caterpillar has as many as 8 generations per year, a single generation taking from 5 weeks in the monsoon to over 2 months in the winter season. There may be 3 to 4 generations during the jute season, of which two are very destructive. (Atwal, 1976; Kabir, 1966).

2.1 Taxonomy:

Common name- Jute hairy caterpillar/Bihar hairy caterpillar

Order-Lepidoptera

Family-Arctiidae

Genus- *Spilarctia*

Species- *Spilarctia obliqua*

Pest status-Major.

In the world, the botanical pesticides have now been recognized as potential pest control measures. Several species of insect pests both in the field and storage have been reported to be controlled by the application of neem oil- a potential sources of antifeedant and inhibitor. A little work has been conducted with the neem based pesticides or its derivatives for the control of jute hairy caterpillar in Bangladesh. Literatures cited below reveal some information's

about the use of neem oil at Bangladesh and other countries against important insect pests.

2.2 Effect of neem oil on mortality, growth and feeding responses

Jagjeet *et al.*(2005) treated pigeon pea seeds with 11 seed protectants, i.e. neem seed kernel powder at 20g, neem oil at 10 ml, mustarded oil and groundnut oil each at 7.5ml, turmeric powder at 3.5g, mustard oil + turmeric powder at 3.75ml+1.75g, groundnut oil +turmeric powder at 3.75ml+1.75g each per one kg of seed, 4m covering with each of seed, dung cake ash, sawdust and wheat husk and mixed them with half kg of seed by shaking it manually. All the seed protectants, except for sawdust and turmeric powder, recorded significantly higher adult mortality than the control after the first day of treatment. Neem oil was effective (64.33% adult mortality) up to 35 DAT and it were followed by mustered oil + turmeric powder; which recorded only 16.33% adult mortality. At the other treatment were no effective.

Zhu *et al.* (2004) observed biological activity of azadirachtin on rice stem borer, *Chilo suppressalis*. After feeding on water-oats treated with 0.75 and 0.50mg azadirachtin/litre, the third instar larvae were completely dead in 3 and 6 days respectively. Mortality of the newly hatched *C. suppressalis* reached 100% within 24h after treatment with 6, 3, and 2mg azadirachtin/lit.

Maisary *et al.* (2004) conducted to examine the effect of neem oil the 2nd and 4th instars and eggs of *Culex pipiens* under laboratory condition. 46.98% of *C. pipiens* were killed upon exposure to 1000ppm of neem oil. The lower concentration (10ppm, 100ppm) showed little efficiency on the eggs. The continuous treatment of the 2nd and 4th instars with neem oil (100ppm) caused high mortality and complete inhibition of the formulation of mature instars.It is concluded that in general, exposure to Neem oil for a short period (24 and 48 hours) is less efficient as compared with continuous.

Neerja and Saroj (2003) investigated that the efficacy oh fresh neem oil in controlling *A. proxima* on Indian mustard. Mustard leaves were dipped in fresh neem oil at 2, 1, 0.5, 0.25, and 0.125% and fed to the larvae. Mortality was observed after 24h of exposure. Pupation was minimum at 2% neem oil, while this was maximum with 0.125% neem oil. Pupation and adult emergence decreased with increasing neem oil concentration.

Pasini *et al.* (2003) showed that the formulation of neem oil was efficient in controlling the adults of red mite of Paraguay tea. Azadirachtin also affected the fecundity of the female mites. However,when the leaves were given azadirachtin, oviposition was not inhibited.

Ogemah *et al.* (2003) observed that neem seed oil at high doses caused more than 80% mortality compared with 4% in the control. Insect population increase was completely inhibited by the doses of more than 7.5ml/kg (Approx. 22.5mg azadirachtin A/kg maize) of neem oil and 3mg of azadirachtin A/kg maize of Neem Azal PC KG 01. Neem oil caused larval mortality in their early developmental stages within the grains.

Rahman *et al.* (2003) conducted an experiment to evaluated five indigenous plants seed oils viz.Castor, Neem, Pithraj , Safflower and Sesame at concentration of 1, 2, 3, 4, and 5% revealed that al the plant seed oils have grain protectant value against lesser meal worm, *A. diaperinus*. The results showed that sesame and neem oil were more effective than Castor, Pithraj, and Safflower oils. The tested seed oils provided good protection for wheat grains.

Gupta (2003) observed that the efficacy of neem products viz., Neem kernel extract in water or cow urine, Neem gold, Neem leaf extract in cow urine and Neem oil in controlling the major pests (*Antigastra catalaunalis* and *Dasineura seasami*)of sesame cv. Flower and capsule damage was lowest with the

application of neem oil and endosulfan, respectively. Application of neem oil resulted in the lowest bud and flower damage (13.3%), and highest grain yield (655 kg/ha) and net profit (Rs. 2633/ha).

Eungwijarupanga *et al.* (2002) tested neem extracts containing 0.185% azadirachtin at 3 concentrations 100ml, 200ml and 300ml diluted in 5 litres of water, These were applied using a thermal fogger to a 15 years old teak (*Tectona grandis*) for control of teak defoliator, *Hybiaea puera.* After application larvae were colleted and reared in the laboratory to observed mortality. One day after fogging mortality started to increase for these treated with 200ml and 300ml/5L concentrations and all larvae died within 6 days when treated with 300ml/5L.

Padmasheela and delvi (2002) tested a commercial formulation of neem oil EC (nimbex, 0.03%) at different concentration viz. 25ppm, 50ppm, 75pmm, and 100ppm for mortality effects against grubs of *Oryctes rhinoceros* (a coconut pest) at laboratory conditions. In feeding toxicity test, neem oil at concentrations of 50ppm, 75ppm, and 100ppm caused 20%, 45% and 90% mortality respectively on exposure up to 96h and 100ppm caused 90.67% mortality of *O. rhinoceros* grubs.

Malinowaki (2002) studied the activity of azadirachtin (10g/litre EC) against 3rd instar *Neodiprion serlifen* larvae, using aqueous emulsion at four different azadirachtin concentration (0.01%, 0.001%, 0.0001% and 0.00001%) larvae were fed with treated pine twigs for three days, and then reared on untreated foliage until pupation. Mortality was significantly increased up to 100%, even at the lowest concentration of azadirachtin (0.00001%).

Qureshi *et al.* (2002) investigated the direct effect of neem extracts on the adult glass beetle, *Costelytra zealandica* where laboratory bioassay showed that neem caused only low mortality even at the highest dose.

Karmakar and Bhole (2001) observed the efficacy and persistent toxicity of some neem products- neem oi8l, and Nimbicidine against adult of *Epilachna dodecastigma*, the treatments with 2% neem oil and 2% Nimbicidine resulted 90.69% and 71.90% mortality respectively.

Shaminathan and Jayaraj (2001) conducted two experiments to evaluated botanical pesticides like Ipomoea and vitavex ,leaf extracts, neem oil and madhuca oil (at 0.3% or 3.0% each) against *Perrisia virgata*. The leaf dip method was used in both experiments and pest mortality was recorded at 24h, 48h, and 72h after treatments. In experiments 1, treatments with 3% neem oil recorded the highest mortality (43.13%). Neem recorded 50% mortality at 72h, and in experiment 2, at 48h, fortified (0.3%) neem oil recorded a maximum mortality of 49.3% and at 72h, fortified neem recorded 63.6% mortality.

Arcos *et al.* (2001) conducted that the effect of neem oil was evaluated by using concentrations of 0.5, 1.0, 1.5, 2.0, and 3.0%).They mainly showed that mortality recorded by ingestion was attributed to starvation. Filter paper soaked with neem oil inhibited feeding of *I. marginipennis*.

The effect of neem (*Azadirachta indica*) oil at 0.25, 0.5, and 1.5% on larvae of *Epilachna vigintioctopunctata* was investigated by Shanmugapriyan and Kingsly (2001). The effects of quinalphos (0.025%) and monocrotophos (0.025%) were also determined for comparison. Neem oil at 1.5% produced the highest mortality of 2^{nd} and 3^{rd} instars (95.23%), and 4^{th} instars (76.19%). Neem oil at 0.25 and 0.5% concentrations resulted in 57.1 and 85.75% mortality in 2^{nd} instars, 47.6 and 85.7% in 3^{rd} instars and 57.1 and 80.9% in 4^{th} instars. Monocrotophos and qunalphos resulted in 95.24% larval mortality.

Rani *et al.* (2000) investigated the efficacy of cotton seed, neem, palm, rice bran and soybean oils as seed coating against pulse beetle, *C. chinensis*

infesting Chickpea and found that neem oil at 1 ml/kg of seeds gave the highest adult mortality (65%) three days after treatment.

Ranjana *et al.* (2000) tested five plant extracts from *Azadirachtin indica* kernels, *Allium sativum* bulbs, *Citrus sinensis* rech, *Citrus limm* peels and *Mangifera indica* leaves each having three concentration (1%, 1.5% and 2%) against pulse beetle, *C. maculatus*. The petroleum ether extract of neem kernel was most effective as 1.5% and 2% level showed 50% and 61.11% mortality.

Menhajul (1999) observed that the effect of neem oil on the third, fourth and fifth instar larvae of Jute hairy caterpillar. He found 30% to 100% larval mortality up to 10% concentration of neem oil. The median lethal dose (LD_{50}) values of neem oil for different larval instar for different concentration were also determined.

Sharma (1999) reported that neem seed (*Azadirachtin indica*) kernel powder (NSKP) at 4% and neem leaf powder (NLP) at 5% protected maize for 5 moths against *Sitophilus oryzae, Sitotroga cerealella, Rhyzopertha dominica* and *Trogoderma granarium*. Neem oil (Neembicidine, 2%) effectively reduced the emergence of F_1 and F_2 progeny of all the pests and completely protected maize up to 9 moths and suggested that neem products can be mixed with stored maize to protect the grains up to 9 months from the attack of these major pests.

Reddy *et al.* (1999) stated that application of four plant neem oil (*Azadirachtin)*, Karanja oil (*Pongamia glubra*), Mahua oil (*Madhuca lalifolia*) and palmolein oil (*Elaeis gaineenis*) at dosages of 0.5% and 1.0% level effectively protected green gram from *C. chinensis*. Neem oil at 1% level was the best protected followed by Palmolein, Karanja and Mahua oils. These oils also exhibited contact toxicity and no adults could survive in neem treated green gram at 5% concentration.

Tabassum et al. (1999) reported that the toxicity of neem compound (Nfc and NC) and an insect growth regulator dimilin (diflubenzuron) were determined against adult of the pulse beetle,*Callosobruchus analis* using filter paper impregnation and glass film method. The LC_{50} values of Nfc, NC and dimilin were 39.20µm/cm^2, 7.17µm/cm^2 and 13.5µm/cm2 respectively, using the filter paper impregnation method, while 10.0µm/cm^2 and 4.9µm/cm^2, respectively, for NC and dimilin using the glass film method.

During conducting a field experiment Mahapatro and Umakanda(1998) found that the green leafhopper(*Nephotettix virescens*) population can be better managed by integrating neem derivatives (neem oil and neem seed extract 0.2% along with 0.1% teepol) at 20 and 70 days after transplanting (DAT) with chemicals such as monocrotophos (0.4 kg a.i./ha) as an intermediate spray at 40 DAT.

Sudipta and Sanjib (1998) reported that larvae of rice moth, *Corcyra cephalonica* (Stainton) were maintained in neem oil (Azadirachtin, 0.03%) absorbed crushed jowar (sorghum) grains in four doses (0.25, 0.50, 0.75,and 1.0 ml; each dose in 20 g of food), with an initial population of 50 newly hatched larvae/100 g of neem absorbed food in each replication. Deformed adults with a prolonged period of development were obtained. Growth inhibition,. developmental disturbances and mortality increased markedly with increased doses.

