EFFICACY OF THREE NON EDIBLE OILS AGAINST EPILACHNA BEETLE

(*EPILACHNA DODECASTIGMA* WIED.) (COLEOPTERA: COCCINELLIDAE)

A THESIS
BY

RUHUL AMIN
Registration No. 1605354
Semester: July – December, 2017

MASTER OF SCIENCE (M.S.)
IN
ENTOMOLOGY

DEPARTMENT OF ENTOMOLOGY
HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY
UNIVERSITY, DINAJPUR - 5200

DECEMBER, 2017

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A Thesis
Submitted to the Department of Entomology
Hajee Mohammad Danesh Science and Technology University, Dinajpur in partial fulfillment of the requirements for the degree of

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DEPARTMENT OF ENTOMOLOGY
HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY UNIVERSITY
DINAJPUR - 5200

DECEMBER 2017

DEDICATED TO

MY BELOVED

PARENTS

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The author

ABSTRACT

The study was conducted to evaluated the efficacy of three non edible oils namely, neem, pithraj and karanja in acetone solvent for their toxicity and antifeedant activity against *Epilachna dodecastigma* Wied. (Coleoptera: Coccinellidae) in the laboratory of the Department of Entomology, HSTU, during March to November, 2017. Four doses viz. 100, 75, 50 and 25 mg/ml along with control was used for mortality of third instar larva and 250, 200, 150 and 100 mg/ml along with a control for adult epilachna beetle. The doses of 100, 75, 50 and 25 mg/ml along with control were also used to determine the antifeedant activity of both third instar larva and adult. The results revealed that all the tested oils had toxic and antifeedant activity against epilachna beetle but pithraj oil was foud the most toxic as compared to others. The mortality and antifeedant activity of three oils and doses with hour after treatments were statistically different. In third instar larva, pithraj oil showed the highest mortality (84.69%) in maximum dose (100 mg/ml) whereas neem oil showed the lowest mortality (24.82%) in minimum dose (25 mg/ml). Similarly, pithraj oil showed the highest mortality (76.67%) in maximum dose (250 mg/ml) and neem oil showed the lowest average mortality (14.33%) in minimum dose (100 mg/ml) against adult beetle. The order of the toxicity of the oils were found as pithraj > karanja > neem. Mortality percentages were also found directly proportional to the hour after treatments. The mortality was found as dose dependent where highest mortality was observed in maximum doses while the lowest in minimum dose in both the larva and adult. The highest antifeedant index was found in pithraj (93.19%) and the lowest in neem (68.81%) against third instar larva. On the other hand, the karanja oil provided the highest antifeedant index (77.99%) while pithraj oil showed the lowest index (62.65%) in adult beetle.

Key words: *Epilachna dodecastigma*, non edible oils, toxicity, antifeedant index.

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CHAPTER I

INTRODUCTION

INTRODUCTION

There are many biotic and abiotic factors those constraints to sustainable production of vegetables. Among the biotic factors, major and common insect pests such as epilachna beetle, fruit fly, red pumpkin beetle, aphid, thrips etc are predominant. They cause variable level of damages to roots, stems, leaves, flower buds, flowers, pods and fruits, i.e. from seedling to entire growing stage. The extent of damage varies from year to year, season to season and locality-to-locality depending on the seasonal abundance of the pests affected and impact of control measures adopted. The area under vegetable production was 992000 acres in 2015-16 with the production of 3877000 M. tons (BBS 2016).

Among insect pests, two species of Epilachna beetle viz., *E. dodecastigma* (Wied.) and *E. vigintioctopunctata* (Fab.) are the serious pests of vegetables in Bangladesh. Out of two species, *E. dodecastigma* is fairly common and causes damage to the cucurbitaceous and solanaceous crops (Anam *et al*. 2006, Rahaman *et al*. 2008, Islam *et al.* 2011).

Epilachna dodecastigma (Wied.) is one of the major important vegetable pests in South-East Asia. It is widely distributed in South and East Asia, Australia, America and the East Indies. Distressing attacks by genus of *Epilachna* have been reported from China on potato, from Sumatra on datura (Den Doop 1919) and from the United States on bean (Dieke 1947).

Both grubs and adults cause severe damage to the host plants and infestation primarily begins just after hatching of egg mass (Murata *et al.* 1994). The nature of damage by the larvae is somewhat distinct from that of the adults. The grubs start their infestation gregariously and later on, they are dispersed close to the next host plants. The grubs feed on the epidermal layer of leaves making concentric markings whereas the adults feed irregularly upon the upper surface of leaves and its grubs also feed on the lower surface of leaves by scraping, causing net like appearance of the leaves that turn brown in color, entirely dry up due to extensive infestation by the growing population and finally defoliate (Pradhan *et al.* 1990). Consequently, vegetative growth and development of the plants are greatly harmed and their economic yield is noticeably declined (Rajagopal and Trivedi 1989). From economic point, the epilachna beetle bears a great significance because it can alone damage up to 80% of the host plants (Rajagopal and Trivedi 1989). The life cycle of the epilachna beetle takes about twenty five days under local conditions and a few generations can occur during a cropping period causing severe damage to the vegetable crop, which may result in an economic loss to the farmer.

Epilachna beetles feed mostly in the morning and evening during the summer and feeding reduces rapidly in the middle of the day and after mid night. The daily fluctuation in the rate of feeding depends mainly on the temperature of the environment which estimates the level of metabolism (Tilavov 1981). The peak infestation of epilachna beetle varies with regions, however in general the peak is found in July to August (Tilavov 1981). Insect pest prevalence and its degree of infestation vary from season to season, place to place due to the environmental changes especially temperature, and host plant species by the reason of its compositional variation (Amitava *et al.* 2002).

Insecticides are the major means of insect control in all crops including vegetables in Bangladesh. Synthetic insecticides are widely used in most developing countries to control insect pests of food crops. Recently, the use of insecticide has considerably increased in vegetables. According to BBS (2016), the use of insecticides was 14266.44 M tons. Synthetic chemical insecticides have been widely preferred to combat this pest problem because of their quick knock-down. But chemical protection suffers so many serious drawbacks such as acute and chronic poisoning of applicators, farm workers, consumers, fishes, birds and other wild life animals etc. (National Research Council 2000, Rohani *et al*. 2001).

The biologically active natural plant products may play a significant role in this regard because they are environmentally safe, biodegradable and cost effective. 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). Plant products contain botanical insecticides or phytochemicals that could be used to repel, deter feeding, or limit reproduction and survival of various insect pest species including coccinellid beetles (Rajagopal and Trivedi 1989, Swaminathan *et al*. 2010).

There are about 2000 plant species that possess pest control properties. Among them neem oil, mahogany seed have extensively been used and has proved its pest controlling efficacy against several insect pests both in field and storage (Heyde *et al.* 1983). Neem and pithraj seed extract has no adverse effects in the agro-ecosystem and its cost of production is likely to be less compared to that of chemical insecticide (Saxena *et al.* 1981). Talukder and Howse (1993) have evaluated toxic effect of pithraj (*Aphanamixis polystachya*) extract on the red flour beetle. It is highly toxic and antifeedant against various insects also. Neem oil is widely used as a pesticide. They also have antifeedant, repellent, growth inhibitory properties, egg laying deterrent and impaired reproductive ability (Schmutterer 1995, Kumar and Babu 1998). The biological efficacy of neem has been proved against a wide range of agricultural pests for more than 20 years (Ostermann 1993). Karanja oil is known to be effective against large number of insect pests such as shoot and fruit borers (Rao *et al*. 2002), leaf miners (Katole *et al*. 1993), aphids (Kulat *et al.* 1997), white flies, plant hoppers (Hiremath *et al*. 1997), leaf hoppers

(Mariappan *et al.* 1988), mites, leaf feeding beetles (Reddy *et al.* 1990), storage pests (Gupta *et al.* 1988), flies (Mathur *et al.* 1990) and mosquito larvae (Sagar *et al.* 1999).

However, exploitation on the use of neem oil against epilachna beetle is widely studied in our country but the oil of pithraj and karanja is very few in Bangladesh. The present study was therefore, undertaken to evaluate the efficacy of pithraj, neem, karanja oils against epilachna beetle, *Epilachna dodecastigma* (Wied.).

Research Objectives: In view of perspectives, the present experiment was conducted to evaluate the effectiveness of pithraj, neem and karanja oil against epilachna beetle. The specific objectives were as follows:

- To evaluate the effect of pithraj, neem and karanja oil on mortality of third instar larva and adult of epilachna beetle.
- To find out the most effective dose of pithraj, neem and karanja oils.
- To determine the antifeedant index of three tested non edible oils against epilachna beetle.

CHAPTER II

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Literature cited below under the following headings and sub-headings reveals information of the test insect and use of botanicals and synthetic pesticide at home and abroad.

2.1 General review of epilachna beetle

2.1.1 Common name

Epilachna beetle

Squash lady beetle

Mexican bean beetle

Eastern lady beetle

2.1.2 Synonyms

Epilachna dodecastigma Wied.

Epilachna vigintioctopuntata Fab.

Epilachna borealis Fab.

Epilachna varivestis Mul.

Epilachna tredecimnotata Lat.

2.1.3 Systematic position

Kingdom: Animalia
Phylum: Arthropoda
Superclass: Hexapoda
Class: Insecta
Subclass: Pterygota
Order: Coleoptera
Suborder: Polyphaga
Superfamily: Cucujoidea
Family: Coccinellidae
Genus: *Epilachna*
Species: *E. dodecastigma* (Wied.)

2.1.4 Origin and distribution

Epilachna beetle is a versatile and widely distributed insect. It is widely distributed in Asia, Europe, Australia, America and West Indies. Severe infestation of this insect has been reported from India, China, Sumatra, Mexico, Guatemala, Florida, Canada and England (Biddle *et al.* 1992).

2.1.5 Host ranges

Epilachna beetle feeds on a variety of host plants mainly among the cucurbitaceae, solanaceae and leguminosae family. This pest has been recorded on many hosts but potato is the principal food. The epilachna beetle has also been recorded on such hosts as brinjal, tomato, tobacco, bottle gourd, sweet gourd, bitter gourd and other cucurbits (Cranshaw 2004).

2.1.6 Nature of damage

Both larval and adult stages feed on the leaves of the plant. The larvae are responsible for great level of injury as are the adults. They usually feed by clinging to the lower surface of the leaves and eating the tender parenchyma, leaving the upper epidermis intact. The upper surface of the leaves quickly dries out after the lower section is injured, giving a lace-like, skeletonized appearance. The leaves finally turned brown and growth of young plants may be completely stopped (Cranshaw 2004).

The 28-spotted potato ladybird or hadda beetle, *Epilachna vigintioctopunctata* Fab. (Coleoptera: Coccinellidae), is one of the devastating pests of vegetable crops in Bangladesh. It is fairly common throughout the country and causes considerable damage to a number of solanaceous, cucurbitaceous and leguminous crops (Alam 1969).

Plate 1: Damage symptom caused by epilachna beetle

2.1.7 Life history

The *E. dodecastigma* (Wied.) has a complete metamorphosis with distinct egg, larval, pupal and adult stages.

Egg: Freshly laid eggs are pale-yellow to orange yellow, elongated, usually in clusters of 5 to 45. An egg is approximately 1.3mm in length and 0.6mm in width. Under laboratory conditions the incubation period varied from 5 to 11 days with an average of 8 ± 2.23

days. The incubation period of was observed as 5.3 days on brinjal, by Varma and Anandhi (2008).

