

**ANTI-OVIPOSITIONAL, OVICIDAL AND LARVICIDAL EFFECT
OF INDIGENOUS PLANT OILS AGAINST PULSE BEETLE**

A THESIS

BY

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Registration No. 1605371

Semester: July-December, 2017

MASTER OF SCIENCE

IN

ENTOMOLOGY



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

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*Submitted to the Department of Entomology
Hajee Mohammad Danesh Science and Technology University,
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**DEPARTMENT OF ENTOMOLOGY
HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY
UNIVERSITY, DINAJPUR**

DECEMBER, 2017

**DEDICATED TO
MY
BELOVED PARENTS**

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The Authoress

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ABSTRACT

The experiment was carried out in the Department of Entomology, Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur during May to November 2017. Pulse beetle, *Callosobruchus chinensis* L., is an important pest of stored chickpea and widely distributed. Chemical insecticides and fumigants are common control used against pulse beetle but it has serious drawbacks. As an alternative control method, plant oils have been successfully used against this pest. The efficacy of six plant oils was assessed for their grain protectant against the attack of *C. chinensis*. All the tested oils were effectively reduced oviposition, adult emergence, seed infestation and weight loss compared to control. Both, kalozira and mustard oils showed best performance to reduce adult emergence, seed infestation and seed weight loss while applied those at the rate of 10% concentration. Both kalozira (95.86%) and mustard (91.05%) oils provided the highest inhibition rate of adult emergence when egg bearing seeds were treated at 10% concentration. Additionally, kalozira (94.47%) and mustard (88.33%) oils also markedly reduced the adult emergence while larva bearing seeds were treated at 10% concentration. Therefore, kalozira and mustard oils would be used for the protection of chickpea seeds from the infestation of *C. chinensis*.

Keywords: Plant oils, pulse beetle, efficacy, damage, chickpea

CONTENTS

CHAPTER	TITLE	PAGE NO.
	ACKNOWLEDGEMENTS	v
	ABSTRACT	vi
	CONTENTS	vii-viii
	LIST OF TABLES	ix
	LIST OF PLATES	x
1	INTRODUCTION	1-3
2	REVIEW OF LITERATURE	4-7
	2.1 The test insect	4
	2.2 Systematic Position	4
	2.3 Life cycle	5
	2.4 Origin and distribution of pulse beetle	5
	2.5 Nature of damage	6
3	MATERIALS AND METHODS	8-13
	3.1 Sources and collection of chickpea seeds	8
	3.2 Source and collection of oils	8
	3.3 Location of the study	9
	3.4 Source and collection of Pulse beetle	9
	3.5 Collection of test materials	9
	3.6 Collection and rearing of test insects	10
	3.7 Data recording procedure	11
	3.8 Ovicidal and larvicidal efficacies of oils	12
	3.9 Statistical analyses	13
4	RESULTS	14-26
	4.1 Effect of plant oils on oviposition	14
	4.2 Effect of oils on adult emergence	15
	4.3 Effect of oils on seed infestation	15
	4.4 Effect of oils on seed weight loss	16
	4.5 Ovicidal efficacy of oils	16
	4.6 Larvicidal activity of oils	17

CONTENTS (Contd.)