Mayabini (1997) studied the efficacy of neem bark decoction, neem based chemicals and neem derivatives (neem oil, leaf extract and leaf decoction) against rice leaf folder *Cnaphalocrosis medinalis.* All were applied as foliar sprays to pot-grown rice plants. Leaf area fed by the larvae was recorded after 48 hours. Neem bark decoction appeared to be a very effective botanical for controlling the rate of feeding and reducing the rate of population.

Naganagouda *et al.* (1997) conducted a field study to determine the efficacy of various insecticides and neem products for the control of *Nilapaevata lugens, Sogatella furcifera, Cnaphalocrosis medinalis* and *Scirpophaga incertulas* on rice. Monocrotophos was the most effective insecticide in terms of giving the highest yield followed by neem oil and Nimbicidin.

Deka and Hazarika(1997) observed that neem(*Azadirachta indica*) seed oil (NSO) acted as a potential antifeedant against adult of the chrysomelid, *Dicladispa armigera*. Under laboratory conditions, daily consumption of fresh rice leaves was 1.05 g, 0.08 mg, which was reduced by 50% when leaves were treated with 6.46% NSO.

Raguraman and Rajasekaran (1997) stated the effect of neem oil and neem seed bitters applied at different concentrations as either high volume, low volume or ultra volume sprays to the rice brown plants hopper,*Nilaparvata lugens.* All neem products affected the orientation, probing and feeding time, food ingested and growth and development of *N. lugens.*

Lowerry *et al.* (1996) reported that neem (*Azadirachta indica*) seed oil (NSO) added to meridic diet at concentration as low as 0.016% reduced pupation and prevented adult eclosion rate of obliquebanded leaf roller (*Choristoneura rosaseana*).At a rate of 0.0016%NSO reduced the fitness of *C. rosaceana*, resulting in longer developmental times, lower adult eclution rates, and reduced egg production compared with controls. Pupation was completely inhibited at concentration of 0.25 and 1.0% for larvae exposed in the fifth or sixth instar, respectively; rates as low as 0.016% reduced pupal weights and elution rates. For larvae transferred to treated diet in the fifth instar, physical abnormalities in the wings of adults occurred at a rate of 0.004% NSO and increased with increasing treatment rates.

Akhtaruzzaman (1996) were tested four concentrations of neem oil viz. 1.0, 2.0, 4.0 and 8.0% by leaf spraying method allowing 5th instar laevae of *Spilosoma obliqua* to feed on treated food for 48 hours. The percent reduction of food consumption at 1.0, 2.0, 4.0 and 8.0% were 41.92, 46.22, 51.29 and 55.63% in 46 hrs and 47.16, 49.72, 56.56 and 67.48% in 48 hrs. Growth inhibition indicated by mortality was 100% at the 4.0% and 8.0% concentration of neem oil.

In a laboratory study Haque *et al.* (1996) found that when first and third instars larvae and adults of *Epilachna dodecastigma* were expresed to 0.25, 0.50, 1.0,or 2.0% neem(*Azadirachta indica*) oil applied on brinjal leaf discs, all the first instar larvae were killed at the concentrations before feeding and the feeding activity of third instar larvae and adults decreased with increasing oil concentration.

Nauman and Islam (1995) found that applications of 3 concentration of oil-free neem seed extracts to cabbage plates in cages did not deter oviposition by individuals of 3 species of noctuid moths, *Trichoplusia ni*, *Peridroma saucia* and *spodoptera litura*. 1% crude oil emulsion significantly reduced the proportion of eggs laid by *S. litura* on treated plants.

Lowery *et al.* (1994) reported that application of formulation neem seed oil effectively controlled several of aphids infesting plants both in laboratory and field. Formulation neem seed oil (NSO) was equally deterrent to first and third instar nymphs and adult strawberry aphids, *Chactosiphon fragaefolii* (Cockerell). Concentration of NSO resulting in 50% feeding deterrence was approximately 1.1% for this species.

Braman (1993) observed antifeedant effects of azadirachtin in nymphal tawny mole crickets, *Scapteriscus vicinus* Scudder, in laboratory tests. Crickets

surviving treatment grow more slowly and tunneled less than their untreated counterparts.

Isman (1993) determined the comparative efficacy of azadirachtin, the major active ingredient in neem, as a feeding deterrent for six species of noctuids of economics importance; the black armyworm, *Actebia fennica* Tansch, bertha armyworm, *Manestra configweata* Walker, variegated cutworm, *Peridroma sancia* Hubner, zebra caterpillar, *Melancra picta* Harr, Asian armyworm, *S. litura* Fab. And the cabbage looper,*Trichoplusia ni* Hubner. Fourth instar larvae of *S. litura* was the most sensitive to the antifeedant effects of azadirachtin whereas *A. fennica* was the least.

Matter *et al* (1993) tested oil from *A. indica, M. azadirach, Cymbopogon citrates* and *Geranium* in the laboratory to evaluate their effect on *Coccinella undecimpunctata* and *aphis gossypii*, Geranium sp. Neem oil was more repellent to *A. gossypii* than the others for antifeedant.

Nesseh *et al.* (1993) tested the repellent effect of neem oil on adults of *Schistocerca gregaria*. They found that *S. gregraria* consumed 100% of the leaves of the untreated plant, while the adults started feeding on treated plant after 24 hours of the application.

The antifeedant properties of the seeds of some meliaceous plants were reported by Chiu-Shin-Foon *et al.* (1993). In their experiments with neem seed oil and petroleum ether extracts of the seed kernels of two species of chinaberry they demonstrated their potentials as strong antifeedant against nymphs of brown plant hopper, *N. lugens*.

Freisewinkel (1993) found the contact effects of neem oil topically sprayed on third instar nymph of *Locusta migratoria migratorioides*. In parallel experiment neem oil was applied directly to the abdomen of the nymphs. The

effectiveness of neem oil given orally was tested by feeding larvae at the beginning of the third instar with maize leaves treated with neem oil.The mortality in feeding experiments was much higher than in spraying or direct application experiments. Treated locusts showed prolonged nymphal developmental and reduced increase in weight.

Nicol (1993) studies the effects the neem seed oil in third instar nymphs of *Schistocerca gregraria*. In cages which were sprayed with neem oil, the locust showed higher mortality rates, delayed nymphal development and morphogenetic effects of antennae, eyes and wings. Moreover, the adults derived from treated nymphs were smaller in size than those in the control.

In laboratory experiments Venkateswarlu *et al.* (1993) studied the effect of neem oil (0.1, 0.25, 0.50, 1.0 and 1.25%) on growth and development of *Lipaphis erysimi*. At concentration of 1.0, 1.25 and 1.50% all the nymphs reared on treated Indian mustard leaves diet before reaching the adult stage. At the lower concentration nymphal survival, fecundity and growth index of the aphid decreased and developmental period increased.

Rao *et al.* (1993) tested, Neemark, Biosol, Repelin and neem oil at 0.5-3.0% against larvae of *Spodoptera litura* in the laboratory. Repellency, antifeedant activity and developmental period increased with increase in concentration of all pesticides. Adult's emergence, growth, survival, larval and pupal weight, number of eggs laid and hatchability of eggs decreased with increase in concentration. Neem oil had the greatest effect, followed by Neemark,Biosol and Repelin.

Isman (1993) stated the efficacy of azadirachtin, the major active ingredient in the botanical insecticide neem, as a larval growth inhibitor and feeding deterrent for six species of noctuids of economic importance: the black army cutworm, *Actebia fennica*, Tansch., the bertha army worm, *Mamestra*

configurata Walker, the variegated cutworm, *Peridroma saucia* Hubner, the zebra caterpillar, *Melanchra picta* Harr., the Asian armyworm, *Spodoptera litura* Fab. and the cabbage looper, *Trichoplusia ni* Hubner. When added to an artificial diet, azadirachtin inhibited normal growth of all species in a dose dependent fashion.

Salsoloy and Embuido (1992) evaluated neem oil for its insecticidal action on cotton bollworm, *Helicoverpa armigera* Hubner. The oil applied along sprayed on cotton and the effects were compared. Neem oil sprayed on cotton gave poor control of the pest.

Becker *et al.* (1992) observed that natural insecticides, neem, contains the active chemical azadirachtin, which disrupt the hormonal changes in *Bemisia tabaci* causing death during moulting.

In laboratory experiments Schmutterer (1992) applied concentration of 10 and 20 ppm/litre of azadirachtin, of an azadirachtin-free fraction and of 100 ppm/litre or an enriched, formulated seed kernel extract of *A. indica,* against the 5^{th} larval of *Pieries brassicae*. Application of neem products against young (1^{st}-3^{rd}) larval instar of *P. brassica*, which may be typical under practical conditions, led to the death of the caterpillars.

Freisewinkel and Schmutterer (1991) showed that the topical application of neem extract at 0.25 to 1.0 ml/m^2 to the 5 nymphal instars of the gregarious phase of *Locusta migratoria migratorioides* led to increased mortality during moults, prolonged development and reduced fitness. Morphogenetic effects were observed on the legs, wings and antennae. A reduction in weight corresponded to reduced feeding activity. Color changes and supernumerary moults suggested tendencies towards soliterization. The earlier the nymphs treated, and the higher the amounts applied, the more distinct the effects.

Salem (1991) found that larval mortality ranged between 14.28% to 78.57% and the percent of eggs hatching ranged between 57.5% and 89.4%, when different concentratration from neem seed oil extract, *A. indica* A. Juss. were tested against the potato tuber moth, *Phthorimaea operculella* Zell and also stated that 100 ppm concentration of neem oil extract was the most effective extract against larval feeding of potato tuber moth, *Phthorimaea operculella* Zell.

Salem (1991a) tested pure neem seed oil against the cotton bollworms, *Pectinophora gossypiella* Saund and *Earias insulana* Boisd. The most active concentration caused reduction in the percentage of infestation nearest to 150 ppm. The percentages of infestation decreased with the increase of neem seed oil concentrations.

Loke *et al.* (1990) evaluated six concentrations (1.25, 2.25, 5.0, 10.0, 20.0, and 40.0 percent) of neem oil in acetone, for contact toxicity against 2nd and 3rd larval instars of *Plutella xylostella* L.Significant mortalities of both larval stages were observed with neem oil concentration of 10 percent and above. Although the lower concentration of neem oil appeared to be sub lethal with regard to contact toxicity effect, physiological and growth disruptive effects, such as retardation of growth (prolonged), delayed adult emergence and abnormal adults but the effects were more pronounced in the younger instar. Subsequent treatment of pupae and adults of *P. xylostella* with neem oil concentrations of 1.25, 2.25. 5.0 and 10.0 percent showed that pupae were generally not affected by the concentrations tested. However, male and female adult moths treated with 2.5 percent and higher concentrations of neem oil had significantly higher mortalities in 48 hours and shorter longevities than the adults in control.

Mishra *et al.* (1990) reported that feeding brinjal leaves treated with 0.025 and 0.05% neem oil to *Epilachna vigintioctopunctata* increased the duration of life stages in the subsequent generation.

Rovesti and Deseo (1990) stated that neem, (*Azadirachta indica*) and its oil, extracts and derivatives including azadirachtin are used as antifeedants, repellents, ovicides and growth regulators; they can also reduce adult fecundity and egg viability.

Karel (1989) evaluated various formulations of neem extracts under field condition to examine the effects on foliar beetle, *Ootheca bennigseni* Weise, a serious pest of beans and other legumes. At concentrations of 2.0 and 4.0%, neem formulations from seed kernels deterred *O. bennigseni*, from feeding on bean leaves. None of the neem formulations protected bean plots completely. In green house studies, 1.o% aqueous leaf and kernel extracts were highly active as an antifeedant against *O. bennigseni*, which consumed little or none of the leaves. In free-choice experiments, bean seedlings treated with 1.0 and 2.0% neem seed kernel extracts were well protected.

Kareem et al. (1987) showed that crude extracts of neem seed kernel (NSK) when evaluated at field, 4% NSK significantly reduced green leaf hopper (GLH), brown plant hopper (BPH), and white backed plant hopper (WBPH) population and leaf folder (LF) damage. This crude water extract caused greater BPH and LF feeding inhibitor and WBPH nymphal emergence.