Larva: Newly hatched larvae or grubs are approximately 1.6 mm in length and light yellow in color. Larvae are soft bodied and covered with six longitudinal rows of stout branched spines on the back. At first the spines are yellow, but later become darken on the tips, and thus more conspicuous. Larva molts four times during the development. Mature larva is approximately 6-7mm in length. The average duration of the 1st, 2nd, 3rd and 4^{th} instar was recorded as 2.5±1.17 days, 4.5±1.92 days, 5.5±2.86 days and 5±2.23 days respectively. During their earlier stages the grubs are gregarious in nature but as they grow older they tend to split into smaller groups.

Pre-pupa

Fully grown 4th instar grubs gradually stop feeding and spend about 1-3 days in pre-pupal stage. The average pre-pupal length and breadth recorded as 5.16±0.57 mm and 2.87±0.40 mm, respectively, by Tyade and Simon (2013).

Pupa: Pupation occurs when mature larvae aggregate and attach themselves, by the posterior end of their bodies, to the underside of the leaves or stem. Larvae pupate in this position. During pupation the larval skin is pushed backwards from the thorax towards the abdomen, where it remains as a whitish, wrinkled mass. It ceases its motion and pupates. Pupa is yellow, spineless, and of the same size and shape as of the adult. The pupal period lasts for 3-5 days with an average of 4±0.79 days. Similar results were obtained by Verma and Anandhi (2008) and Qamar *et al.,* (2009).

Adult: The adult is oval in structure, about 6-7 mm in length. The color of newly emerged adult is straw or cream-yellow. Shortly after emergence, twelve black spots of variable size appear on fore wings. The adults darken with age until they become an orange brown with a bronze tinge, at that time the black spots are less conspicuous.

The males are slightly smaller than the females. Males can be distinguished from females by having a small notch on the ventral side of the last abdominal segment. The adults are strong fliers and travel long distances searching for new fields. The beetles over winter in moist, protected places and remain dormant until spring (Roberts and Douce 1999).

2.2 *Azadirachta indica*

Neem is an evergreen, tall, fast-growing tree, which can reach a height of 25m and 2.5m in girth. It has an attractive crown of deep green foliage and masses of honey scented flowers. A full-grown tree can produce 30 to 100 kg of fruits, depending on rainfall, soil type, and ecotype. About 50 kg of fruit yields 30 kg of seed, which gives 6 kg of oil and 24 kg of seed cake. Neem has more than 100 unique bio-active compounds, which have potential applications in agriculture, animal care, public health, and for even regulating human fertility.

Maria *et al.* (2005) evaluated ethanolic extract of aerial parts of *Artemisia annua* and artemisinin as anti-insect products. Complete mortality in *Epilachna paenulata* and 50% mortality in *Spodoptera eridania* were observed with extract at 1.5 mg/cm^2

Abdel-Moniem *et al.* (2004) reported that neem anal- T/ S (neem seed kernel extract containing 1% azadirachtin), neudosan (potassium soap + rape oil) and spruzitflussig (natural pyrethrum + pyrethrin + piperonylbutoxide) at different concentrations were evaluated against the second and fourth instar larvae of *Epilachnac htysomelina* under laboratory conditions. In case of acute effects, the highest mortality was recorded when larvae were offered squash leaves treated with the highest concentration (2%) of neem-azal T/S, whereas the lowest mortality recorded after treating them with the lowest concentration (0.25%). On the other hand, the second instar larvae were more susceptible to any compound at any concentration than the fourth instar larvae. Neudosan 1%

formulation showed the highest toxicity to both second and fourth instar larva. As the concentration of this compound decreased, the mortality percentage of the treated larvae decreased.

Rajesh *et al.* (2003) observed the toxicity of neem formulations viz. bio-neem, nimbicidine, neemarin and neemazal (0.5, 1.0 and 1.5%) to *Spodoptera litura* infesting tobacco under laboratory conditions. Larval mortality at 24 h after application with 5% neem formulations was not significant. After 72h, neemarin at 1.5% resulted the highest larval mortality (90%) followed by neemazal (83.35%), bioneem (63.33%) and nimbicidine (53.33%).

Abdel-Moniem (2003) studied the efficacy of some neem products against adults of the melon ladybird beetle, *Epilachna chrysomelina* under laboratory and semi field conditions at 30 ± 5°C and 70 ± 5% R.H. The data recorded at 24, 48, 72 and 96 h after treatment revealed that neem products were significantly superior to the untreated control. Two percent nivaar (1.5% azadirachtin) seemed to be the most effective treatment and significantly superior to all other treatments followed by 2% neemazal-T (3% azadirachtin) and 2% neemazal-T (1% azadirachtin). The least effective treatment was observed using 1% neemazal-T.

Swaran *et al.* (2002) found neem oil micro-emulsion significantly more bio-active than its macro-emulsion against *Helicoverpa armigera* in respect of increasing larval mortality.

Singh (2002) conducted an experiment on French bean in summer season with nine treatments comprising combinations of three fertilizer and three pesticides namely neemax @ 1.0 kg/ha, garlic extract @ 10 L/ha and endosulfun @ 1.2 L /ha. He found that the mortality of the bean beetle, *Epilachna varivestis* was more in case of neemax compared to endosulfun.

Shanmugapriyan and Kingsly (2001) reported the effects of neem oil at 0.25, 0.5 and 1.5% on larvae of *E. vigintioctopunctata.* Neem oil at 1.5% concentration caused the highest mortality of third (95.23%) and fourth instar (76.19%) larvae. Neem oil at 0.25 and 0.5% concentrations resulted in 57.1 and 85.7 % mortality of second instar, 47.6 and 85.7 % of third instar and 57.1 and 80.9% of fourth instar larvae.

Karmakar and Bhole (2001) observed the efficacy and persistent toxicity of some neem products such as neem oil, neembicidine and neemark against adult of *Epilachna dodecastigma* (Weid). The treatments with 2% neem oil, 2% neembicidine and 2% neemark resulted 100%, 90.69% and 71.90% mortality, respectively.

Imtiaz *et al.* (2001) observed the effects of neem leaf extracts and Cyhalothrin on adult *Sitophilus onyzae*. Glass film method was adopted to determine the LC_{50} rate. After plotting a graph between mortality and concentration, the LC_{50} was found 0.53 and 0.44 microgram/sq.cm, respectively.

Aziz and Kelany (2001) investigated the effects of two neem *(Azadirachta indica)* formulations viz. neemazal-T (1% Azadirachtin) and neemazal-T (5% azadirachtin) on some biological aspects of the two-spotted spider mite, *Tetranychus urticae* under laboratory conditions. Neemazal-T was highly effective which showed high mortality and reduced the number of eggs laid by females.

Bream *et al.* (1995) tested azadirachtin and jojoba oil on development and morphogenesis of the red palm weevil, *Rhynchophorus ferrugineus.* Nine dose-levels of jojoba oil (joj) or six doses of azadirachtin (azt) were topically applied on the pre-pupae of *R. ferrugineus.* The dose range of joj was 0.200- 0.001 pg/insect and of azt was 0.500-0.001 jag/insect. With regard to azt, the lethal action increased by the age of pupae. By each extract, increased water loss may act as one of the importance causes of pupal death. Pre-pupal maximal body weights decreased, irrespective of the extract, and were reflected on small growth index of pre pupae and pupae.

Nadia and Schmidt (1992) tested margosan-O, a commercial preparation of neem seed kernel extract for its effects as toxicant, deterrent and sterilant against the bean aphid, *Aphis jabae* and compared with the German product neem-azal S. Second and fourth nymphal instars of *A. jaba,* placed on bean plants treated with different concentrations of margosan-O or neem-azal S, were adversely affected and very high percentage of mortality occurred after 96 hours.

2.3 *Aphanamixis polystachya*

All the plant extracts were found to be effective repellents and having feeding deterrent action against the beetle.

Podder *et al.* (2013) were assessed the effectiveness of pithraj seed extract, bishkatali leaf extract, neem oil, mehagony oil, and diazinon 60 EC for managing epilachna beetle, *Epilachna dodecastigma* (Wied.) . Pithraj, biskatali Neem and mahogany treated plots showed significant variation over untreated control in aspects of percent population reduction of epilachna beetle.

Rahman *et al.* (2001) conducted an experiment to evaluate the seed oils of castor (*R. communis*), neem (*A. indica*), pithrai (*A. polystachya*), safflower (*Carthamus tinctorius*) and sesame (*Sesamum indicum*) against *Alphitobius diaperinus*. Adult insects were fed on wheat grains with the mentioned oils at concentrations of l, 2, 3, 4 and 5%. Insect mortality was recorded at 24, 48, 72 and 96 hours after treatment (HAT). Significant toxicity was obtained with neem, pithraj, safflower and sesame oils. LC_{50} values indicated that sesame oil was most toxic at 24 HAT (3.63%) and 48 HAT (2.27%), while pithraj oil was most toxic at 72 HAT (2.236%) and 96 HAT (1.91%).

Talukder and Howse (2000) observed the Sub-fractions of an acetone extract of *Aphanamixis polystachya* (Meliaceae) seeds were evaluated for their feeding deterrent effects on adult *Tribolium castaneum* (Coleoptera: Tenebrionidae). The results show that a sub-fraction A- 142 was highly feeding deterrent to *T. castaneum*. This fraction was isolated and analyzed by Gas Chromatography-Mass Spectrometry (GC-MS). Four compounds were identified. 2-methoxy-2-hydroxy propanoic acid might be the active compound against *T. castaneum*.

Islam and Shahjahan (2000) conducted experiments to evaluate the toxicity of five botanicals, neem (*A. indica*), marigold (*T. erecta*), durba (*Cynodon dactylon*), castor (*R. communis*), pithraj (*Aphanamixis polystachya*) and one insecticide (Malathion) against rice weevil (*S. oryzae* L.) and red flour beetle (*T. castaneum* Herbst.). The results showed that the water extracts of all the five botanicals were effective in controlling the rice weevil and red flour beetle infesting stored grains. Among the botanicals, neem was found to be the most toxic ranking next to Malathion. The order toxicity was as follows: Malathion>neem>pithraj> marigold>durba.

Talukder and Howse (1995) stated that the ground leaves, bark and seeds of *A. polystrachya* provided protection of wheat flour by reducing F_1 progeny of *T. castaneum*.

Talukder and Howse (1993) have noted toxic effect of pithraj (*Aphanamixis polystachya*) extract on the red flour beetle.