Solsoloy and Solsoloy (1987) stated that cottons bolls treated with 2% neem oil emulsified with 1.0% surfactant inhibited the feeding of even starved larvae of cotton bollworm. The amount of frass they excreted was significantly lower than the control which was sprayed with acetone. Similarly, the larvae were relatively lighter and smaller. These results showed the antifeedant effect of the oil.

Unchalle (1987) observed the efficacy of neem oil on rice leaf hopper, *Nephoteti virscens*. The repellent property of neem oil was found to increase along with the increasing oils concentration. Neem oil at 7% concentration and above was observed to reduce the population density of treated 3rd instar nymphs of green leaf hopper to more than 50%. Twenty five percent neem oil was found to decrease mortality of female green leafhopper to lower than 50% after 6 days of spraying.

Kareem and Durairaj (1987) evaluated crude extracts of neem seed kernel (NSK) and neem cake (NC) in water and neem oil (NO) emulsion along with two synthetic insecticides in field as foliar sprays for the control of major insect pests of rice . NSK 4% significantly reduced green leaf hopper (GLH), brown plant hopper (BPH), and white backed plant hopper (WBPH) populations and leaf folder ()LF damages in two fields trials proving either on per with or next in efficacy to fethion and phosphamidon spraying.

Saxena (1987) reported that neem seed derivatives were promising against sucking insects-the green leaf hopper, *N. virescens* Distant, the brown plant hopper, *Nilaparvata luigens* Stal, the white backed plant hopper, *Sogatella fercifera* Horvath, and the rice bug, *L. oratorius* Fbricus, and foliage feeders-the rice leaf folder, *C. medinalis* Guenee, the ear-cutting caterpillar *Mythimna separate* Walker, and the rice army worm, *S. maurita · acronynctoides* Boisduval. Isect growths were inhibited on neem treated plants. Contact with or ingestion of neem derivatives disrupted growth of insect pests but emergence of parasites was not affected.

Chiu-Shin-Foon (1987) evaluated the potential of *Azadirachta indica, M. toosendan*,and *M. azadirach, Tripterygium wilfordii* and *T. hypoglaucum*, and regulators, in laboratory and field experiments. Fifth instar *Pieris rapae* larvae topically treated with 20% neem oil at 2 micro liter/larvae developed properly

and died after population. In field trials, spray application of a 0.3% neem seed extract (ATZ-VR-K) was effective against *P. rapae* larvae for almost 21 days. The extract was also effective against *Plutella xylostella*. Azadirachtin disrupted the growth of corn borers and other pests.

Saxena and Khan (1986) monitored feeding behavior of *N. virescens* on rice plants kept in an arena permeated with the odour of neem seed oil. The garlicky odour of neem oil disrupted the normal feeding behavior of Cicadellids. Phloem feeding by *N. virescens* on rice plants kept in an arena permeated with odour of 6, 12 or 25% neem oil was significantly reduced.

Saxena (1985) found that insects fed far les, grew poorly and laid fewer eggs on rice plants treated with the oil, cake, extracts such as azadirachtin, and their formulation. Contact with or ingestion of neem seed derivatives disrupted growth of insect pests. Neem oil alone or in combination with seed oil of clustered apple (*Annona Squamosa* L.) was effective in reducing the survival of *N. virescens* and its transmission of grassy and ragged stunt viruses.

Jotwani and Srivastava (1984) reported that antifeeding properties of neem seed kernel suspensions against desert locust, *Schitocerca gregaria* Forsk; Castor hairy caterpillar, *Cuproctis lunata* W., Tobacco caterpillar, *Spodoptera litura* F., Bihar hairy caterpillar, *Utethesia pulchella* L. Grasshoppers, *Acrida exaltata* W. Antifeedant properties of *A. indica, M. azadirach, M. toosendan* were investigated by Shin-Foon and Zhang (1984) on fifth instar larvae of *S. litura* and were sensitive to neem treated leaf discs in choice tests. Shin-Foon and Zhang (1984) observed that the leaf discs treated with neem oil was protected more than 97.5% when offered to fifth instar off the fall army worm, *Spodoptera litura* F.

Ladd *et al.* (1984) stated that the application if azadirachtin from seeds of Indian neem tree to larvae of Japanese beetle, *Popilla japonica* Newnam,

completely disrupted subsequent normal development to the adult stages. Calculated LD_{50} and LD_{90} values of topically applied azadirachtin were 0.1 and 0.4 µg/larvae respectively. Azadirachtin also increased in duration of immature stages.

Schmutterer *et al.* (1984) investigated that topical application of nem oil on last instars *N. lugens, S. furcifera* and *N. virescens* nymphs resulted in their premature death. Seventy seven to 100% mortality of *S. furcifera* was caused by neem oil.

Islam (1983) revealed that oil neem as well as extracts of leaves and seeds of *A. indica, M. azedirach, A. ruhituka* and *A. reticulata* with haxen diethyl ether, 95% ethanon and acetone showed potential as antifeedant or feeding deterrents for the control of brown rice plant hopper (BPH), rice green leaf hopper (RGLH), rice hispa (RH) and lesser rice weevil (LRW). The young seedling of rice sprayed with 8-12% of crude and emulsified neem oil also significantly reduced feeding in brown hopper and green rice leaf hopper. Aqueous and methanol extracts of neem and chinaberry also deterred feeding in adult pulse beetle and early instar larvae of Jute hairy caterpillar.

Mariappan and Saxena (1983) reported that custard-apple oil, neem oil and their mixtures were effective in reducing the survival of the green leaf hopper, *N. virescens* distant and its transmission of the rice tungro virus (RTV).

Heyde *et al.* (1983) found that 2 to 4 days exposure of *Sogatella furcifera* to plants treated with 500 ppm of neem seed kernel extracts resulted in 75% mortality whereas in the control, mortality was only 5%. On third instar, *N. lugens* nymphs, a combination of foliar and topical application induced higher mortality (75%) than either application alone (30%).

Schmutterer *et al.* (1983) studied the morphogenetic effects of four partially purified fractions of neem seed extracts and two methanolic seed extracts on larvae of rice ear cutting caterpillar, *Mythimna separeta* Walker, and the rice leaf folder, *C. medinalis*, larvae fed for 24 hours. On rice leaf cuts dipped in different solution of the partially purified fractions and methanolic extracts exhibited pronounced development abnormalities and mortalities in succeeding larval instars and in pupal and adults stages.

In the laboratory and green house tests, Reed *et al.* (1982) reported that suspensions containing low concentrations of azadirachtin and salanin compounds isolated from ethanolic extracts of neem seeds, (*A. indica*) possessed feeding deterrents against the spotted cucumber beetle, *Diabrotica undecimpunctata* howardi Barber, and striped cucumber beetle, *Acalyma vittatum* F.

Feuerhake *et al.* (1982) dissolved neem seed extract in water by means of emulsifier and these formulations were tested against *Leptinotarsa decemlineata*; *Pieries brassica* and *Mamestra brassicae*. A concentration of 100 mg/kg was the most effective extract that caused 100% mortality in all the pest species.

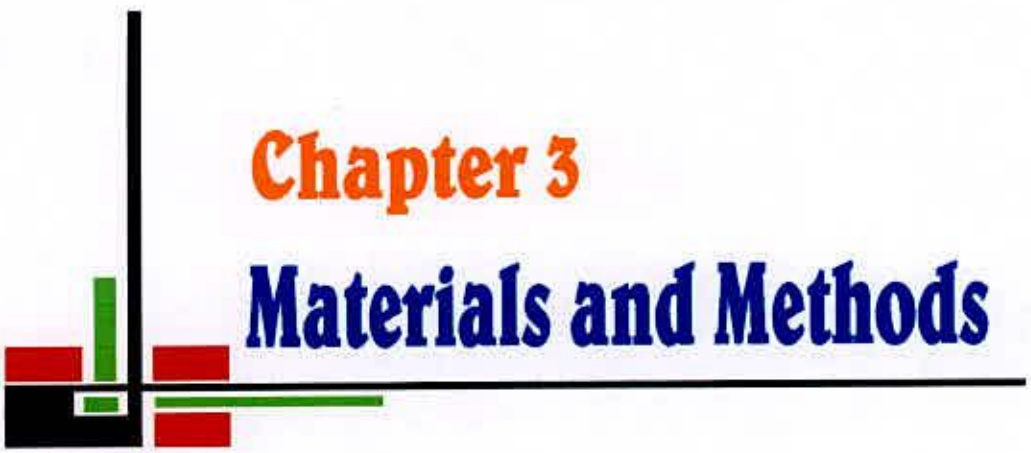
Redknap (1981) found various neem suspensions to control the leaf eating larvae of *Epilachna chrysomelina* on cucumber, *Cucumis sativus* and leaf eating flea beetles, *Prodagica niforma* and *P. syastedti* on sorrel, *Hibiscus sabdariffa*. In addition, citrus seedlings sprayed with neem suspension were protected from the larvae of *Papilio demoleous*

Kareem (1981) observed the neem oil as an antifeedant for certain phytophagous insects and bruchid of pulses. Feeding and gallery formation by *Nephantis serinopa* Meyer. By their larvae were inhibited by 1% neem seed kernel extract. Seed extract had significant antifeedant effect on *Spodoptera*

litura F. larvae on sweet potato. A 3% aqueous neem seed kernel extract reduced the damage by *Crocidolomia binotalis* Zell. and *Phyllotreta downsii* on radish whereas 2% neem seed kernel powder mixed with seeds of pigeon pea and green gram effectively checked *Callosobruchus chinessis* L Also reported that 25% mortality in *Plutella xylostella* larvae fed on leaves treated with 3% neem oil. High mortality was induced at higher concentrations.

Saxsena *et al.* (1981a) observed that neem oil acted as a potent antifedant for controlling brown plant hopper, *Nilaparvata lugens* Stal both in the laboratory and field when plants were sprayed with 12% neem oil. The quantity of food ingested/female/day was significantly reduced. Feeding duration decreased by 0.93 min/h for every 0.1% increase in oil concentration. Later Heyde *et al.* (1983) further confirmed the antifeeeding properties of neem oil against *N. lugens*, White backed plant hopper, *Sogatella furicifera* Horvath and rice green leaf hopper, *Nephotetttix virescens* Distant. Another observation he found that neem oil caused high mortality of the first instar larvae of *N. lugens*. Saxena *et al.* (1981b) found that fifth istar larvae of *C. medinalis* treated topically with neem oil or confined on treated rice leaves showed high developmental abnormalities and mortality

Saxena *et al.* (1981b) revealed that food consumption by the larvae of rice leaf roller, *Cnaphalocrosis medinalis* Guenee was significantly reduced when they fed rice leaves sprayed with 12% or higher concentration of crude emulsified neem oil.



Chapter 3

Materials and Methods

Chapter III

MATERIALS AND METHODS

To evaluate the efficacy of neem oil and neem leaf extract on the development and feeding response of *Spilarctia obliqua* (Walker), experiments were carried out in the laboratory of the Department of Entomology, Bangladesh Jute Research Institute, Manik Mia Avenue, Dhaka during the period from April to August 2005 with $27^0 \pm 1^0$C and in average 13:11 (light: dark).

3.1 Collection of neem oil:

Neem oil was collected from local Boro bazar of Mymensingh district (Plate 1). From this stock, a series of concentrations (0.5, 1.0, 2.5 and 5%) were prepared with distilled water containing 0.1% Nikalin. Nikalin was used as emulsifier.

3.2 Preparation of leaf extract:

Neem leaves were collected from BJRI campus (Plate 2). Leaf extract of neem (1:20) was prepared by using blender machine with the ratio of 20gm fresh leaves and 400ml of water and filtered through linen cloth to get 1: 20 neem leaf extract followed by Zebitz (1986).

3.3 Food selection and source of supply

S. obliqua is a polyphagous pest occurs in all seasons of the year, which chooses different host plants viz. jute, bean, soybean, cabbage etc. as its food. On the basis of their availability jute plants (BJRI, var: CVL-1) were selected to serve as food for the test insect for this study. The fresh jute leaves were collected daily from the plots of Bangladesh Jute Research Institute Campus and SAU campus and fed to the pest which never sprayed with chemicals.

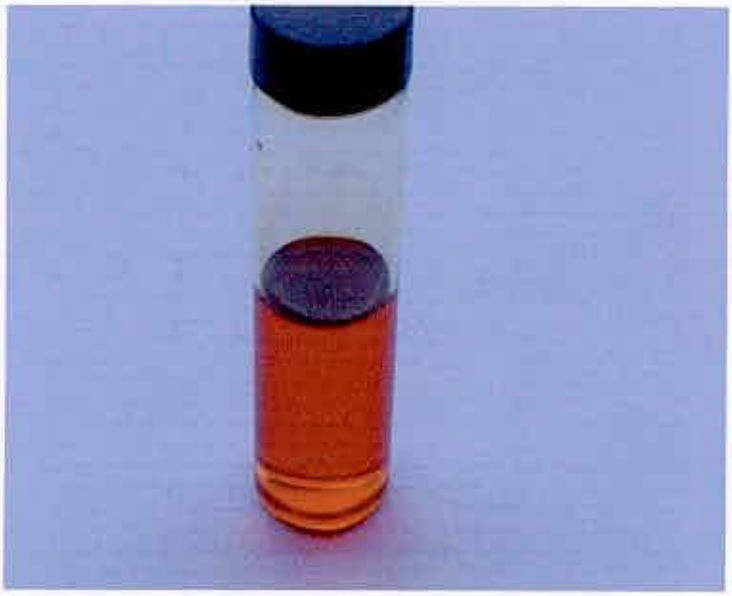


Plate 1. Pure neem oil

Plate 2. Neem leaves

3.4 Stock culture of the test insect

To get adequate supply of the test insect, a stock culture of jute hairy caterpillar larvae was established in the laboratory. The mature larvae of *S. obliqua* were collected from the jute fields and were reared in the laboratory to get adult male and female. The adult male and female were allowed to mate freely in a breeding cage on potted plants. On completion of mating, the female adults started laying eggs in clusters on leaves in potted plants. During mating and oviposition, 10% sugar solution in small cotton pads were supplied as food sources for adult's moths. The newly hatched larvae were allowed to feed on jute leaves in beaker and fresh jute leaves were supplied daily and overcrowding was avoided for healthy growth of the larvae.

3.5 Food treatment

Collected fresh and healthy jute leaves were treated separately with different concentrations of neem oil viz. 0.5, 1.0, 2.5, 5.0 and leaf extract (1:20) by dipping method. These treated leaves were air dried before offering to the insects. In control treatment, leaves were treated with distilled water containing 0.1% Nikalin.

3.6 Treatments

The treatments included in the study were as follows:

T_1 = Neem leaf extract (1:20),

T_2 = Neem oil (0.5%),

T_3 = Neem oil (1.0%)

T_4 = Neem oil (2.5%)

T_5 = Neem oil 5.0%

T_6 = Untreated control

3.7 General information of neem plant

Common name: Neem

English name: Neem

Scientific name: *Azadirachta indica*

Plant part use: Leaf, Seed kernel

3.8 Experimental design:

The experiment was laid out in Completely Randomized Design (**CRD**) with three replications.

3.9 Data recording:

The following parameters were considered for evaluating the effectiveness of each treatment against *S. obliqua*.

1. Mortality Counting at 10 DAT
2. Mortality Counting at 12 DAT
3. Mortality Counting at 14 DAT
4. Duration of larval instars
5. Duration of pupal instar
6. Measurement of food consumption on different instars

3.10 Dose-mortality response

The effect of neem oil and leaf extract on the larval mortality of *S. obliqua* was determined by exposing third, fourth and fifth instars larvae to jute leaves treated with neem oil at 0.5, 1.0, 2.5, 5.0% concentrations and neem leaf extract (1:20). For this purpose, newly moulted third to fifth instars larvae were separately placed in beaker (300 ml) covered with linen net containing treated food. Five larvae were maintained in each beaker for each concentration and were replicated three times. The larvae were transferred to new beaker containing fresh treated food daily. Control treatments were done side by side for each instar separately. The larval mortality was recorded in every 24 hours.

3.11 Growth response

The efficacy of neem oil on the growth and development of *S. obliqua* was observed by exposing fourth instar larvae to the treated and untreated jute leaves. These leaves were treated with neem oil at 0.5% concentration and were exposed to the larvae either for 48 hours (48 hrs treatment) or continuously throughout the larval period (continuous treatment). From laboratory culture, newly moulted fourth instar larvae were individually transferred into beaker (250 ml) containing treated and untreated leaves. Twenty-five beakers were maintained for each treatment and one Beaker was used for one larva. The larvae were transferred daily into new beaker containing fresh food and unconsumed leaves were removed along with faces. Rearing of the larvae was continued till death or pupation. The duration of each larval instar, total larval period, abnormal growth of larvae or pupae, percent pupation, pupal period, and percent adult emergence were carefully recorded.

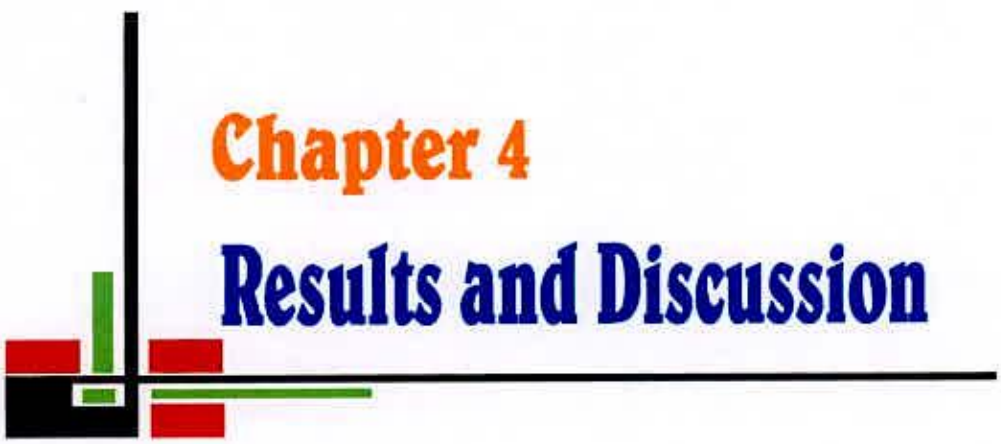
3.12 Feeding response

Similar to growth response, the efficacy of crude neem oil on the feeding response of *S. obliqua* was also observed by exposing fourth instar larvae to the treated and untreated leaf (average leaf area 900 sq. mm). Three to four leaves were offered in a beaker to each larva and 25 beakers were maintained for each treatment- 48 treatments and continuous treatment and control treatment. Fresh leaves were offered daily and the food consumption by the larvae was measured at every 24 hours by using a square millimeter graph paper.

3.13 Statistical analysis

The recorded data were analyzed by single factor Completely Randomized Design (CRD) and mean values were separated by Duncan's Multiple Range Test (DMRT). (Gomez and Gomez, 1984). For toxicity test, data were analyzed by probit analysis originally designed by Finny (1971) using MSTAT Statistical Package Programme. Before analysis, mortality data were corrected by Abbott's (1925) formula as follows:

$$\text{Corrected mortality (\%)} = \frac{\text{Treatment mortality} - \text{Control mortality}}{100 - \text{Control mortality}} \times 100$$



Chapter 4

Results and Discussion

Chapter IV

RESULTS AND DISCUSSION

The comparative effectiveness of neem oil at different concentrations and neem leaf extract on the mortality, growth and feeding responses of *S. obliqua* have been presented in the following sections:

4.1 Effect of neem oil and leaf extract on the larval mortality of *S. obliqua*

The effect of neem oil at different concentrations and neem leaf extract on the mortality of different larval instars (third to fifth) of *S. obliqua* was observed when larvae were fed on neem products treated food.

4.1.1 Cumulative mortality

The daily cumulative mortality of different larval instars of *S. obliqua* occurred at different concentrations of neem oil are presented in Figures 1-3. It was found that mortality increased proportionately with the increased of concentration of neem oil and exposure period. Maximum percent mortality of all larval stages was observed with neem oil at 5.0 % concentration The lower concentration of neem oil viz. 0.5, 1.0, 2.5 and leaf extract (1:20) are also markedly reduced the survival of the larvae as compared to the larvae fed on untreated leaves. A large number of larvae were found to die during moulting and before pupation.

The cumulative mortality of 3rd instar larvae was highest (93.33%) in treatment T_5 during the study period (Figure 1) and other treatments (T_1, T_2, T_3 and T_4) also had the significant effect on cumulative larval mortality. The similar results were also observed for 4th (Figure 2) and 5th (Figure 3) instars larvae during the study period. The 4th and 5th instar larvae had the highest cumulative mortality 80% and 66.67% respectively.

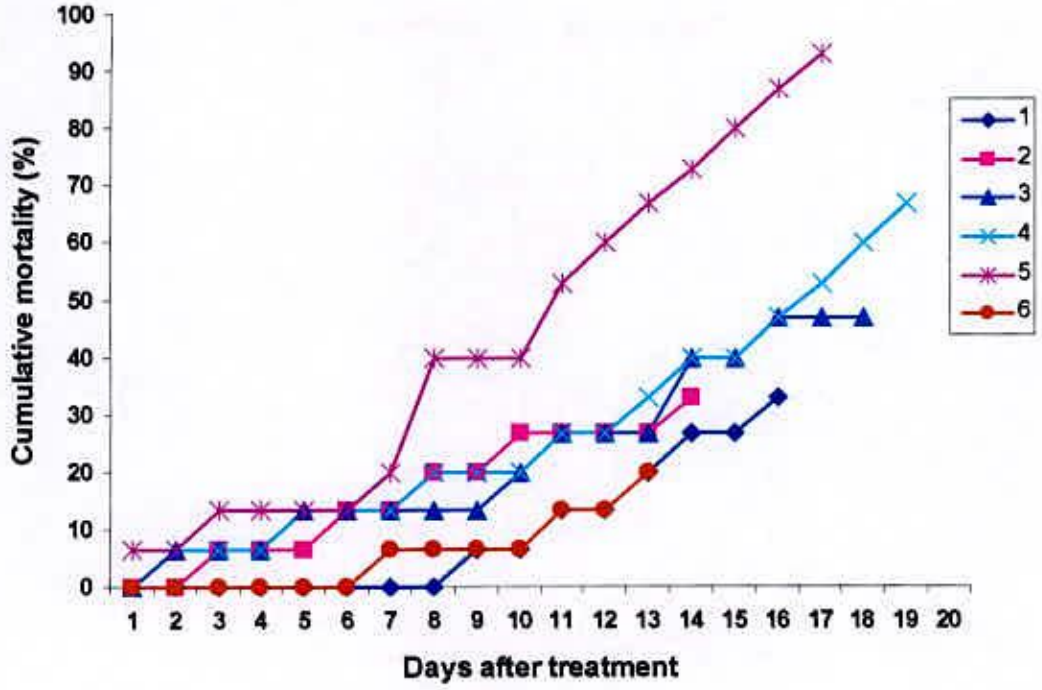


Figure 1. Effect of neem products on percent cumulative mortality of 3rd instar larvae of *S. obliqua*