2.4 *Pongamia pinnata*

Mahesh *et al.* (2016) determine the antifeedant and larvicidal activity of *Pongamia pinnata* chemical compositions were assayed for their toxicity test against polyphagous field pest, *Spodoptera litura*. Chemical constituents of twenty compounds were identified in the *P. pinnata* compounds representing to 99.96%. Phytol, 1,2-Benzenedicarboxylic acid, diisooctyl ester, n-Hexadecanoic acid and 3,7,11,15-Tetranethyl-2-hexadecen-l-ol compounds of *P. pinnata* are evaluated for their antifeedant properties against *S. litura* reared in the laboratory on the host castor leaf. Experiments to evaluate antifeedant activity of *P.pinnata* compounds conducted under no-choice method. In no-choice condition only treated leaves were provided as food to the larvae. The larvicidal activity of *P. pinnata* was determined against *S. litura* at concentrations of 10, 20, 30, 40 and 50 ppm. Data pertaining to the experiments clearly revealed that four compounds of *P. pinnata* showed varying degree of feeding deterrency. Phytol, 1,2-Benzenedicarboxylic acid, diisooctyl ester, n-Hexadecanoic acid and 3,7,11,15-Tetranethyl-2-hexadecen-l-ol compounds of *P. pinnata* showed similar but less profound trends in antifeedant activity against fourth instar larvae of *S. litura*. Antifeedant activity of Phytol compound of *P. pinnata* was 100% on treated leaf disc against *S. litura* at 2.5 ppm concentration. The LC_{50} and LC_{90} values of Phyto compound of *P. pinnata* were 24.51 ppm and 42.40 ppm, respectively.

Phytol compound of *P. pinnata* exerted strong antifeedant and larvicidal activity to the *S. litura*. The results indicate moderate level of antifeedant and larvicidal activity against fourth instar larvae of *S. litura*.

Mohanchander *et al.* (2013) was evaluates the nature of feeding damage of *Epilachna vigintioctopunctata* on leaves of *Solanum melongena* and antifeedant activities of essential oil formulations against *E. vigintioctopunctata.* Investigations were made of on the morphological and anatomical damage caused by the feeding activity of the adults and grubs. Maximum antifeedant activities 80.06% were observed in formulation Ш (composed of karanja , neem, zinger and emulsifier)

Kumar *et al.* (2006) was found the Methanolic extracts of crude seed oil of *Pongamia pinnata* (Karanj) showed the maximum growth reduction and antifeedancy against the larvae of *S. litura* (EC_{50} : 0.11 and 0.21% respectively) and was also most toxic to *Trogoderma granarium* (EC_{50}: 35.1 µg/insect) whereas karanj leaves (EC_{50}: 19.9 µg/insect) exhibited the toxicity against *Tribolium castaneum*. Crude seed oil of karanj showed the maximum repellency (EC_{50}: 0.2 mg/ cm^2) against *T. granarium*.

CHAPTER III

MATERIALS AND METHOD

MATERIALS AND METHODS

3.1 Location of the study

The experiment was carried out to evaluate the efficacy of three non edible oils against epilachna beetle (*Epilachna dodecastigma* Wied.) (Coccinellidae: Coleoptera) in the laboratory of the Department of Entomology, Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur, Bangladesh during March to November, 2017.

3.1.1 Tested materials

Three non edible oils of pithraj, neem and karanja were used as test materials (Plate 2). The oils were collected from the local market of Dinajpur town.

Plate 2: Oils of pithraj, karanja and neem (From left to right side respectively).

3.2 Collection and rearing of *E. dodecastigma*

In order to meet the supply of the test insect, a stock culture of large number of grubs and adult beetles of *E. dodecastigma* was maintained in the laboratory and in the field. Adult males and females were collected from heavily infested sweet gourd and sponge gourd plants of HSTU campus. In the laboratory, the adult beetles were sexed and confined in pairs in the Petri dishes for mating and egg laying. Fresh and healthy leaves were supplied everyday to meet up the food requirements in the laboratory. After oviposition, adult beetles were transferred into glass jar (1000 ml) and the eggs were left undisturbed for hatching. Immediately after hatching the larvae were transferred to several glass jars (500 ml) and allowed to feed on fresh leaves. Ten larvae were maintained per glass jar. The newly emerged adults were again sexed and confined in pairs in the Petri dishes for mating and egg laying. The process was continued for the whole period of the study. Fresh sponge gourd leaves were supplied regularly to maintain stock culture.

3.3 Determination of the larval instar

Third instar larva or grub of epilachna beetle was determined by number of moulting i.e. larval exuviae (old cuticle / skin). Moulting of larva of *E. dodecastigma* took place three times. The larva of first instar with the marginal outlines oblong in shape and general body color was light yellow. Tubercles had distinct scoli. In the larva of second instar the scoli were more branched than those of first instar. The scoli of third and final instar larva were more branched than those of second instar (Rahman M. S. 2008). The average duration of the third instar was recorded as 5.5±2.86 days (Tara and Sharma 2017).

3.4 Food sources

Sponge gourd was cultivated in the research field of the Department of Entomology for continuous supply of food. The dust free fresh leaves were used as food for the epilachna beetle in the laboratory everyday.

3.5 Dose preparation

3.5.1 For mortality

Four doses of 100, 75, 50 and 25 mg/ml of three selected oils were prepared by diluting with acetone solvent for the third instar larva and 250, 200, 150, and 100 mg/ml for adult.

3.5.2 For antifeedant activity

Four doses of 100, 75, 50 and 25 mg/ml of three selected oils were prepared by diluting with acetone solvent for both third instar larva and adult.

Prior to conducting study, a pilot experiment was done to obtain the appropriate dose.

3.6 Insect bioassay test

3.6.1 Toxicity test

Direct toxicity test of three non edible oils against *E. dodecastigma* was conducted by dipping the larvae and adult in four concentrations just for a moment. Ten insects was used in each treatment and each concentration and replicated thrice. Only solvent with equal number of insect were treated as control. Insect mortality was recorded at 24, 48, and 72 hours after treatment. The percentage of the mortality was corrected by the Abbott's (1987) formula.

$$P = \frac{p' - C}{100 - C} \times 100$$

Where,

P= Percentage of corrected mortality

P'= Observed mortality (%)

C= Mortality (%) at control.

3.6.2 Antifeedant activity test

Antifeedant activity of three non edible oils was assayed against third instar larvae and adult of *E. dodecastigma* using a leaf disc (6 cm). The experiment was done in Petri dishes (9 cm diameter). Moist cotton sheet covered with filter paper was kept at the bottom of each Petri dish in order to maintain optimum humidity and to keep the leaves fresh. The leaf disc was treated by dipping method with different concentration of each oil. Both adults and third instar larva were taken from stock culture and released singly per petridish to treated food (Plate 3). Fifteen replications of each treatment and each dose along with control treatments were maintained. Data on food consumption was measured by using square millimeter graph paper. Antifeedant activity was assessed by calculating the formula as stated by Isman *et al.* (1990).

$$\text{Antifeedant Index} = \frac{\text{Leaf area consumed in control} - \text{Leaf area consumed in treated}}{\text{Leaf area consumed in control} + \text{Leaf area consumed in treated}} \times 100$$

Plate 3: Antifeedant activity test of three non edible oils against third instar larva of epilachna beetle

3.7 Statistical analysis

The data from the experiment was statistically analyzed by Completely Randomized Design (CRD) using MSTAT-C statistical software. The mean values were adjusted by Duncan's New Multiple Range Test (DMRT). The observed mortality data were subjected to probit analysis.

CHAPTER IV

RESULTS AND DISCUSSION

RESULTS AND DISCUSSION

The experiment was conducted to find out the toxicity effect and antifeedant activity of three non edible botanical oils against *E. dodecastigma*. The results are presented and discussed under the following sub-headings:

4.1 Probit analysis of third instar larva of *E. dodecastigma* for direct toxic effect

The probit analysis for the mortality of third instar larva of epilachna beetle was performed for the estimation of LD_{50} values, chi-square values and their 95% fiducial limits at 24, 48 and 72 HATs are presented in Table 1. The LD_{50} values of pithraj (58.57 mg/ml), karanja (96.23 mg/ml) and neem oils (751.69 mg/ml) at 24 HAT indicated that pithraj oil was the most toxic followed by karanja and neem oil. Considering the LD_{50} values at 48 HAT of pithraj (29.84 mg/ml), karanja (40.15 mg/ml) and neem oil (254 mg/ml), indicated that pithraj oil was the most toxic followed by karanja and neem oil. Among the three non edible oils, pithraj also performed the highest toxicity as compared to the LD_{50} values at 72 HAT (12.41 mg/ml). From the non-significant chi-square values it was observed that all the equations fitted well with no heteregenity. The mortality data of third instar larva of epilachna beetle are also shown Appendices 1-9.

Table 1: Probit analysis of three non edible oils against third instar larva of *E. dodecastigma* after 24, 48 and 72 HATs

Treatments (oils)	No. of insect used	LD_{50} values (mg/ml)	95 % fiducially limits		χ^2 values with 2 df
			Lower	Upper	
		24 HAT			
Pithraj	30	58.57	44.687	76.769	0.58459
Neem	30	751.69	17.226	32802.09	0.20560
Karanja	30	96.23	35.176	263.283	0.02621
		48 HAT			
Pithraj	30	29.84	19.109	46.611	0.68355
Neem	30	254.16	29.117	2218.66	0.16773
Karanja	30	40.15	10.10848	159.4317	0.19170
		72 HAT			
Pithraj	30	12.41	4.235	36.403	1.79179
Neem	30	55.40	29.367	104.529	0.26618
Karanja	30	24.86	15.237	40.557	0.89299

HAT = Hours after treatment values were based on four doses, three replications of 10 insects each.

χ^2 = Goodness of fit

The tabulated value of χ^2 =5.99 with 2 df at 5 % level.

4.2 Probit analysis of adult *E. dodecastigma* for direct toxic effect

The probit analysis of the adult mortality at 24, 48 and 72 HATs using three botanical oils are presented in Tables 2. At 24 HAT, the LD_{50} values of pithraj (260.66 mg/ml), karanja (342.56 mg/ml) and neem oil (443.54 mg/ml) indicated that pithraj oil was the most toxic and neem oil was the least toxic. The LD_{50} values of pithraj (170, and 132.62 mg/ml), karanja (208.78 and 140.09 mg/ml) and neem (348.20 and 241.56 mg/ml) at 48 and 72 HAT repectively indicated that pithraj oil noted as most toxic followed by karanja and neem oil. The chi-square values were insignificant at 5 % level of probability of three non edible oils at different HAT and mortality data of the adults also did not show any heterogeneity. The mortality data of adult beetles are also shown in Appendices 10-18.

From the above probit results it was observed that all the tested three non edible oils would more or less effective against epilachna beetle but pithraj oil had the most effective followed by the karanja and neem oil. The lowest LD_{50} values of pithraj oil showed the highest toxic effects against both the larvae and adult of epilachna beetle. The present finding agreed with Rahman *et al.* (2001) where they found that the seed oils of *A. polystachya* was most toxic at 72 HAT (2.236%) and 96 HAT (1.91%) against *Alphitobius diaperinus* on wheat grains. Islam and Shahjahan (2000) conducted experiments to evaluate the toxicity of five botanicals, pithraj (*Aphonamixis polystachya*), neem (*A. indica*), marigold (*T. erecta*), durba (*Cynodon doctylon*), castor (*R. communis*), and one insecticide (Malathion) against rice weevil (*S. oryzae* L.) and red flour beetle (*T. castaneum* Herbst.). They found that the pithraj extract have significant toxic effect against those insects.