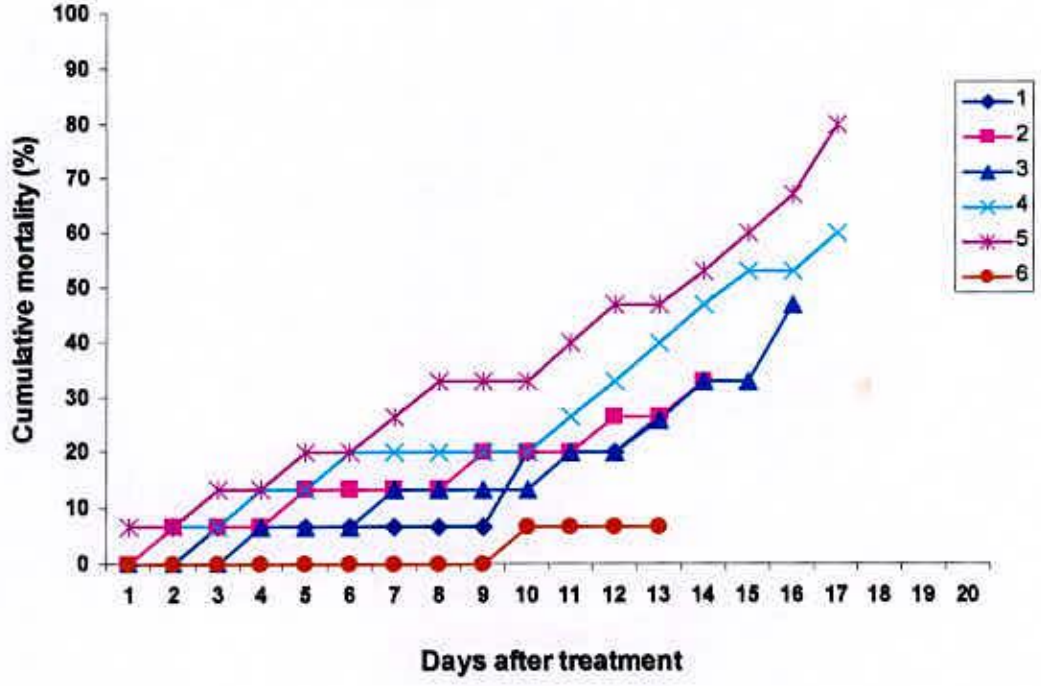


Figure 2. Effect of neem products on per cent cumulative mortality of 4th instar larvae of *S. obliqua*

In all cases mortality was increased with time of exposure during the study period. The longer the exposure period the greater the toxic effect of neem derivatives on larval mortality of the pest. The effect of neem products also varied with concentration, the higher the concentration the stronger the effect. From the above results, it is evident that neem products have significant effect on the larval mortality of *S. obliqua* and the effect being observed in the present study is varied with concentration and time of exposure. The same results were also observed by Ogemah *et al.* (2003). They observed that neem seed oil at high doses caused more than 80% mortality compared with 4% in the control.

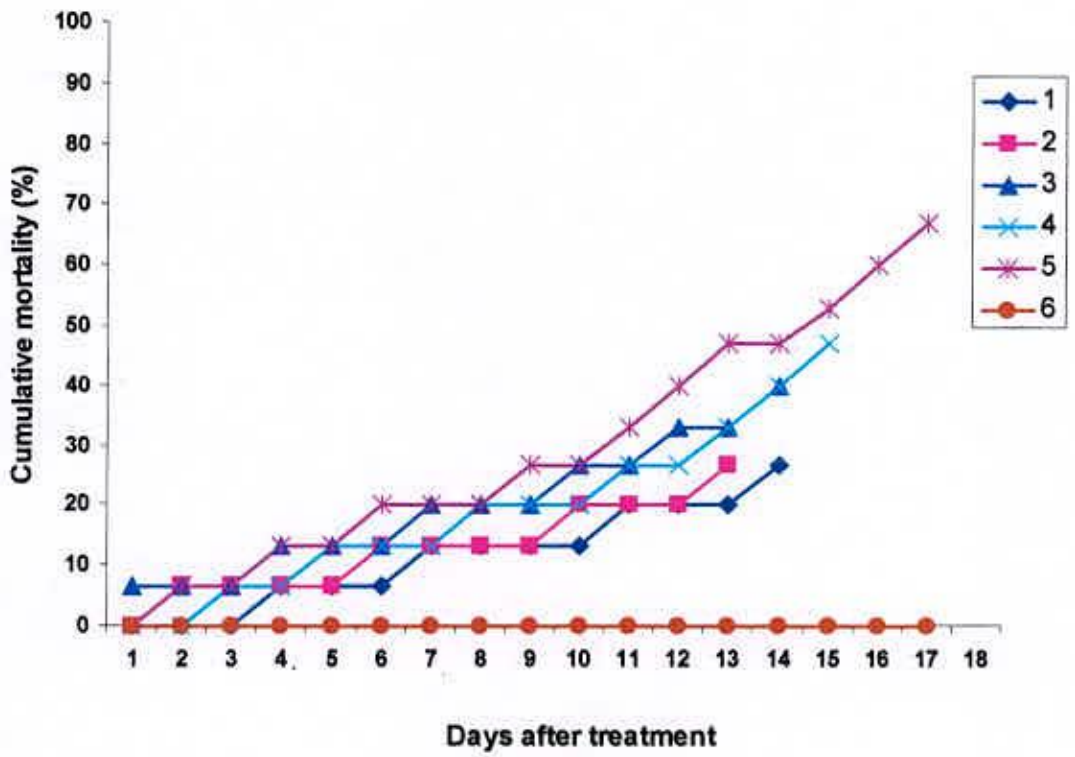


Figure 3. Effect of neem products on per cent cumulative mortality of 5th instar larvae of *S. obliqua*

4.1.2 Toxicity (LD_{50}) measurement

The median lethal doses (LD_{50}) of neem oil and leaf extract against different larval instars (3rd, 4th and 5th) of S. *obliqua* are presented below. Neem products tested against 3rd instar larvae of *S. obliqua* demonstrated considerable toxicity but varied with the time of exposure. The toxic effect increased with increase of exposure time. Thus as shown in Table 1, the LD_{50} of neem products against 3rd instar larvae was found to be the lowest (1.5695 mg) at 14 DAT exposure while it was maximum (18.0175 mg) at 10 DAT exposure. The relationship between the toxicity and the concentrations of neem products at different exposure has been expressed by the regression equation as shown in Table 1. The regression equation (Y= a + bx) expressed a positive relationship between the concentration and the toxicity. The regression coefficient (b) was always higher at higher exposure.

Table 1. LD_{50} value (50%) of neem products for 3rd instar larvae of *S. obliqua* at different days after treatment

Exposure (days)	Heterogeneity (X^2)	DF	Regression equation	Probability	LD_{50} (mg)
10 DAT	0.3416	3	Y=4.2245+0.6175X1.2556	0.9971	18.0170
12 DAT	0.9901	3	Y=4.4532+0.8597X0.6360	0.9901	4.3252
14 DAT	2.2335	3	Y=4.5762+2.1644X0.1957	0.9346	1.5695

DAT= Days after treatment

Similar to the 3rd instar larvae, neem products had a quite good toxicity against 4th instar larvae and varied in the toxic effect depending on the exposure time as shown Table 2. The LD_{50} against 4th instar larvae was found to be the lowest (1.1606 mg) at 14 DAT exposures while it was the highest (39.9104 mg) at 10 DAT exposures. This indicates that the larger the exposure period, the more the

toxic effect. These results also indicate the regression coefficients (b) were always higher at higher exposure.

Table 2. LD_{50} value (50%) of neem products for 4th instar larvae of *S. obliqua* at different days after treatment

Exposure (days)	Heterogeneity (X^2)	DF	Regression equation	Probability	LD_{50} (mg)
10 DAT	0.2154	3	Y=4.3797+0.2890X2.1458	0.9982	39.9104
12 DAT	0.3323	3	Y=4.5361+0.7825X0.5928	0.9957	3.9156
14 DAT	1.8118	3	Y=4.9480+0.8035X0.0646	0.9974	1.6160

DAT= Days after treatment

The neem products tested against 5th instar larvae, showed considerable toxic effect depending on the time of exposure. The LD_{50} value was minimum (2.2126 mg) at 14 DAT exposure reached to a maximum of (54.8666 mg) at 10 DAT exposure as shown in Table 3. Menhajul (1999) found that the median lethal dose (LD_{50}) value of neem oil against third, fourth and fifth instar larvae were 5.0172 mg and 3.488 mg in 3rd instar, 5.9325 mg and 4.0081 mg, in 4th instar and 6.2411 mg and 4.5383 mg in 5th instar at 12 and 14 DAT respectively on bean leaves during the winter season. Thus, the LD_{50} values as found in the present study were different from the findings reported by Menhajul (1999) but it was logical because he conducted experiment on different host plants and different season.

Table 3. LD_{50} value (50%) of neem products for 5th instar larvae of *S. obliqua* at different days after treatment

Exposure (days)	Heterogeneity (X^2)	DF	Regression equation	Probability	LD_{50} (mg)
10 DAT	0.6379	3	Y=4.4195+0.3337X1.7393	0.9982	54.8666
12 DAT	0.7603	3	Y=4.7553+0.5288X0.4626	0.9984	2.9014
14 DAT	1.3120	3	Y=4.7912+0.6052X0.3449	0.9984	2.2126

DAT= Days after treatment

Comparative toxicity of Neem products against different larval instar of *S. obliqua* at different exposure time has been shown in Figure 4. The graph reveals that there exist a very sharp distinction among the different instars of the insects in respect to toxic action of neem products and also a high degree of positive correlation between the toxicity and the dose of the neem products. The difference in correlation between the toxicity and the dose of neem products among different instars of *S. obliqua* may be attributable to the difference in vulnerability of the different instars of the insect. The results suggested that neem products were more toxic to 3rd instar larvae than 4th and 5th instar larvae. Similar to the difference in toxicity against different instars of *S. obliqua* observed in the present study, difference in toxicity to different instars of *S. obliqua* was reported by Menhajul (1999).

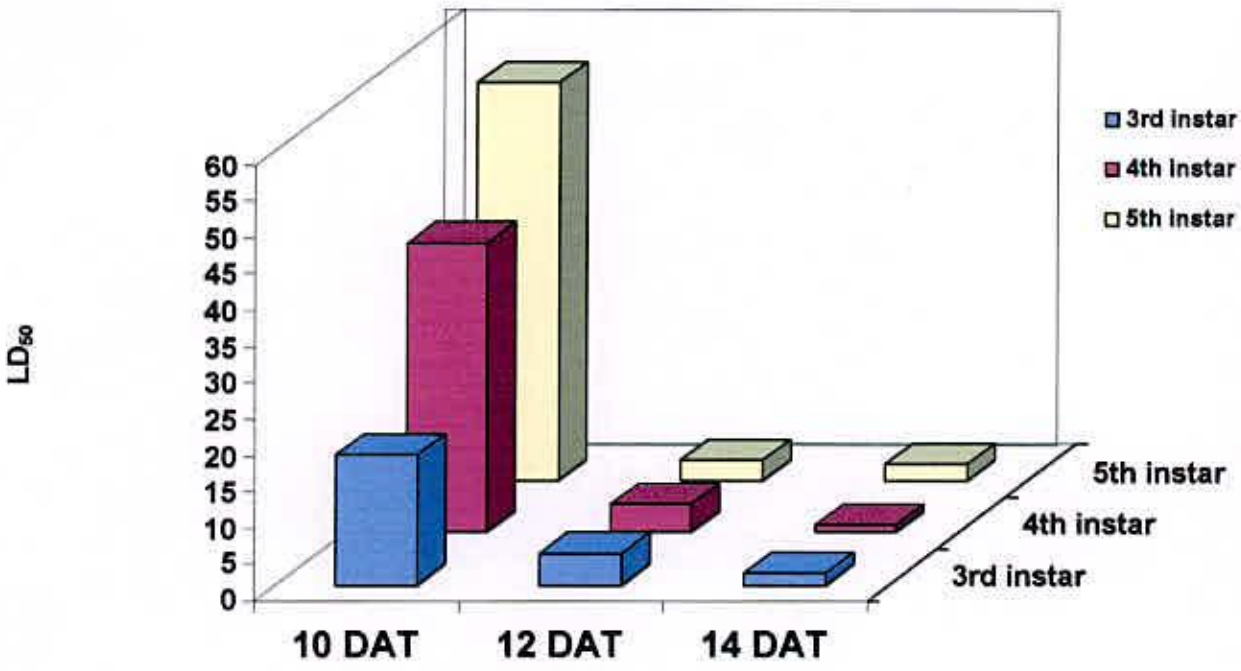


Figure 4. Comparative toxicity of neem products against different larval instars of *S. obliqua* at different exposure time.

4.1.3 Mortality counts on 3rd, 4th and 5th instar larvae after different time of exposure

The effect of neem products was tested against third, fourth, and fifth instars larval mortality of *S. obliqua* at 10 DAT, 12 DAT and 14 DAT. The highest mortality (33.33%) was observed in T_5 (neem oil 5%) and T_4 (neem oil 2.5%) after 10 days of treatment (Table 4) which was statistically similar to T_3 (26.67%), and T_2 (20.00%). The lowest mortality was observed in T_1 (0.00%), which was similar to T_1 and T_2. Among the treatments, T_5 and T_4 and T_3 were significantly more effective in controlling the 3rd instar larvae of *S. obliqua* compared to T_2, T_1 and T_6. The T_1 (neem extract, 1: 20) treatment was found to be statistically similar to those untreated control treatment, indicating its ineffectiveness in controlling this pest after 10 days of treatment.

Table 4. Effectiveness of some neem products on the mortality 3rd instar larvae of *S. obliqua* after 10, 12 and 14 days of treatment

Treatment	10 DAT	12 DAT	14 DAT
T_1	0.000 b	26.67 bc	33.33 c
T_2	20.00 ab	26.67 bc	33.33 c
T_3	26.67 a	33.33 abc	46.67 bc
T_4	33.33 a	53.33 ab	66.67 b
T_5	33.33 a	60.00 a	93.33 a
T_6	0.000 b	13.33 c	20.00 c
LSD (0.01%)	20.36	27.81	26.29
CV%	19.92	25.81	7.84

DAT= Days after treatment
Within column means followed by same letter (s) are not differ significantly from each other by DMRT test (1% level).

The mortality counts recorded after 12 days of treatment indicated that T_5 gave 60% mortality of the pest, which was statistically similar to T_4 (53.33%) and T_3 (33.33%) but significantly different from T_2 (26.67%) and T_1(26.67%) (Table 4). The mortality recorded after 14 days of treatment indicated that T_5 gave the highest mortality (93.33%) of test insects whereas mortality was the lowest (20.00%) in T_6 (Table 4). T_3 and T_4 were found to be statistically similar and

significantly different from T_1 in controlling this pest. No significant difference was found between T_1, T_2 and T_6 (untreated control) or statistically similar to those treatments, indicating their effectiveness in controlling this pest after 14 days of treatment.

The effect of neem products was determined against 4th instar larvae at 10 DAT, 12 DAT and 14 DAT. After 10 days of treatment, the highest mortality (33.33%) was observed in treatment T_5 and T_4 (Table 5), which was statistically similar with T_3 (20.00%), T_2 (20.00%) and T_1 (20.00%). The lowest mortality was observed treatment T_6 (0.00%) which is statistically similar with T_1. Therefore T_5, T_4 and T_3 had similar effect on larval mortality of *S. obliqua* at 10 DAT. After 12 days of treatment, the highest mortality was observed in treatment T_5 (60.00%), which was statistically similar with T_4 (46.67%) followed by T_3 (33.33%), T_2 (26.67%) and T_1 (20.00%) (Table 5). The lowest mortality was observed treatment T_6 (6.67%) which statistically different from T_5 and T_4. Therefore T_5 and T_4 had same effect on larval mortality at 12 DAT.

Table 5. Effectiveness of some neem products on the mortality 4th instar larvae of *S. obliqua* after 10, 12 and 14 days of treatment

Treatment	10 DAT	12 DAT	14 DAT
T_1	20.00 ab	20.00 cd	33.33 bc
T_2	20.00 ab	26.67 bcd	33.33 bc
T_3	26.67 a	33.33 bc	46.67 ab
T_4	33.33 a	46.67 ab	60.00 ab
T_5	33.33 a	60.00 a	80.00 a
T_6	0.00 b	6.67 d	6.67 c
LSD (0.01%)	20.36	23.51	37.18
CV%	17.03	21.34	21.73

DAT= Days after treatment
Within column means followed by same letter (s) did not differ significantly from each other by DMRT test (1% level).

Incase of 14 DAT, T_5 gave the highest mortality (80.00%) of the test insects which was statistically similar with T_4 (60.00%) and T_3 (46.67%), but

significantly different from T_2 (33.33%) and T_1 (33.33%). No significant difference was found between T_3 and T_4 but significant difference was observed only when T_3 and T_4 are compared with untreated control (T_6). Therefore, T_5 is more effective in controlling this pest at 14 DAT.

Table 6. Effectiveness of some neem products on the mortality 5th instar larvae of *S. obliqua* after 10, 12 and 14 days of treatment

Treatment	10 DAT	12 DAT	14 DAT
T_1	20.00 ab	26.67 b	26.67 b
T_2	26.67 a	26.67 b	26.67 b
T_3	26.67 a	40.00 ab	40.00 b
T_4	33.33 a	46.67 ab	46.67ab
T_5	40.00 a	60.00 a	66.67 a
T_6	0.00 b	0.00 c	0.00 c
LSD (0.01%)	20.36	20.36	23.51
CV%	16.28	13.08	13.94

DAT= Days after treatment
Within column means followed by same letter (s) are not significantly differ from each other by DMRT test at 1% level.

The mortality counts of the 5th instar larvae after 10 days of treatment indicated that the highest mortality (40.00%) in T_5 treatment followed by T_4 (33.33%), T_3 (26.67), T_2 (26.67)and T_1 (20.00%). All treatments were statistically similar and significantly effective in controlling this pest when compared the lowest mortality of the treatment T_6 (0.00%). In case of 12 days, highest mortality was observed in treatment T_5 (60.00%) and the lowest mortality in treatment T_6 (0.00%). Both treatments T_4 (46.67%) and T_3 (40.00%) were found to be statistically very similar and significantly differed from untreated control. After 14 days of treatment, the highest mortality was found treatment T_5 (66.67%) which is statistically similar with the T_4 (46.67%). Treatment T_3 (40.00%), T_2 (26.67%) and T_1 (26.67%) were statistically similar in controlling this pests. The lowest mortality was found in the treatment T_6 (0.00%).

The effect of neem products was determined against 3rd, 4th and 5th instar larvae to view the average mortality, pupal recovery and adult emergence. In 3rd instar result showed that the highest average mortality (93.33%) was found in treatment T_5 and the lowest mortality (20.00%) found in treatment T_6 (Table 8). No significant difference was found between T_3 (46.67%) and T_4 (66.67%) treatment but significant difference was observed with T_6 (untreated control). Therefore, T_5 was significantly more effective in controlling this pest when compared with other treatment (Table 7).

Table 7. Effect of neem products on the larval mortality, pupal recovery and adult emergence *of S. obliqua* at 3rd instar larvae

Treatment	Average mortality (%)	Pupal recovery (%)	Adult emergence (%)
T_1	33.33 c	66.67 a	66.67 ab
T_2	33.33 c	66.67 a	60.00 ab
T_3	46.67 bc	53.33 ab	40.00 bc
T_4	66.67 b	33.33 b	20.00 cd
T_5	93.33 d	6.67 c	6.67 d
T_6	20.00 c	80.00 a	80.00 a
LSD (0.01%)	26.29	26.29	26.29
CV%	21.56	20.62	23.14

Within column means followed by same letter (s) are not significantly differ from each other by DMRT test (1% level).

In pupal recovery, treatment T_5 showed the lowest percentage i.e. 66.67% than the other treatment. T_1, T_2 and T_6 showed the better performance to pupal recovery. So that, T_5 is the significantly more effective treatment in controlling this pest. Similarly, the adult emergence from 3rd instar larvae T_5 (6.667%) is the lowest performance and highest performance showed T_6 (80.00%). Both treatment T_5 and T_4 were found to be statistically similar and significantly more effective when compared with other treatment.

In case of 4th instar, the highest mortality was found in treatment T_5 (80.00%) while the lowest mortality was observe in T_6 (6.67%). Treatment T_4, T_3, T_2 and T_1 had similar effect on larval mortality. In pupal recovery, the highest number

was found in treatments T_6 (93.33%), which was statistically similar to T_1 and T_2 but significantly different from other treatments. The lowest pupal emergence was found in treatment T_5 (20.00%) which was significantly different from T_1 and T_2 (Table 8).

Table 8. Effect of neem products on the larval mortality, pupal recovery and adult emergence *of S. obliqua* at 4th instar larvae

Treatment	Average mortality (%)	Pupal recovery (%)	Adult emergence (%)
T_1	33.33 bc	66.67 ab	60.00ab
T_2	33.33 bc	66.67 ab	60.00 ab
T_3	46.67 ab	46.67 bc	40.00 bc
T_4	60.00 ab	40.00 bc	26.67 bc
T_5	80.00 a	20.00 c	13.33 c
T_6	6.67 c	93.33 a	93.33 a
LSD (0.01%)	37.18	42.39	35.27
CV%	21.73	20.16	20.16

Within column means followed by same letter (s) are not significantly differ from each other by DMRT test (1% level).

Table 9. Effect of neem products on the larval mortality, pupal recovery and adult emergence *of S .obliqua* at 5th instar larvae

Treatment	Average mortality (%)	Pupal recovery (%)	Adult emergence (%)
T_1	26.67 b	73.33 b	73.33 ab
T_2	26.67 b	73.33 b	66.67 b
T_3	40.00 b	60.00 b	53.33 bc
T_4	46.67 ab	53.33 bc	40.00 bc
T_5	66.67 a	33.33 c	26.67 c
T_6	0.00 c	100.00 a	100.0 a
LSD (0.01%)	23.51	23.51	31.11
CV %	13.94	8.45	11.46

Within column means followed by same letter (s) are not significantly differ from each other by DMRT test (1% level).

In 5th instar, the highest mortality was found T_5 (66.67%) followed by T_4 (46.67%) and T_3 (40.00%). The highest pupal recovery (100.00%) was found in T_6 (untreated control), which was significantly different from other treatments. The adult emergence (100.00%) was also the highest in T_6 (untreated control)

that was similar with T_1 but significantly different from other treatments. The lowest pupal recovery and adult emergence were observed in treatment T_5 which were significantly different from T1, T_2 and T_6 (Table 9).

The results indicated that neem oil caused larval mortality of *S. obliqua* and the mortality was dose dependent. Menhajul (1999) found that larval mortality ranged from 35% to 100% when different concentrations of neem seed oil were tested against *S. obliqua*. Maisary *et al.* (2004) conducted to examine the effect of neem oil the 2nd and 4th instars of *Culex pipiens* under laboratory condition. 46.98% of *C. pipiens* were killed upon exposure to 1000ppm of neem oil. The continuous treatment of the 2nd and 4th instars with neem oil (100ppm) caused high mortality and complete inhibition of the formation of mature instars. Ogemah *et al.* (2003) observed that neem seed oil at high doses caused more than 80% mortality compared with 4% in the control. The effect of neem (*Azadirachta indica*) oil at 0.25, 0.5, and 1.5% on larvae of *Epilachna vigintioctopunctata* was investigated (Shanmugapriyan and Kingsly, 2001). They found neem oil at 1.5% produced the highest mortality of 2nd and 3rd instars (95.23%), and 4th instars (76.19%). Neem oil at 0.25 and 0.5% concentrations resulted in 57.1 and 85.75% mortality in 2nd instars, 47.6 and 85.7% in 3rd instars and 57.1 and 80.9% in 4th instars. Salem (1991) found that the larval mortality ranged between 14.28 to 78.57% when different concentrations of neem seed oil were tested against the potato tuber moth, *Phthorimaea operculella* Zell. Loke *et al.* (1990) observed significant mortalities with neem oil against the larvae of *Plutella xylostella* L. The larval mortality of different insects caused by neem and neem products have also been reported by several researchers (Sudipta and Sanjib, 1998; Freisewinkel, 1993; Nicol, 1993; Schmutterer, 1992). The result obtained from the present investigation revealed that T_5 at the concentration was found to be the most effective bio pesticide in controlling the larvae of *S. obliqua*.