Table 2: Probit analysis of three non edible oils against adult *E. dodecastigma* after 24, 48 and 72 HATs

Treatments (oils)	No. of insect used	LD $_{50}$ values (mg/ml)	95 % fiducially limits		χ^2 values with 2 df
			Lower	Upper	
		24 HAT			
Pithraj	30	260.66	205.5114	330.6143	1.066199
Neem	30	443.54	187.204	1050.857	0.634801
Karanja	30	342.56	211.1468	555.7587	1.153246
		48 HAT			
Pithraj	30	170.90	153.1502	190.7145	0.9182644
Neem	30	348.20	180.5794	671.4194	2.439016
Karanja	30	208.78	172.1019	253.2725	0.6161671
		72 HAT			
Pithraj	30	132.62	119.4554	147.2531	1.264736
Neem	30	241.56	168.4337	346.4262	1.088863
Karanja	30	140.09	123.2913	159.1805	4.044356

HAT = Hours after treatment values were based on four doses, three replications of 10 insects each.

χ^2 = Goodness of fit

The tabulated value of χ^2 =5.99 with 2 df at 5 % level.

4.3 Direct toxicity of three non edible oils against third instar larva of *E. dodecastigma*

Toxicity effect of three non edible oils, doses and their interaction at different hour after treatment (HAT) against the third instar larva of *E. dodecastigma* are presented in the Tables 3-5. The average mortality percentage of epilachna beetles differed significantly (P>0.05) among all the oils applied. The mortality percentage at 24 hours after treatment, the pithraj oil showed highest mortality (39.04%) whereas neem oil has the lowest mortality (12.67%). Similarly, the highest (54.37%, 67.48%) and the lowest (24.67%, 40.74%) mortality was observed in pithraj and neem oil, respectively at 48 and 72 HAT. The average mortality percentage of epilachna beetle at 24, 48 and 72 HATs also indicated the highest morality (53.63%) in pithraj oil whereas neem oil perfomed the lowest mortality (26.02%) (Table 3). Insect mortality increased proportionally with the time interval. On the basis of insect mortality the order of the toxic effect of three non edible oils against epilachna beetle were found as pithraj>karanja>neem.

Table 3: Direct toxic effect of three non edible oils against third instar larva of *E. dodecastigma* at different HATs

Botanical oils used	Insect mortality (%) in different time intervals			Average mortality (%)
	24HAT	48HAT	72HAT	
Pithraj	39.04 a	54.37 a	67.48 a	53.63 a
Karanja	34.67 a	42.00 b	58.59 b	45.09 b
Neem	12.67 b	24.67 c	40.74 c	26.02 c
Control	0.00 c	0.00 d	0.00 d	0.00 d
LSD	8.217	9.527	6.691	6.871
CV (%)	38.27	31.66	16.13	22.16
P-value	0.001	0.001	0.001	0.001

HAT = Hour after treatment, within column values followed by different letter (s) are significantly different by DMRT at 5 % level of probability.

Mortality of epilachna beetle was also differed significantly (P>0.05) among all the concentrations level at different times of interval (Table 4). The highest mortality (80.49%) was observed in the maximum doses (100 mg/ml) whereas the lowest was found in control treatment (3.33%) at 72 HAT. The highest average mortality (63.62%) was also found at the maximum dose (100mg/ml) of selected oils and the lowest average mortality (38.72%) was found at the lowest dose of 25mg/ml.

Table 4: Direct toxic effect of doses of three non edible oils against third instar larva of *E. dodecastigma* at different HATs (Dose effect)

Doses (mg/ml)	Insect mortality (%) in different time intervals			Average mortality (%)
	24HAT	48HAT	72HAT	
100	48.40 a	61.98 a	80.49 a	63.62 a
75	38.15 ab	52.96 ab	73.58 ab	54.90 ab
50	33.70 b	47.28 bc	65.43 b	48.81 b
25	22.59 c	38.40 c	55.19 c	38.72 c
Control	1.111 d	1.111 d	3.333 d	1.851 d
LSD	10.61	12.30	8.638	8.870
CV (%)	38.27	31.66	16.13	22.16
P-value	0.001	0.001	0.001	0.001

HAT = Hour after treatment, within column values followed by different letter (s) are significantly different by DMRT at 5 % level of probability.

The interaction effect of non edible oils, their doses and different time interval indicated that there was a significant different among the toxicity of the tested oils when applied against the third instar larva of epilachna beetle (Table 5). In 24 HAT, the highest mortality (71.85%) was found in pithraj oil at the highest dose (100 mg/ml) whereas neem oil showed the lowest mortality (10%) at lowest dose (25 mg/ml). Similarly, at 48 HAT, the highest mortality (85.93%) was observed in pithraj oil at the maximum dose (100 mg/ml) but neem oil showed the lowest mortality (23.33%) at the lowest dose (25 mg/ml). In 72 HATs, pithraj oil showed the highest mortality (96.30%) at the maximum dose (100 mg/ml) and lowest mortality (55.19%) was found in neem oil at the lowest dose (25 mg/ml).Average value indicated that the highest percent mortality (84.69%) was found in pithraj oil at highest dose (100 mg/ml) whereas neem oil showed the lowest mortality (24.82%) at lowest dose (25 mg/ml), which was statistically different from all other non edible oils at different dose levels. The ANOVA tables are also shown in Appendices 19-21.

Table 5: Direct toxic effect of three non edible oils of different doses against third instar larva *E. dodecastigma* at different HAT (Interaction of oils, doses and time)

Botanical oils used	Doses (mg/ml)	Insect mortality (%) in different time intervals			Average mortality (%)
		24HAT	48HAT	72HAT	
Pithraj	100	71.85 a	85.93 a	96.30 a	84.69 a
	75	51.11 b	72.22 ab	85.93 ab	69.75 ab
	50	44.44 b	65.19 abc	79.26 b	62.97 bc
	25	24.44 cde	45.19 c-g	72.59 bc	47.41 c-f
	Control	3.333 fg	3.333 hi	3.333 f	3.333 i
Karanja	100	50.00 b	60.00 bcd	86.67 ab	65.55 b
	75	46.67 b	53.33 b-e	79.26 b	59.75 bcd
	50	43.33 bc	50.00 b-f	71.85 bc	55.06 b-e
	25	33.33 bcd	46.67 c-g	51.85 de	43.95 d-g
	Control	0.0000 g	0.0000 i	3.333 f	1.110 i
Neem	100	23.33 def	40.00 d-g	58.52 cd	40.62 e-h
	75	16.67 d-g	33.33 efg	55.56 de	35.19 fgh
	50	13.33 d-g	26.67 fg	45.19 de	28.39 gh
	25	10.00 efg	23.33 gh	41.11 e	24.82 h
	Control	0.0000 g	0.0000 i	3.333 f	1.110 i
LSD		18.37	21.30	14.96	15.36
CV (%)		38.27	31.66	16.13	22.16
P-value		0.0359	0.1695	0.0228	0.0322

HAT = Hour after treatment, within column values followed by different letter (s) are significantly different by DMRT at 5 % level of probability.

4.4 Direct toxicity of three non edible oils against adult *E. dodecastigma*

Direct toxicity effect of three non-edible oils, doses and their interactions at different time intervals against the adult of *E. dodecastigma* are presented in the Tables 6-8. Average mortality percentage of epilachna beetle differed significantly among all the tested oils (P>0.05). The mortality percentage of the epilachna beetle at 24 hours after treatment indicated that pithraj oil showed the highest (20.67%), while neem oil performed the lowest mortality (16.00%) followed by karanja . Similarly, at 48 hours after treatment pithraj oil also showed the highest (39.33%) while neem oil provided the lowest mortality (20.67%). After 72 hours of treatment pithraj also showed the highest (54.00%) but the lowest mortality (29.33%) implied in neem oil. The average mortality percentage of epilachna beetle at 24, 48 and 72 hours after treatment showed that pithraj oil observed the highest morality (38.00%), meanwhile neem oil referred the lowest mortality (22.00%) (Table.6). On the basis of insect mortality, the order of the toxic effect of three non edible oils against epilachna beetles were found as pithraj > karanja > neem.

Table 6: Direct toxic effect of three non edible oils against adult *E. dodecastigma* at different HAT

Botanical oils used	Insect mortality (%) in different time intervals			Average mortality (%)
	24HAT	48HAT	72HAT	
Pithraj	20.67 a	39.33 a	54.00 a	38.00 a
Karanja	16.00 b	30.67 b	49.33 b	32.00 b
Neem	16.00 b	20.67 c	29.33 c	22.00 c
Control	0.00 c	0.00 d	0.00 d	0.00 d
LSD	4.584	4.447	4.447	3.496
CV (%)	35.01	19.73	13.48	15.29
P-value	0.0716	0.001	0.001	0.001

HAT = Hour after treatment, within column values followed by different letter (s) are significantly different by DMRT at 5 % level of probability.

Mortality of adult epilachna beetle was also differed significantly among all the doses at different time interval (Table 7). In 24, 48 and 72 HAT, the highest mortality was found 40.00%, 62.22% and 83.33% respectively at the highest dose (100 mg/ml) whereas the lowest mortality 7.77%, 14.44% and 25.56% respectively at the lowest dose (25 mg/ml). The average value indicated that the highest mortality (61.85%) was counted in 100 mg/ml and lowest mortality (15.92) was found in 25 mg/ml. Highest dose possessed the highest mortality while lowest dose possessed the lowest mortality.

Table 7: Direct toxic effect of doses of three non edible oils against adult *E. dodecastigma* at different HAT (Dose effect)

Doses (mg/ml)	Insect mortality (%) in different time intervals			Average mortality (%)
	24HAT	48HAT	72HAT	
250	40.00 a	62.22 a	83.33 a	61.85 a
200	25.56 b	47.78 b	67.78 b	47.04 b
150	14.44 c	26.67 c	44.44 c	28.52 c
100	7.778 d	14.44 d	25.56 d	15.92 d
Control	0.00 e	0.00 e	0.00 e	0.00 e
LSD	5.917	5.741	5.741	4.514
CV (%)	35.01	19.73	13.48	15.29
P-value	0.001	0.001	0.001	0.001

HAT = Hour after treatment, within column values followed by different letter (s) are significantly different by DMRT at 5 % level of probability.

Interaction effects of non edible oils, doses, and time are presented in table 8. The result revealed that there was a significant different among the tested oils when applied against the adult of epilachna beetle (P>0.05). Average mortality indicated that the highest mortality (76.67%) was found in pithraj oil at maximum dose (250 mg/ml) whereas neem oil showed the lowest mortality (14.33%) at lowest dose (100 mg/ml), which was statistically different from all the oil at different dose level. The ANOVA tables are also shown in Appendices 22-24.

Table 8: Direct toxic effect of three non edible oils and doses against adult *E. dodecastigma* at different HATs (Interaction of oils, doses and time)

Botanical oils used	Doses (mg/ml)	Insect mortality (%) in different time intervals			Average mortality (%)
		24HAT	48HAT	72HAT	
Pithraj	250	53.33 a	80.00 a	96.67 a	76.67 a
	200	26.67 bcd	70.00 b	93.33 a	63.33 b
	150	16.67 def	33.33 e	56.67 c	35.55 d
	100	6.676 fg	13.33 g	23.33 e	14.44 f
	Control	0.0000 g	0.0000 h	0.0000 f	0.0000 g
Karanja	250	33.33 b	60.00 c	96.67 a	63.33 b
	200	30.00 bc	50.00 d	73.33 b	51.11 c
	150	10.00 efg	26.67 ef	46.67 c	27.78 de
	100	6.667 fg	16.67 fg	30.00 de	17.78 f
	Control	0.0000 g	0.0000 h	0.0000 f	0.0000 g
Neem	250	33.33 b	46.67 d	56.67 c	45.55 c
	200	20.00 cde	23.33 efg	36.67 d	26.67 e
	150	16.67 def	20.00 fg	30.00 de	22.22 ef
	100	10.00 efg	13.00 g	20.00 e	14.33 f
	Control	0.0000 g	0.0000 h	0.0000 f	0.0000 g
LSD		10.25	9.943	9.943	7.818
CV (%)		35.01	19.73	13.48	15.29
P-value		0.0189	0.001	0.001	0.001

HAT = Hour after treatment, within column values followed by different letter (s) are significantly different by DMRT at 5 % level of probability.

The tested three non edible oils showed toxic effect against epilachna beetle, but pithraj oil could be most effective. The present finding agreed with Rahman *et al.* (2001) who found the lethal effects of *Aphanamixis polystachya, A. indica, Carthamus tinctorius, R. communis and Sesamum indicum* oils on *Alphitobius diaperinus.* The major active constituent of pithraj is aphanamixin, which is well known for toxic effects on insects (Talukder and Howse 1995). Podder *et al.* (2013) were assessed the effectiveness of pithraj seed extract, bishkatali leaf extract, neem oil, mehagony oil, and diazinon 60 EC for managing epilachna beetle, *Epilachna dodecastigma* (Wied.) where pithraj extracts showed significant toxic effect compared with other extraxts and oils.

4.5 Probit regression lines for third instar larva

The probit regression lines for the bio-efficacy of three non edible oils against third instar larvae of epilachna beetle at 24, 48 and 72 HAT are presented in figures 1- 3. The calculated probit regression equation for three oils against epilachna beetle at 24 HAT were Y=1.908x+2.761, Y=2.176x+1.937 and Y=0.790x+3.623 for pithraj, karanja and neem oils, respectively (Figure 1). Among them, the regression line for pithraj oil showed the highest probit mortality and neem oil showed the lowest probit mortality. The probit regression equation at 48 HAT were Y=1.865x+2.234, Y=0.501x+4.188 and Y=0.782x+3.126 for pithraj, karanja and neem oils, respectively (Figure 2). Compairing among three tested oils at 48 HAT for probit mortality, pithraj oil showed the highest probit mortality whereas neem oil showed the lowest. Pithraj oil showed the highest mortality also at 72 HAT and the probit regression lines were Y=1.908x+2.761, Y=2.176x+1.937 and Y=0.790x+3.623 for pithraj, karanja and neem oils, respectively (Figure 3).

The insect mortality rate showed positive correlation with the doses in all treatments. The probit regression lines of three botanical oils showed a clear linear relationship between probit mortality and their log doses and the regression lines become sleeper as doses increased.

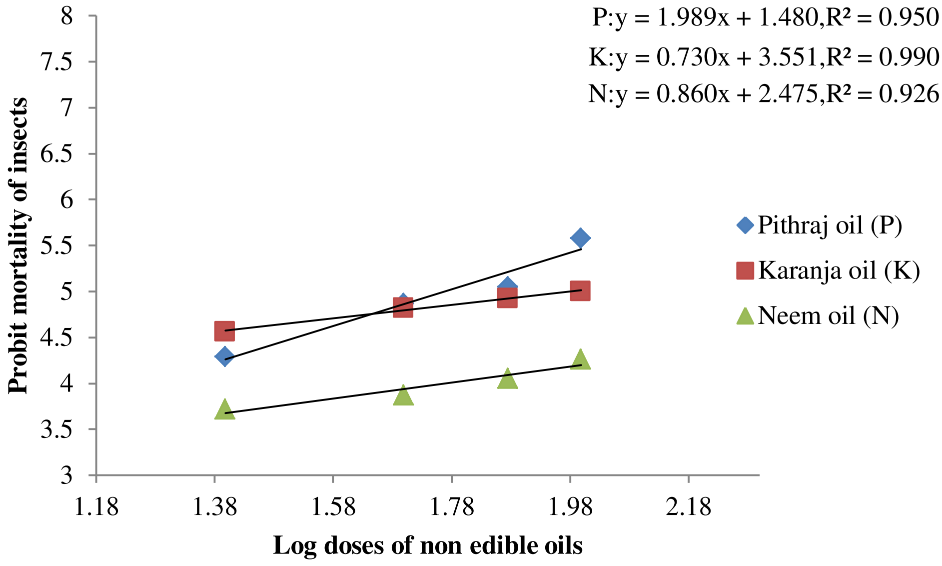

Fig.1. Relationship between probit mortality and log doses of three non edible oils on third instar larva of *E. dodecastigma* at 24 HAT

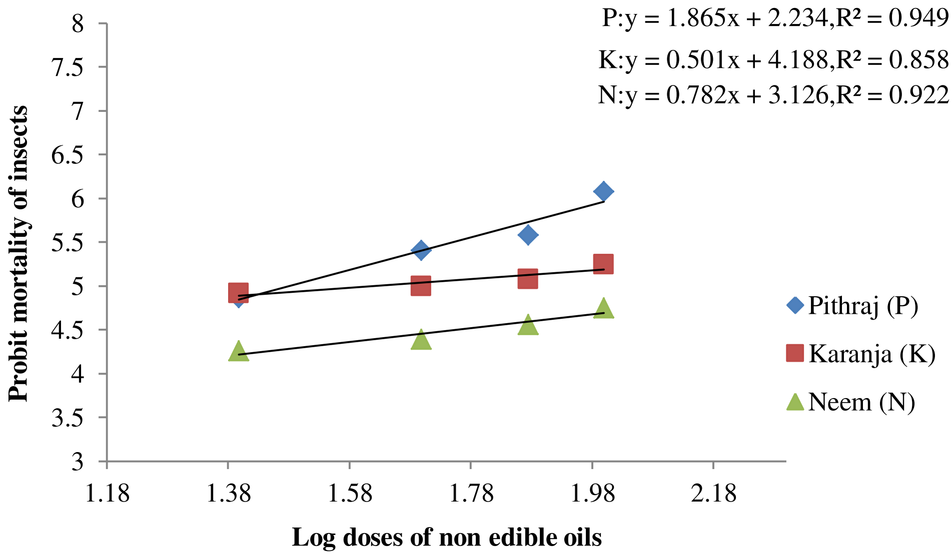

Fig.2. Relationship between probit mortality and log doses of three non edible oils on third instar larva of *E. dodecastigma* 48 HAT

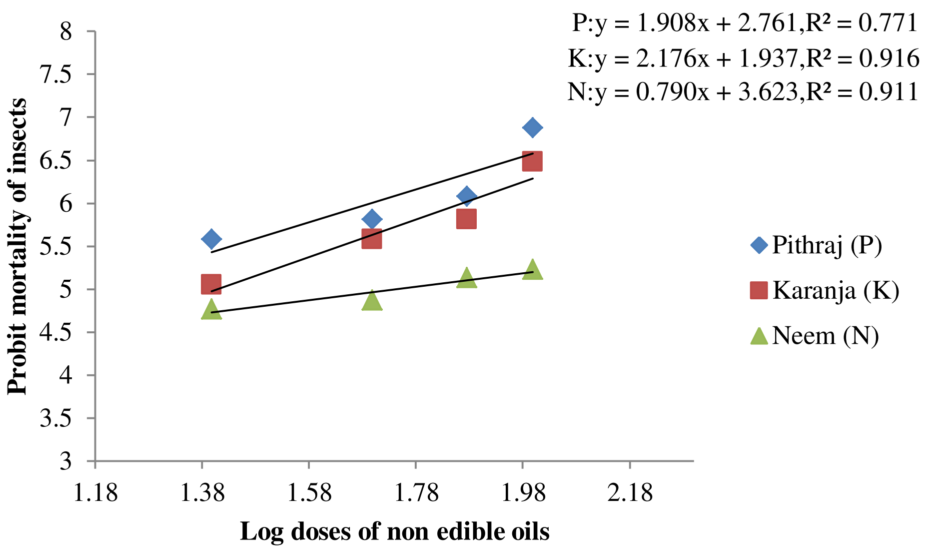

Fig.3. Relationship between probit mortality and log doses of three non edible oils on third instar larva of *E. dodecastigma* 72 HAT.

4.6 Probit regression lines for adult epilachna beetle

The probit regression lines for the bio-efficacy of three oils (pithraj, karanja and neem) against adult epilachna beetle at 24, 48 and 72 HAT are presented in figures 4-6.

The calculated probit regression equations for tested oils against epilachna beetle at 24 HAT were Y=3.708x-3.968, Y=2.899x-2.362 and Y=2.035x-0.439 for pithraj, karanja and neem oils, respectively (Figure 4). Among the three lines, pithraj oil showed the highest but neem oil showed the lowest probit mortality. The probit regression lines for three oils against epilachna beetle at 48 HAT are given in the figure 5. The regression equations were Y=5.205x-6.60, Y=3.146x-2.308 and Y=1.902x+0.127 for pithraj, karanja and neem oils respectively. Comparing the three oils, pithraj oil showed the highest probit mortality while neem oil showed the lowest. The regression equation of pithraj oil was Y=6.919x-9.651 and showed the highest probit mortality but neem oil (Y=2.136x-0.091) showed the lowest when the probit regression lines were compared at 72 HAT (Figure 6).

The probit regression lines of three oils showed a clear linear relationship between probit mortality and their log doses and the regression lines become sleeper as doses increased.

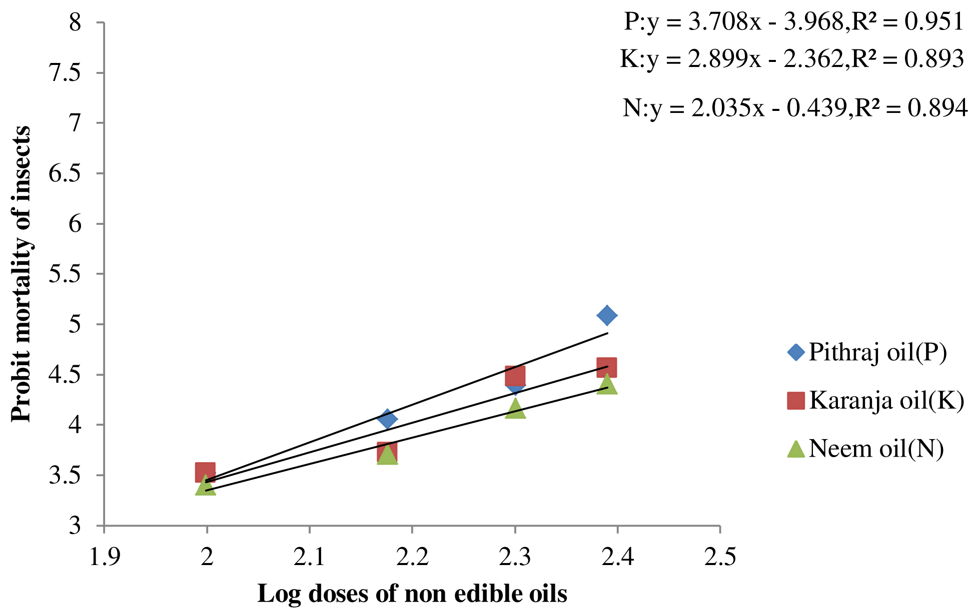

Fig.4. Relationship between probit mortality and log doses of three non edible oils on adult *E. dodecastigma* 24 HAT