4.2 Effect of neem oil on the growth and development of *S. obliqua*

The effect of neem oil on the growth response of *S. obliqua* was evaluated by exposing fourth instar larvae to jute leaves treated with neem oil at 0.5% concentration either for 48 hours (48 hrs treatment) or continuously throughout the larval period (continuous treatment). Observations based on the effect of neem oil on the larval and pupal development of *S. obliqua* are presented in Table 10. In continuous treatment the larval period was prolonged compared to control and 48 hours treatment which were found effective to the larvae with regard to growth and development. The longest larval period was recorded as11.32 days in continuous treatment, whereas the lowest larval period was recorded as 11.08 days in control treatment. In 48 hours treatment, the larval period was recorded as 11.16 days. Pupal period slightly prolonged with the 48 hours treatment of neem oil.

Table 10. Duration of larval and pupal stages (days) of *S. obliqua* fed on neem oil treated and untreated jute leaves

Concentration (%)	Larval instar (days)			Total larval duration (days) (Mean ± SD)	Pupal period (days) (Mean ± SD)	Total Developmental period (days) (Mean ± SD)
	4th instar	5th instar	6th instar			
Control	3.12	3.04	4.92	11.08±0.20	10.04±0.20	21.16±0.47
0.5 (Continuous)	3.20	3.28	4.84	11.32±0.56	10.00±0.00	21.72±0.94
0.5 (48 hrs)	3.12	2.92	5.00	11.16±0.47	10.24±0.52	21.44±0.82
LSD (0.01%)	0.7759	0.8749	1.016	0.9733	0.6967	1.661
CV%	11.41	13.17	9.34	4.03	3.20	3.59

Within column means followed by same letter (s) did not differ significantly from each other by DMRT test (1% level)

The mean pupal period was recorded as 10.04, 10.00 and 10.24 days in control, continuous and 48 hours treatments, respectively. The mean developmental

period was 21.16, 21.72 and 21.44 days in control, continuous and 48 hours treatments, respectively (Table10).

The results on the growth and development of *S. obliqua* revealed that when the larvae were allowed to feed on neem oil at 0.5% concentration either for 48 hours or continuously throughout the larval period, they were slightly affected because of lower doses. Few numbers of larvae were unable to moult and they took longer time to complete the instar. In continuous treatments, the larvae which were pupated, their pupal period was longer. Many researchers have done their work on the growth and development of different insect pests. Neerja and Saroj (2003) evaluated an experiment and found that pupation was minimum at 2% neem oil. Pupation and adult emergence decreased with increasing Neem oil concentrations. Loke *et al.* (1990) reported that although the lower Neem oil concentrations appeared to be sub lethal with regard to contact toxicity effect, physiological and growth disruptive effects, such as retardation of growth (prolonged), delayed adult emergence and abnormal adults but the effects were more pronounced in the younger instar. Subipta and Sanjib (1998) reported that when larvae of *Corcyra cephalonica* were maintained in Neem oil absorbed food, with an initial pupation of 50 newly hatched larvae/100 g Neem absorbed food, deformed adults with a prolonged period of development were obtained. Growth inhibition, developmental disturbances and mortality increased markedly with increased dose. Growth inhitory or regulatory properties of Neem oil were reported by Lowery *et al.* (1996) on *Choristoneura rosaceana*: Venkateswarlu *et al.* (1993) on *Lipaphis erysimy*; Rao *et al.* (1993) on *Spodoptera litura*; Isman (1993) on *Actevia fennica* Tansch, *Mamestra configurata* Walker, *Periadroma saucia* Hubner, *Melanchra picta* Harr, *S. litura* Fab, *Trichoplusia ni* Hubner; Schmutterer(1992) on *Pieris brassicae*. The present findings on *S. obliqua* are almost similar to findings on various insects mentioned above. It appeared that the possible use of neem oil could hinder the normal growth patterns of insects, thereby preventing their survival.

4.3 Effect of neem oil on the morphological abnormalities of *S. obliqua*

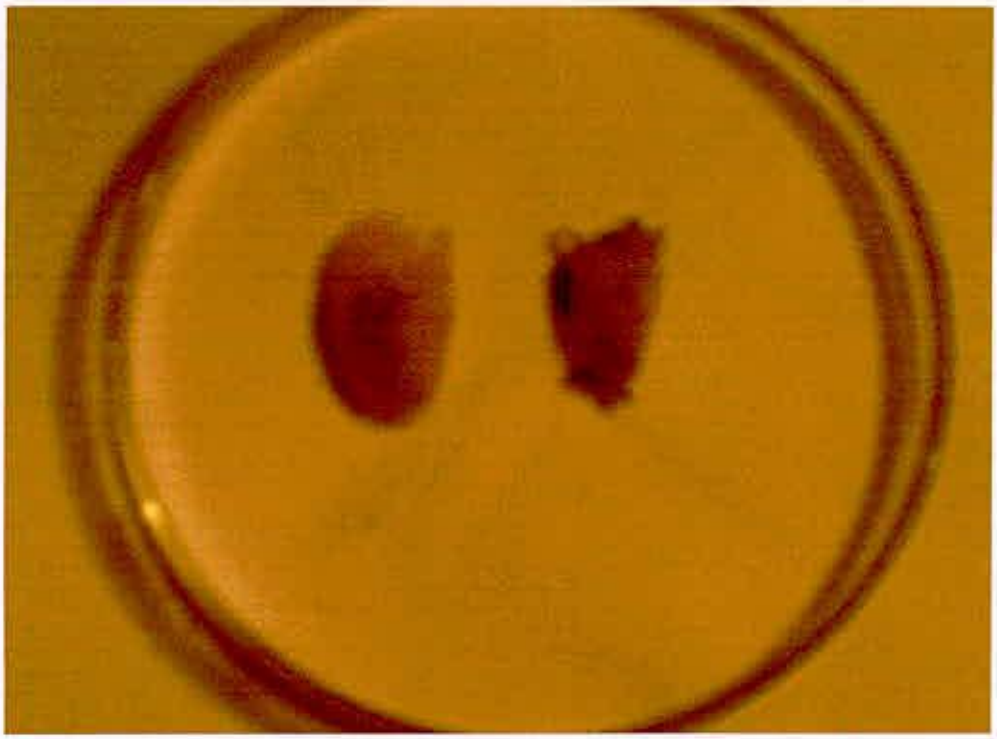


Plate 3. Larval-pupal intermediates of *S. obliqua* developed from treated larvae which failed to pupate.

Plate 4. Pupae of *S. obliqua* developed from treated and untreated larvae

Plate 5. Adult moths of *S. obliqua* developed from treated and untreated larvae

When fourth instar larvae of *S. obliqua* were fed on neem oil treated food; they showed morphological abnormalities in larval, pupal and adult stages. Some of the treated larvae passed through supernumerary moults and some larvae produced larval-pupal intermediates which never pupated (Plate 3). Some of the treated larvae were found to have black spots on the body surface, hairless or less hairy bodies, light and dark color and markedly smaller in size. Morphogenetic defects of larvae mainly occurred after ecdysis. The pupal abnormalities were found mostly on the pupal bodies, creaking of pupa and emission of transparent sticky liquid from of pupa. Pupae and adults developed from treated larvae were smaller in size compared to control. Most of the adults developed from larvae were weak and with malformed or crippled wings (Plates 4 and 5).

Several authors reported the morphogenetic abnormalities caused by the neem oil and other products. Lowery *et al.* (1996) found the morphogenetic abnormalities of neem seed oil on *Choristoneumera rosaceana* in the wings; Loke et al.(1990) observed the physiological and growth disruptive effect on *Plutellla xylostella* L.; Schmutterer *et al.* (1983) studied the morphogenetic effects of neem seed extracts on *Mythimna separata* and *Cnaphalocrosis medinalis;* Freisewinkel and Schmutterer (1991) showed the morphogenetic effects of neem oil on *Locasta migratoria migratorioides* in the legs, wings and antennae. Becker *et al.* (1992) observed that neem contains the active chemical azadirachtin, which disrupted hormonal changes in *Bemisia tabaci.* Similar results were also observed in the present study on *S. obliqua.* It may so happen that the chemical compounds present in the neem oil might be responsible for the abnormalities of the insects. Therefore, the findings found in the present study confirmed the results obtained by the other researchers.

4.4 Effect of neem oil on the food consumption of *S. obliqua*

The consumption of jute leaves treated with neem oil at 0.5% concentration and exposed to fourth instar larvae either for 48 hours (48 hrs treatment) or continuously throughout the larval period (continuous treatment) were found to vary among the treatments. In continuous treatment, the rate of food consumption was lower compared to control and 48 hours treatments. There was no significant difference in food consumption between 48 hours treatment and control. The mean food consumption in continuous and 48 hours treatments were 20193.00 and 29924.64 sq.mm leaf area compared to 27748.60 sq.mm in control (Table 11). Over control the percent decreased of food consumption was 27.22 in continuous treatment and increase was 7.84 in 48 hours treatment, respectively (Table 11).

Table 11. Effect of neem oil on food consumption of jute leaves by *S. obliqua*

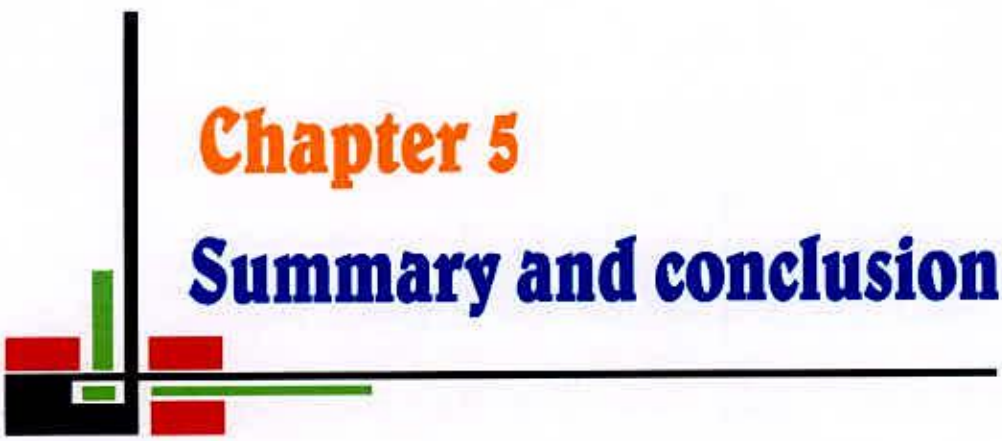
Concentration (%)	Food consumption (mm^2)			Total food consumption (mm^2)	Mean ±SD (sq. mm)	Percent decrease (-)/ increase (+) over control
	4^{th} instar	5^{th} instar	6^{th} instar			
Control	30885	110529	552301	693715	27748.60 ±7449.81	-
0.5 (continuous)	25395	95985	383445	504825	20193.0±4584.62	-27.22
0.5 (48 hrs)	40240	115395	592481	748116	29924.64±6504.02	+7.84

LSD (0.01%) = 13600
CV% = 24.25
Within column means followed by same letter (s) are not significantly different from each other by DMRT test (1% level).