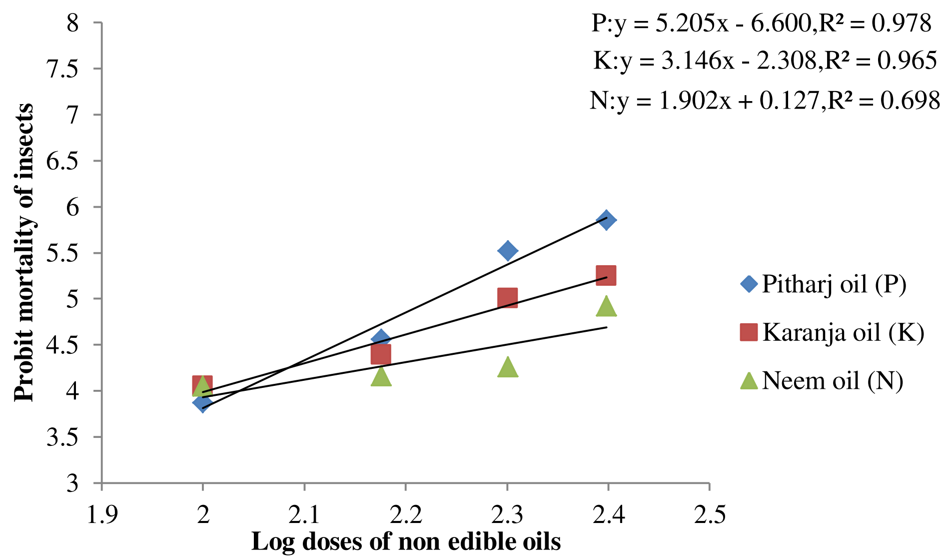

Fig.5. Relationship between probit mortality and log doses of three non edible oils on adult *E. dodecastigma* 48 HAT

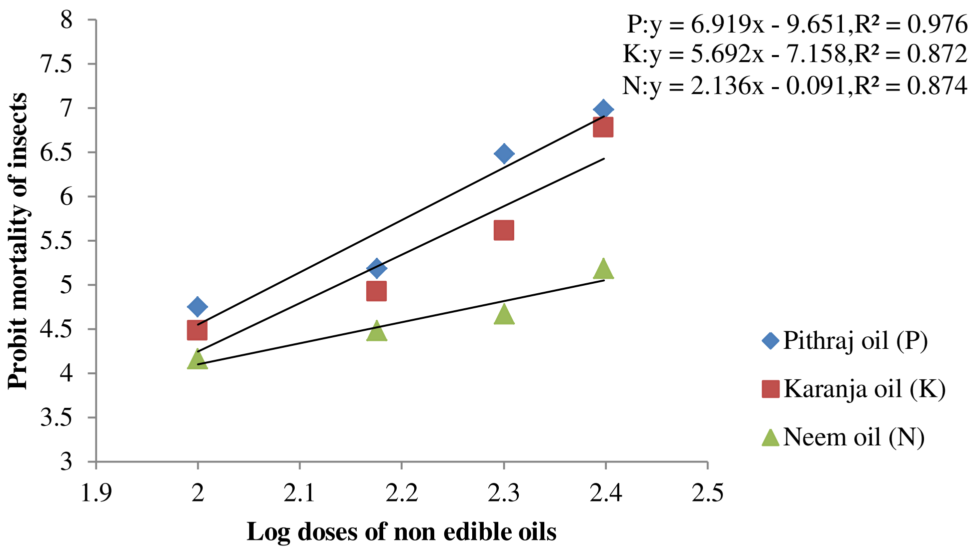

Fig.6. Relationship between probit mortality and log doses of three non edible oils on adult *E. dodecastigma* 72 HAT

4.7 Antifeedant activity of oils on *E. dodecastigma*

4.7.1 Antifeedant activity of oils against third instar larva

The antifeedant effect of three non edible oils, doses, and their interaction on third instar larvae of epilachna beetle differed significantly with treatments (Table 9-11). The present result revealed that pithraj, karanja and neem oils have different antifeedant activity against epilachna beetle.

Among the three oils, pithraj oil showed lowest leaf consumed area (113.9 mm^2) followed by karanja (114.5 mm^2) and neem oil (180.6 mm^2). On the basis of antifeedant index (AI %), it was found that pithraj oil showed the highest (52.99 %) but neem oil provided the lowest (22.51 %) antifeedant index (Table 9).

Table 9: Antifeedant activity of three non edible oils against third instar larva of *E. dodecastigma* at 6 HAT

Treatments (oils)	Consumed leaf area (mm^2)	Antifeedant index (%)
Neem	180.6 b	22.51 b
Karanja	114.5 c	52.61 a
Pithraj	113.9 c	52.99 a
Control	254.0 a	-
LSD	5.215	2.511
CV (%)	11.88	16.31
P-value	0.001	0.001

Within column values followed by different letter (s) are significantly different by DMRT at 5 % level of probability.

Among the four doses, the lowest consumed area of leaf (81.60 mm^2) was observed in 100 mg/ml dose while the highest (254.0 mm^2) in the control. Among the doses significantly differed over control. On the basis of antifeedant index, it was found that 100 mg/ml indicated the highest (52.95 %) but 25 mg/ml showed the lowest (34.82%) antifeedant index (Table 10.).

Table 10: Antifeedant activity of three non edible oils against third instar larva of *E. dodecastigma* at 6 HAT (Dose effect)

Doses (mg/ml)	Consumed leaf area (mm^2)	Antifeedant index (%)
100	81.60 e	52.95 a
75	100.4 d	44.99 b
50	118.7 c	38.05 c
25	127.0 b	34.82 d
Control	254.0 a	-
LSD	6.733	2.899
CV (%)	11.88	16.31
P-value	0.00	0.001

Within column values followed by different letter (s) are significantly different by DMRT at 5 % level of probability.

The interaction effect of oils and doses indicated that there was a significant difference in antifeedant activities against the third instar larva of epilachna beetle (Table 11). 100 mg/ml of Pithraj oil showed the lowest consumed area of leaf (56.07 mm^2) but at 25 mg/ml provide the highest consumed area of leaf (187.0 mm^2) found in neem oil. In the antifeedant index, it was found that pithraj oil showed the highest antifeedant index (63.86%) at 100 mg/ml and neem oil showed the lowest (15.11%) at 25 mg/ml. In control condition the consumed area of leaf was 254.0 mm^2.

ANOVA on antifeedant activity of third instar larva of epilachna beetles are given in Appendices 25-26.

Table 11: Antifeedant activity of three non edible oils and doses against third instar larva of *E. dodecastigma* at 6 HAT (Interaction of oils and doses)

Treatments (oils)	Dose (mg/ml)	Consumed leaf area (mm^2)	Antifeedant index (%)
Neem	100	129.0 d	32.62 e
	75	152.9 c	25.28 f
	50	180.3 b	17.01 g
	25	187.0 b	15.11 g
Karanja	100	59.73 h	62.37 a
	75	72.93 g	55.32 b
	50	89.93 e	47.69 d
	25	96.00 e	45.07 d
Pithraj	100	56.07 h	63.86 a
	75	75.40 fg	54.38 bc
	50	86.00 ef	49.44 cd
	25	98.07 e	44.29 d
	Control	254.0 a	-
LSD		11.66	5.021
CV (%)		11.88	16.31
P-value		0.00	0.00

Within column values followed by different letter (s) are significantly different by DMRT at 5 % level of probability.

From the above results it was observed that these three oils would be effective to control third instar larva of epilachna beetle but pithraj oil would be the most effective. The finding of antifeedant activity of tested oils was coincide by Talukder and Howse (1995). They reported that pithraj seed extract have feeding deterent action against *T. castaneum*. Lidert *et al.* (1987) reported the isolation and identification of rohitukin and rohituka-7, prieurianin- type limonoids from the seeds of pithraj which were shown to be insect antifeedants. Jacobson (1990) also reviewed the insect feeding inhibitory properties of aphanin, a tetracyclic triterpene hemizectal, iso- lated from the seed extract of pithraj. Talukder and Howse (2000) observed the sub-fractions of an acetone extract of *Aphanamixis polystachya* (Meliaceae) seeds were evaluated for their feeding deterrent effects on adult *Tribolium castaneum* (Coleoptera: Tenebrionidae). The results showed that a sub-fraction A- 142 was highly feeding deterrent to *T. castaneum*.

4.7.2 Antifeedant activity of oils against adult epilachna beetle

The antifeedant effect of three non edible oils on adult epilachna beetle is presented in Table. 12-14. The present result revealed that pithraj, karanja and neem oils have antifeedant activity against adult epilachna beetle.

Among the three oils karanja oil showed lowest leaf consumed area (74.61 mm^2) followed by neem (75.99 mm^2) and pithraj (104.9 mm^2). On the basis of antifeedant index, it was found that karanja oil possessed highest (54.73 %) and pithraj oil showed the lowest (35.55 %) antifeedant index (Table 12).

Table 12: Antifeedant activity of three non edible oils against adult of *E. dodecastigma* at 6 HAT

Treatments (oils)	Consumed leaf area (mm^2)	Antifeedant index (%)
Neem	75.99 c	53.53 a
Karanja	74.61 c	54.73 a
Pithraj	104.9 b	35.55 b
Control	171.1 a	-
LSD	3.603	2.475
CV (%)	13.14	14.32
P-value	0.0	0.00

HAT = Hour after treatment

Within column values followed by different letter (s) are significantly different by DMRT at 5 % level of probability.

Among the four doses, the lowest leaf consumed area (41.04 mm^2) was observed in 100 mg/ml while the highest (171.1 mm^2 in the control. In view of antifeedant index, the highest (61.46 %) was found in 100 mg/ml but the lowest (34.55%) antifeedant index was observed in 25 mg/ml (Table 13).

Table 13: Antifeedant activity of doses of three non edible oils against adult *E. dodecastigma* at 6 HAT

Doses (mg/ml)	Consumed leaf area (mm^2)	Antifeedant index (%)
100	41.04 e	61.46 a
75	55.67 d	52.89 b
50	70.31 c	42.84 d
25	87.73 b	34.55 d
Control	171.1 a	-
LSD	4.651	2.858
CV (%)	13.14	14.32
P-value	0.0	0.001

HAT = Hour after treatment

Within column values followed by different letter (s) are significantly different by DMRT at 5 % level of probability.

The interaction effect of tested oils and doses indicated that there was a significant difference in antifeedant activity against adult of epilachna beetle (Table 14.). The lowest leaf consumed area (37.00 mm^2) was observed in maximum dose (100 mg/ml) of karanja oil while the highest (137.1 mm^2) in 25 mg/ml dose of pithraj oil. In antifeedant index, the highest antifeedant index (64.39%) was found in 100 mg/ml of karanja oil but the lowest (11.15%) in 25 mg/ml of pithraj oil. In the control treatments the leaf consumed area was 171.1 mm^2.

ANOVA on antifeedant activity of adult beetles are given in Appendices 27-28.