The consumption of food by the larvae of *S. obliqua* treated with crude neem oil was found to reduce considerably in continuous treatment as compared to the untreated leaves. During continuous feeding antifeedant effect of neem is higher than 48 hrs treatment. Therefore food consumption is higher in 48 hrs

treatment. The lower food consumption might be due to the antifeedant properties of neem oil against different insect pests. Padmasheela, and Delvi, (2002) observed that Neem oil EC, deterrent feeding in terms of percent reduction of body weight at or above 25 ppm. But significantly feeding deterrence was observed in higher concentration against *oryctes rhinoceros*. Saxena *et al.* (1981a) reported that neem oil act as a potent antifeedant for controlling brown plant hopper, *Nilaparvata lugens*. Islam (1983) observed that crude neem seed oil as well as extracts of leaves and seeds of *A. indica* was a potential antifeedant or feeding deterrent for the control of brown plant hopper (BPH), rice green leaf hopper (RGLH), rice hispa and rice weevil. He also found that extract of neem seed kernels deterred feeding by adults of pulse beetle and early instar larvae of jute hairy caterpillar. The antifeedant activity and feeding deterrent properties of neem oil have been also reported by several researchers (Deca and Hazarika 1997, Raguraman *et al.* 1997, raman 1993, Isman 1993, Chiu-Shin-Foon *et al.* 1993, Matter *et al.* 1993, Rovesti and Desio 1990)

The reduction of feeding due to the presence of neem oil or leaf extract in the food was also recorded by Saxena *et al.* (1981b) on *Cnaphalocrosis medinalis* Guenee, Reed et al. (1982) on spotted cucumber beetle, *Acalyma vittatum* F.; Red Knap (1981) on the leaf eating larvae, *Epilachna chrysomelina*, leaf eating flea beetlea, *Podagrica uniforma* and *P. syastedti*; Saxena and Khan (1986) on *N. virescens;* Shin-Foon and Zhang (1984) on fall armyworm, *Spodoptera litura* F; Nesseh *et al.* (1993) on adults on *Schistocerca gregaria*; Salem (1991a) on *Phthorimaea operculella* Zell., Unchalle (1987) on *N. virescens*. Solsoloy and Solsoloy (1987) on cotton bollworm, Karel (1989) on *Ootheca bennigseni* Weise, Salem (1991b) on *pectinophora gossypiella* Saund and *Earias insulana* Boisd; Mayabini (1997) on *Cnaphalocrosis medinalis*, Haque *et al.* (1996) *Epilachna dodecastigma.*



Chapter 5

Summary and conclusion

Chapter V

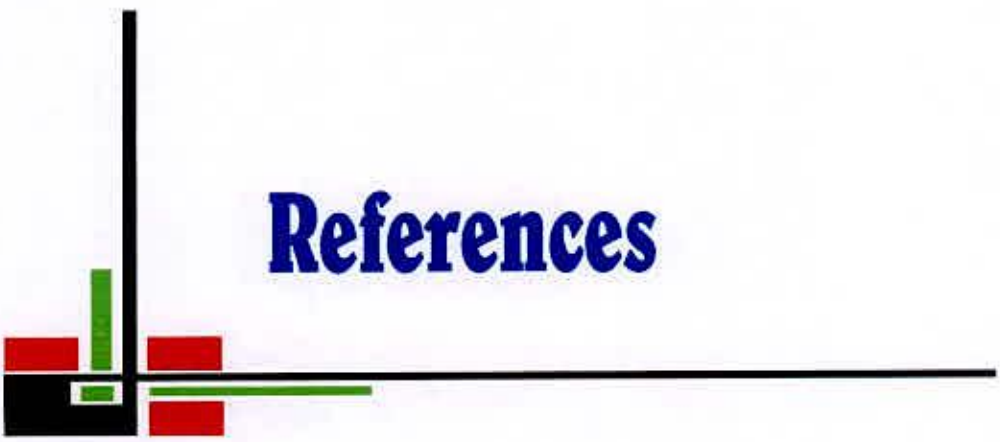
SUMMARY AND CONCLUSION

The effect of neem oil and neem leaf extract on mortality, growth and food consumption of jute hairy caterpillar, *Spilarctia obliqua* (Walker) were studied in the laboratory of Department of Entomology, Bangladesh Jute Research Institute, during April to August 2005. From the present study it was found that neem oil and leaf extract had significant effects on the mortality, growth and feeding responses against *S. obliqua*. For mortality test third, fourth and fifth instars larvae were exposed to treated jute leaves at 0.5, 1.0, 2.5 and 5.0% concentrations of neem oil emulsified with 0.1% Nikalin and neem leaf extract (1: 20) and compared with untreated (control). The larval mortality was recorded at different days after treatment (DAT) and highest mortality of all larval stages occurred at higher two (2.5% and 5.0%) concentrations. The lower concentrations of neem oil, viz. 0.5%, 1.0% and neem extract (1: 20) also markedly reduced the survival of the larvae as compared to control treatment. The median lethal dose (LD_{50}) of neem oil for different instars larvae at 0.5, 1.0, 2.5 and 5.0% concentrations and neem extract (1: 20) were also determined. The results indicated that neem oil caused larval mortality of *S. obliqua* and the mortality was dose dependent.

The effect of neem oil on the growth and feeding response *of S. obliqua* were evaluated by exposing fourth instar larvae to food treated with neem oil at 0.5% concentration either for 48 hours (48 hrs treatment) or for continuously throughout the rest of larval period (continuous treatment).The results on the growth and development of *S. obliqua* showed that the neem oil caused morphogenetic effects in all stages, when the larvae consumed treated leaves. Some treated larvae were found to have hairless and black spots on the body,

which might be due to the effects of neem oil on hormonal processes. Neem oil significantly prolonged larval and pupal periods; some of the treated larvae pass through supernumerary moults and did not pupate. Pupae developed from the treated larvae were smaller and adults emerged from these pupae were malformed or had crippled wings. The effect of neem oil on the feeding response also indicated that neem oil markedly reduced the amount of food consumption when fed continuously and acted as a potent antifeedant against *S. obliqua*.

In Bangladesh, crop losses occurred due to damage and deterioration by various insect pests in the field which led to import of large amount of insecticides every year for their control. The advent of synthetic insecticides, even the botanical insecticides that were in use went into oblivion. However, the longer persistence and extensive use of the synthetics resulted in a number of problems such as the development of resistance in target pests, elimination of parasites and predators resulting in secondary pest outbreaks, toxicity to higher animals, and environmental pollution. Neem oil is an indigenous plant product, a low cost alternative and a promising known botanical insect control agent. Its disrupting effects on mortality, development and feeding responses offer many opportunities to save the crops from different insect pests effectively and economically without hampering the ecological balance and agro-ecosystem. The present study will presumably help in future research and to improve the effectiveness of botanical products as insecticides for the benefit of agriculture sector. Though few works have been done on neem oil against different insect pests, research should be continued as it can be used as one of the components of IPM programme.



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APPENDICES

Appendix I. Duration of larval and pupal stages (days) of *S. obliqua* fed on untreated jute leaves (starting feeding from 4th instar)

Replication	4th instar(days)	5th instar (days)	6th instar (days)	Total larval instar(days)	Pupal period(days)	Total period(days)
1	3	3	5	11	10	21
2	3	3	5	11	10	21
3	3	3	5	11	10	21
4	4	4	4	12	10	22
5	3	3	5	11	10	21
6	3	3	6	12	11	23
7	3	3	5	11	10	21
8	3	3	5	11	10	21
9	3	3	5	11	10	21
10	3	3	5	11	10	21
11	3	3	5	11	10	21
12	4	3	4	11	10	21
13	3	3	5	11	10	21
14	3	3	5	11	10	21
15	3	3	5	11	10	21
16	3	3	5	11	10	21
17	3	3	5	11	11	22
18	3	3	5	11	10	21
19	4	3	4	11	10	21
20	3	3	5	11	10	21
21	3	3	5	11	10	21
22	3	3	5	11	10	21
23	3	3	5	11	10	21
24	3	3	5	11	10	21
25	3	3	5	11	10	21
Total	78	76	123	277	251	529
mean	3.12	3.04	4.92	11.08	10.00	21.16

Appendix II. Duration of larval and pupal stages (days) of *S. obliqua* fed on 0.5% (continuous) treated jute leaves with neem oil (starting feeding from 4th instar)

Replication	4th instar(days)	5th instar(days)	6th instar (days)	Total larval instar(days)	Pupal period (days)	Total period (days)
1	3	3	5	11	10	21
2	3	3	5	11	11	22
3	4	4	3	11	10	21
4	3	3	5	11	10	21
5	3	3	5	11	11	22
6	4	4	4	12	10	22
7	4	3	5	12	11	23
8	3	3	5	11	11	22
9	3	4	5	12	12	24
10	3	3	5	11	10	21
11	4	3	4	11	12	23
12	3	3	6	12	11	23
13	3	3	5	11	10	21
14	3	3	5	11	10	21
15	3	3	5	11	10	21
16	3	3	5	11	10	21
17	3	3	5	11	10	21
18	3	4	6	13	10	23
19	3	3	5	11	10	21
20	4	3	5	12	10	22
21	3	4	4	11	10	21
22	3	3	5	11	10	21
23	3	3	5	11	10	21
24	3	5	4	12	11	23
25	3	3	5	11	10	21
Total	80	82	121	283	250	543
Mean	3.20	3.28	4084	11.32	10.00	21.72

Appendix III. Duration of larval and pupal stages (days) of *S. obliqua* fed on 0.5 %(48 hrs) treated jute leaves with neem oil (starting feedin from 4th instar)

Replication	4th instar (days)	5th instar (days)	6th instar (days)	Total larval instar (days)	Pupal period (days)	Total period (days)
1	3	3	5	11	10	21
2	3	3	5	11	10	21
3	4	3	5	12	10	22
4	3	3	5	11	10	21
5	3	3	5	11	10	21
6	4	4	4	12	11	23
7	3	3	5	11	10	21
8	3	3	5	11	10	21
9	3	3	5	11	10	21
10	3	3	5	13	10	23
11	3	3	5	11	10	21
12	3	2	6	11	11	22
13	3	3	5	11	10	21
14	3	3	5	12	12	24
15	3	3	5	11	10	21
16	3	3	5	12	11	23
17	3	3	5	11	10	21
18	3	3	5	11	10	21
19	4	2	5	11	10	21
20	3	3	5	11	10	21
21	3	3	5	11	10	21
22	3	3	5	11	10	21
23	3	3	5	11	10	21
24	3	2	5	11	11	22
25	3	3	5	11	10	21
Total	78	73	125	279	256	536
Mean	3.12	2.92	5.00	11.16	10.24	21.44

Appendix IV. Total food consumption (mm^2) of S. oblique fed on neem oil treated and untreated jute leaves at 0% (control),0.5%(continuous) and 0.5% (48 hrs) concentration (starting feeding from 4^{th} instar).

Replication No.	Total food consumption at different concentrations		
	Control	0.5% (Continuous)	0.5% (48 hrs)
1	18500	25000	35400
2	30060	8125	35280
3	35875	20530	35700
4	30275	22100	32115
5	36200	12600	37117
6	19333	13400	35338
7	30200	17300	15615
8	28225	19545	30665
9	16520	25435	35650
10	20535	30255	30422
11	38500	20230	22611
12	20500	20500	12119
13	24045	20340	30475
14	20045	21350	25605
15	40625	25675	22640
16	24291	21500	27650
17	20625	12550	33120
18	40001	15200	32025
19	30005	23250	33020
20	23250	21200	30500
21	25122	23295	25822
22	35530	25235	28219
23	30615	22535	30640
24	35211	24050	40245
25	20122	20625	30123
Total	693715	504825	748116
Mean	27748.6	20193.0	29924.64

Appendix V. Analysis of varience of the data of mortality at different larval instar of difference days after treatment of *S. obliqua*

Source of variance	d.f	MEAN SUM OF SQUARE								
		3rd instar larvae			4th instar larvae			5th instar larvae		
		10 DAT	12 DAT	14 DAT	10 DAT	12 DAT	14 DAT	10 DAT	12 DAT	14 DAT
Treatment	5	715.55**	942.22*	2168.88**	462.22**	1088.88**	1906.66**	568.88**	1280.0**	1515.55**
Error	12	66.67	244.44	111.11	66.66	88.88	222.22	66.66	66066	88.88

** = Significant at 1% level
*= Significant at 5% level