Table 14: Antifeedant activity of three non edible oils and doses against adult *E. dodecastigma* at 6 HAT (Interaction of oils and doses)

Treatments (oils)	Dose (mg/ml)	Consumed leaf area (mm^2)	Antifeedant index (%)
Neem	100	41.00 gh	61.48 ab
	75	48.93 fg	55.70 cde
	50	55.93 ef	50.71 ef
	25	63.00 de	46.23 f
Karanja	100	37.00 h	64.39 a
	75	47.93 fg	56.23 cd
	50	54.00 f	52.03 de
	25	63.07 de	46.26 f
Pithraj	100	45.13 gh	58.52 bc
	75	70.13 d	46.74 f
	50	101.0 c	25.77 g
	25	137.1 b	11.15 h
	Control	171.1 a	-
LSD		8.056	4.949
CV		13.14	14.32
P-value		0.0	0.00

HAT = Hour after treatment

Within column values followed by different letter (s) are significantly different by DMRT at 5 % level of probability.

From the above results it was observed that among the tested three oils would be effective to control adult epilachna beetle but karanja oil will be the most effective. The present result agreed with the findings of Kumar *et al.* (2006). They observed that crude seed oil of *P. pinnata* showed the maximum growth reduction and antifeedancy against *Spodoptera litura*. Karanja seed oil is known to contain active metabolites such as karanjin, pongamol, glabrin, and pinnatin etc. Karanjin is effective against large number of insects (Mathur *et al*. 1990). Phytol, 1, 2-Benzenedicarboxylic acid, di-iso-octyl ester, n-Hexadecanoic acid and 3, 7, 11, 15-Tetranethyl-2-hexadecen-l-ol compounds of *P. pinnata* are evaluated for their antifeedant properties against *S. litura* (Mahesh *et al.* 2016).

CHAPTER V

SUMMARY AND CONCLUSION

SUMMARY AND CONCLUSION

The experiment was carried out in the laboratory of the Department of Entomology, Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur during March to November 2017 to find out the effectiveness of pithraj, karanja and neem oil against epilachna beetle. Acetone solvent was used to dilute the three non edible oils at doses of 100, 75, 50, 25 mg/ml for third instar larva and 250, 200, 150, 100 mg/ml for adult beetle in order to determine their mortality and 100, 75, 50, 25 mg/ml doses were used for both third instar larva and adult beetle to evaluate their antifeedant activity.

In case of third instar larva, the lowest LD_{50} values was observed in pithraj oil viz. 58.57, 29.84, 12.41 mg/ml whereas neem oil was found highest values of 751.69, 254.16, 55.40 mg/ml at 24, 48, 72 HAT respectively indicated that pithraj oil showed the highest toxic effects. Similarly, in adult, the lowest LD_{50} values was found in pithraj oil 260.66 , 170.90, 132.62 mg/ml whereas neem oil possessed highest LD_{50} values of 443.54, 348.20, 241.56 mg/ml at 24, 48, 72 HAT, respectively indicated that neem oil showed the lowest toxicity. The chi-square values of these oils at different HAT are significant at 5% level of probability and did not show any heterogeneity of the insect mortality.

The average mortality of third instar larva of epilachna beetle at 24, 48, and 72 HAT indicated that pithraj oil showed the highest mortality (53.63%) among three non edible oils whereas neem oil showed the lowest (26.02%). Similarly for adult beetles, pithraj oil showed the highest (38.00%) whereas neem oil showed the lowest mortality (22.00%) at 24, 48, and 72 HAT respectively. Mortality percentage was also found directly proportional to the time after treatment. The order of the toxicity of three oils were pithraj>karanja>neem.

Non edible oils of pithraj, karanja and neem showed antifeedant activity against epilachna beetle after 6 hour treatment. For third instar larva, the highest (52.99%) was observed in pithraj oil whereas neem oil showed the lowest antifeedant index (22.51%). in case of adult epilachna beetle, the highest antifeedant index (54.73%) was found in karanja oil but pithraj oil showed the lowest (35.55%).

From the present research findings it can be concluded that pithraj oil was the most effective among tested oils on mortality against both larva and adult epilachna beetle. In case of antifeedant activity, pithraj and karanja oil showed highest feeding deterant action in third instar larva and adults respectively. Effectiveness of selected non edible oils increased with the increase of concentration. Therefore, to control epilachna beetle an integrated approach should be developed and promoted where the non edible oils could be a vital tool with other methods.

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APPENDICES

APPENDICES

Appendix 1: Doses mortality of third instar larva of epilachna beetle treated with pithraj oil in acetone solvent at 24 HAT

dose	logdose	Number	kill	% kill	corr%	emp probit	expt probit	wrk probit	weight	final probit
100	1.999	30	22	73.333	72	5.58	5.458	5.564	18.03	5.461
75	1.875	30	16	53.333	52	5.05	5.209	5.072	18.81	5.213
50	1.699	30	14	46.667	45	4.87	4.860	4.890	18.81	4.864
25	1.399	30	8	26.667	24	4.29	4.262	4.286	15.09	4.266

Y = 1.490924 + 1.985123 X χ^2value is .584589 with 2 df

LD_{50} is 58.57157 95% confidence limits are 44.68746 to 76.7694

Appendix 2: Doses mortality of third instar larva of epilachna beetle treated with pithraj oil in acetone solvent at 48 HAT

dose	logdose	Number	kill	% kill	corr%	emp probit	expt probit	wrk probit	weight	final probit
100	1.999	30	26	86.667	86	6.08	5.964	6.098	14.13	5.956
75	1.875	30	22	73.333	72	5.58	5.731	5.574	15.96	5.728
50	1.699	30	20	66.667	66	5.41	5.402	5.402	18.03	5.408
25	1.399	30	14	46.667	45	4.87	4.842	4.890	18.81	4.859

Y = 2.315172 + 1.820396 X χ^2value is .6835566 with 2 df

LD_{50} is 29.84416 95% confidence limits are 19.10861 to 46.61112

Appendix 3: Doses mortality of third instar larva of epilachna beetle treated with pithraj oil in acetone solvent at 72 HAT

dose	logdose	Number	kill	% kill	corr%	emp probit	expt probit	wrk probit	weight	final probit
100	1.999	30	29	96.667	97	6.88	6.412	6.759	9.06	6.444
75	1.875	30	26	86.667	86	6.08	6.217	6.026	11.10	6.245
50	1.699	30	24	80	79	5.81	5.943	5.832	14.13	5.964
25	1.399	30	22	73.333	72	5.58	5.474	5.564	18.03	5.484

Y = 3.256632 + 1.593573 X χ^2value is 1.791789 with 2 df

LD_{50} is 12.4165 95% confidence limits are 4.235107 to 36.40272

Appendix 4: Doses mortality of third instar larva of epilachna beetle treated with neem oil in acetone solvent at 24 HAT

dose	logdose	Number	kill	% kill	corr%	emp probit	expt probit	wrk probit	weight	final probit
100	1.999	30	7	23.333	23	4.26	4.196	4.284	14.13	4.211
75	1.875	30	5	16.667	17	4.05	4.089	4.037	13.17	4.099
50	1.699	30	4	13.333	13	3.87	3.937	3.878	12.15	3.940
25	1.399	30	3	10	10	3.72	3.678	3.73	9.06	3.669

Y = 2.4111 + .9001612 X χ^2value is 0.2056065 with 2 df

LD_{50} is 751.0926 95% confidence limits are 17.2258 to 32802.09

Appendix 5: Doses mortality of third instar larva of epilachna beetle treated with neem oil in acetone solvent at 48 HAT

dose	logdose	Number	kill	% kill	corr%	emp probit	expt probit	wrk probit	weight	final probit
100	1.999	30	12	40	40	4.75	4.691	4.74	18.03	4.683
75	1.875	30	10	33.33	33	4.56	4.593	4.54	17.43	4.585
50	1.699	30	8	26.67	27	4.39	4.455	4.39	16.74	4.447
25	1.399	30	7	23.33	23	4.26	4.220	4.25	15.09	4.211

Y = 3.115448 + .7835598 X χ^2value is 0.167753 with 2 df

LD_{50} is 254.1656 95% confidence limits are 29.11675 to 2218.66

Appendix 6: Doses mortality of third instar larva of epilachna beetle treated with neem oil in acetone solvent at 72 HAT

dose	logdose	Number	kill	% kill	corr%	emp probit	expt probit	wrk probit	weight	final probit
100	1.999	30	18	60	59	5.23	5.202	5.254	18.81	5.210
75	1.875	30	17	56.67	55	5.13	5.104	5.115	19.02	5.108
50	1.699	30	14	46.67	45	4.87	4.965	4.865	19.02	4.963
25	1.399	30	13	43.33	41	4.77	4.728	4.766	18.48	4.717

Y = 3.571776 + .8191482 X χ^2value is 0.266176 with 2 df

LD_{50} is 55.40492 95% confidence limits are 29.36715 to 104.5286

Appendix 7: Doses mortality of third instar larva of epilachna beetle treated with karanja oil in acetone solvent at 24 HAT

dose	logdose	Number	kill	% kill	corr%	emp probit	expt probit	wrk probit	weight	final probit
100	1.999	30	15	50	50	5	5.012	5	19.11	5.013
75	1.875	30	14	46.666	47	4.92	4.921	4.915	19.02	4.919
50	1.699	30	13	43.333	43	4.82	4.793	4.818	18.48	4.786
25	1.399	30	10	33.333	33	4.56	4.573	4.544	17.43	4.560

Y = 3.50933 + .751597 X χ^2value is 0.02621365 with 2 df

LD_{50} is 96.23603 95% confidence limits are 35.17647 to 263.2831

Appendix 8: Doses mortality of third instar larva of epilachna beetle treated with karanja oil in acetone solvent at 48 HAT

dose	logdose	Number	kill	% kill	corr%	emp probit	expt probit	wrk probit	weight	final probit
100	1.999	30	18	60	60	5.25	5.191	5.24	19.02	5.175
75	1.875	30	16	53.33	53	5.08	5.129	5.065	19.02	5.120
50	1.699	30	15	50	50	5	5.040	5	19.11	5.042
25	1.399	30	14	46.67	47	4.92	4.889	4.942	18.81	4.909

Y = 4.290979 + .4421351 X χ^2value is 0.1917036 with 2 df

LD_{50} is 40.14487 95% confidence limits are 10.10848 to 159.4317

Appendix 9: Doses mortality of third instar larva of epilachna beetle treated with karanja oil in acetone solvent at 72 HAT

dose	logdose	Number	kill	% kill	corr%	emp probit	expt probit	wrk probit	weight	final probit
100	1.999	30	28	93.33	93	6.48	6.289	6.383	11.1	6.193
75	1.875	30	24	80	79	5.81	6.017	5.759	13.17	5.946
50	1.699	30	22	73.33	72	5.58	5.634	5.58	16.74	5.599
25	1.399	30	16	53.33	52	5.05	4.979	5.04	19.02	5.005

Y = 2.24653 + 1.973136 X χ^2value is 0.8929996 with 2 df

LD_{50} is 24.85871 95% confidence limits are 15.23676 to 40.55692

Appendix 10: Doses mortality of adult epilachna beetle treated with pithraj oil in acetone solvent at 24 HAT

dose	logdose	Number	kill	% kill	corr%	emp probit	expt probit	wrk probit	weight	final probit
250	2.398	30	16	53.33	53	5.08	4.925	5.065	19.02	4.93
200	2.301	30	8	26.67	27	4.39	4.565	4.376	17.43	4.56
150	2.176	30	5	16.67	17	4.05	4.101	4.056	14.13	4.08
100	1.999	30	2	6.67	7	3.52	3.449	3.54	7.14	3.41

Y = -4.262041 + 3.833501 X χ^2value is 1.066199 with 2 df

LD_{50} is 260.6627 95% confidence limits are 205.5114 to 330.6143

Appendix 11: Doses mortality of adult epilachna beetle treated with pithraj oil in acetone solvent at 48 HAT

dose	logdose	Number	kill	% kill	corr%	emp probit	expt probit	wrk probit	weight	final probit
250	2.398	30	24	80	80	5.85	5.883	5.8	15.09	5.855
200	2.301	30	21	70	70	5.52	5.378	5.5	18.48	5.353
150	2.176	30	10	33.33	33	4.56	4.728	4.558	18.48	4.706
100	1.999	30	4	13.33	13	3.87	3.811	3.873	11.10	3.795

Y = -6.5623 + 5.178501 X χ^2value is 0.9182644 with 2 df

LD_{50} is 170.9034 95% confidence limits are 153.1502 to 190.7145

Appendix 12: Doses mortality of adult epilachna beetle treated with pithraj oil in acetone solvent at 72 HAT

dose	logdose	Number	kill	% kill	corr%	emp probit	expt probit	wrk probit	weight	final probit
250	2.398	30	29	96.67	97	6.88	6.939	6.844	4.62	6.84
200	2.301	30	28	93.33	93	6.48	6.269	6.383	11.10	6.19
150	2.176	30	17	56.67	57	5.18	5.405	5.159	18.03	5.36
100	1.999	30	7	23.33	23	4.26	4.186	4.284	14.13	4.18

Y = -9.213879 + 6.696336 X χ^2 value is 1.264736 with 2 df

LD_{50} is 132.628 95% confidence limits are 119.4554 to 147.2531

Appendix 13: Doses mortality of adult epilachna beetle treated with neem oil in acetone solvent at 24 HAT

dose	logdose	Number	kill	% kill	corr%	emp probit	expt probit	wrk probit	weight	final probit
250	2.398	30	10	33.33	33	4.40	4.442	4.57	16.74	5.464
200	2.301	30	6	20	20	4.16	4.245	4.15	15.09	4.255
150	2.176	30	4	13.33	13	3.70	3.991	3.88	12.15	3.986
100	1.999	30	3	10	10	3.40	3.632	3.73	9.06	3.607

Y = -0.7004915 + 2.153625 X χ^2 value is 0.634801 with 2 df

LD_{50} is 443.5364 95% confidence limits are 187.204 to 1050.857

Appendix 14: Doses mortality of adult Epilachna beetle treated with neem oil in acetone solvent at 48 HAT

dose	logdose	Number	kill	% kill	corr%	emp probit	expt probit	wrk probit	weight	final probit
250	2.398	30	14	46.67	47	4.92	4.689	4.929	18.03	4.708
200	2.301	30	7	22.33	23	4.26	4.504	4.264	17.43	4.511
150	2.176	30	6	20	20	4.16	4.266	4.150	15.09	4.258
100	1.999	30	5	16.67	17	4.05	3.931	4.062	12.15	3.901

Y = -0.1564822 + 2.028649X χ^2 value is 2.439016 with 2 df

LD_{50} is 348.2019 95% confidence limits are 180.5794 to 671.4194

Appendix 15: Doses mortality of adult epilachna beetle treated with neem oil in acetone solvent at 72 HAT

dose	logdose	Number	kill	% kill	corr%	emp probit	expt probit	wrk probit	weight	final probit
250	2.398	30	17	56.67	57	5.18	5.030	5.175	19.11	5.032
200	2.301	30	11	36.67	37	4.67	4.823	4.682	18.81	4.823
150	2.176	30	9	30	30	4.48	4.556	4.460	17.43	4.553
100	1.999	30	7	23.33	23	4.26	4.180	4.284	14.13	4.174

Y = -0.1396833 + 2.156794X χ^2 value is 1.088863 with 2 df

LD_{50} is 241.5572 95% confidence limits are 168.433 to 346.4262

Appendix 16: Doses mortality of adult epilachna beetle treated with karanja oil in acetone solvent at 24 HAT

dose	logdose	Number	kill	% kill	corr%	emp probit	expt probit	wrk probit	weight	final probit
250	2.398	30	10	33.33	33	4.56	4.589	4.54	17.43	4.599
200	2.301	30	9	30	30	4.48	4.309	4.49	15.96	4.316
150	2.176	30	3	10	10	3.72	3.946	3.74	12.15	3.950
100	1.999	30	2	6.66	7	3.52	3.436	3.54	7.14	3.435

Y = -2.417605 + 2.926382 X χ^2 value is 1.153246 with 2 df

LD_{50} is 342.5589 95% confidence limits are 211.1468 to 555.7587

Appendix 17: Doses mortality of adult epilachna beetle treated with karanja oil in acetone solvent at 48 HAT

dose	logdose	Number	kill	% kill	corr%	emp probit	expt probit	wrk probit	weight	final probit
250	2.398	30	18	60	60	5.25	5.236	5.28	18.81	5.255
200	2.301	30	15	50	50	5.00	4.931	4.99	19.02	4.939
150	2.176	30	8	26.67	27	4.39	4.538	4.38	17.43	4.531
100	1.999	30	5	16.67	17	4.05	3.984	4.06	12.15	3.957

Y = -2.567088 + 3.262117 X χ^2 value is 0.6161671 with 2 df

LD_{50} is 208.7791 95% confidence limits are 172.1019 to 253.2725

Appendix 18: Doses mortality of adult epilachna beetle treated with karanja oil in acetone solvent at 72 HAT

dose	logdose	Number	kill	% kill	corr%	emp probit	expt probit	wrk probit	weight	final probit
250	2.398	30	29	96.67	97	6.88	6.302	6.656	10.08	6.239
200	2.301	30	22	73.33	73	5.61	5.810	5.562	15.09	5.761
150	2.176	30	14	46.67	47	4.92	5.175	4.915	19.02	5.146
100	1.999	30	9	30	30	4.48	4.281	4.490	15.09	4.279

Y = -5.564382 + 4.921882 X χ^2 value is 4.044356 with 2 df

LD_{50} is 140.0913 95% confidence limits are 123.2913 to 159.1805

Appendix 19: ANOVA table of the direct toxic effect of three non edible oils against the third instar larva of epilachna beetle at 24 HAT

K value	Source	Degrees of freedom	Sum of squares	Mean square	F value	Probability
2	Oil	2	5992.369	2996.185	24.6749	0.0000
4	Dose	4	11705.580	2926.395	24.1002	0.0000
6	Oil×Dose	8	2379.960	297.495	2.4500	0.0359
-7	Error	30	3642.787	121.426		
	Total	44	23720.696			

Co efficient of variation: 38.27%

Appendix 20: ANOVA table of the direct toxic effect of three non edible oils against the third instar larva of epilachna beetle at 48 HAT

K value	Source	Degrees of freedom	Sum of squares	Mean square	F value	Probability
2	Oil	2	6679.308	3339.654	20.4626	0.0000
4	Dose	4	19965.218	4991.304	30.5825	0.0000
6	Oil×Dose	8	2076.702	259.588	1.5905	0.1695
-7	Error	30	4896.230	163.208		
	Total	44	33617.457			

Co efficient of variation: 31.66%

Appendix 21: ANOVA table of the direct toxic effect of three non edible oils against the third instar larva of epilachna beetle at 72 HAT

K value	Source	Degrees of freedom	Sum of squares	Mean square	F value	Probability
2	Oil	2	5563.828	2781.914	34.5601	0.0000
4	Dose	4	33945.518	8486.379	105.4273	0.0000
6	Oil×Dose	8	1740.579	217.572	2.7029	0.0228
-7	Error	30	2414.852	80.495		
	Total	44	43664.776			

Co efficient of variation: 16.13%

Appendix 22: ANOVA table of the direct toxic effect of three non edible oils against the adult epilachna beetle at 24 HAT

K value	Source	Degrees of freedom	Sum of squares	Mean square	F value	Probability
2	Oil	2	217.778	108.889	2.8824	0.0716
4	Dose	4	8831.111	2207.778	58.4412	0.0000
6	Oil×Dose	8	848.889	106.111	2.8088	0.0189
-7	Error	30	1133.333	37.778		
	Total	44	11031.111			

Co efficient of variation: 35.01%

Appendix 23: ANOVA table of the direct toxic effect of three non edible oils against the adult epilachna beetle at 48 HAT

K value	Source	Degrees of freedom	Sum of squares	Mean square	F value	Probability
2	Oil	2	2617.778	1308.889	36.8125	0.0000
4	Dose	4	22564.444	5641.111	158.6562	0.0000
6	Oil×Dose	8	2648.889	331.111	9.3125	0.0000
-7	Error	30	1066.667	35.556		
	Total	44	28897.77			

Co efficient of variation: 19.73%

Appendix 24: ANOVA table of the direct toxic effect of three non edible oils against the adult epilachna beetle at 72 HAT

K value	Source	Degrees of freedom	Sum of squares	Mean square	F value	Probability
2	Oil	2	5151.111	2575.556	72.4375	0.0000
4	Dose	4	39497.778	9874.444	277.7188	0.0000
6	Oil×Dose	8	4182.222	522.778	14.7031	0.0000
-7	Error	30	1066.667	35.556		
	Total	44	49897.778			

Co efficient of variation: 13.48%

Appendix 25: ANOVA table of the antifeedant activity (consumed leaf area in mm^2) of three non edible oils against the third instar larva of epilachna beetle at 6 HAT

K value	Source	Degrees of freedom	Sum of squares	Mean square	F value	Probability
2	Oil	2	220639.209	110319.604	420.3328	0.0000
4	Dose	4	833726.000	208431.500	794.1525	0.0000
6	Oil×Dose	8	58382.213	7297.777	27.8055	0.0000
-7	Error	210	55116.133	262.458		
	Total	224	1167863.556			

Coefficient of variation: 11.88%

Appendix 26: ANOVA table of the antifeedant index of three non edible oils against the third instar larva of epilachna beetle at 6 HAT

K value	Source	Degrees of freedom	Sum of squares	Mean square	F value	Probability
2	Oil	2	36710.181	18355.090	378.3444	0.0000
4	Dose	3	8730.381	2910.127	59.9850	0.0000
6	Oil×Dose	6	73.397	12.233	0.2521	0.0000
-7	Error	168	8150.392	48.514		
	Total	179	53664.350			

Coefficient of variation: 16.31%

Appendix 27: ANOVA table of the antifeedant activity (consumed leaf area in mm^2) of three non edible oils against the adult epilachna beetle at 6 HAT

K value	Source	Degrees of freedom	Sum of squares	Mean square	F value	Probability
2	Oil	2	43858.996	21929.498	175.0643	0.0000
4	Dose	4	469039.760	117259.940	936.0920	0.0000
6	Oil×Dose	8	37480.427	4685.053	37.4010	0.0000
-7	Error	210	26305.733	125.265		
	Total	224	576684.916			

Coefficient of variation: 13.14%

Appendix 28: ANOVA table of the antifeedant index of three non edible oils against the adult epilachna beetle at 6 HAT

K value	Source	Degrees of freedom	Sum of squares	Mean square	F value	Probability
2	Oil	2	13852.424	6926.212	146.9211	0.0000
4	Dose	3	18570.793	6190.264	131.3099	0.0000
6	Oil×Dose	6	6138.911	1023.152	21.7034	0.0000
-7	Error	168	7919.921	47.142		
	Total	179	46482.049			

Coefficient of variation: 14.32%