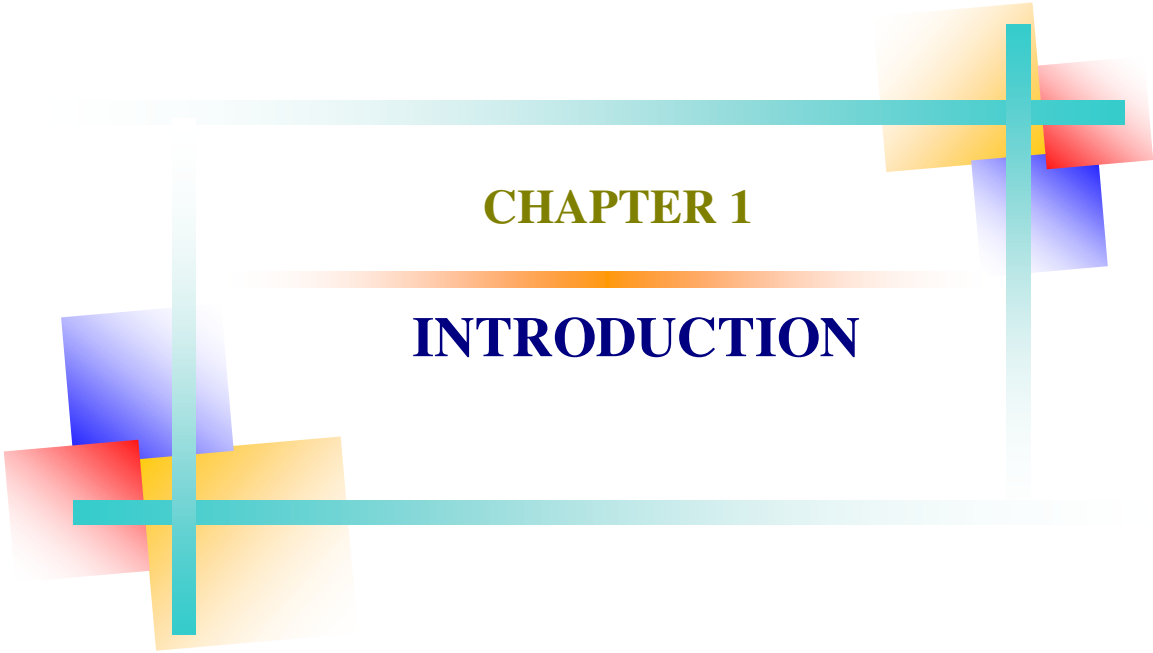
CHAPTER	TITLE	PAGE NO.
5	DISCUSSION	27-30
6	SUMMARY AND CONCLUSION	31-32
	REFERENCES	33-39

LIST OF TABLES

TABLE NO.	TITLE	PAGE NO.
1	List of indigenous plant oils against <i>C. chinensis</i> in chickpea seeds	10
2	Effect of plant oils on oviposition, adult emergence, seed infestation, and weight loss done by <i>C. chinensis</i>	19
3	Effect of different concentrations on oviposition, adult emergence, seed infestation, and weight loss done by <i>C. chinensis</i> .	20
4	Effects of plant oil concentrations on the oviposition, adult emergence, seed infestation, and weight loss done by <i>C. chinensis</i>	21
5	Effect of plant oils on number of adults of <i>C. chinensis</i> emerged from egg bearing chickpea seeds.	22
6	Effect of different doses on number of adults of <i>C. chinensis</i> emerged from egg bearing chickpea seeds.	22
7	Effect of different indigenous oils with 3 concentrations on number of adults of <i>C. chinensis</i> emerged from egg bearing chickpea seeds.	23
8	Effect of plant oils on number of adults of <i>C. chinensis</i> emerged from larvae bearing chickpea seeds.	24
9	Effect of different concentrations on number of adults of <i>C. chinensis</i> emerged from larvae bearing chickpea seeds.	25
10	Effects of plant oil and this concentrations on number of adults of <i>C. chinensis</i> emerged from larvae bearing chickpea seeds.	26

LIST OF PLATES

PLATE NO.	TITLE	PAGE NO.
1	Six different edible plant oils used in experiment against <i>C. chinensis</i>	8
2	Adult female and male of <i>C. chinensis</i>	9
3	Mass culture of pulse beetle, <i>C. chinensis</i>	11

An abstract geometric design featuring two large, light blue squares that overlap each other. A horizontal teal line and a vertical teal line intersect at the center of the composition. Several smaller, semi-transparent squares in yellow, red, and blue are scattered around the central intersection, some overlapping the larger squares and the teal lines.

CHAPTER 1

INTRODUCTION

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INTRODUCTION

Pulse is one of the most important leguminous crops in the world (Aslam *et al.*, 2002) and many varieties of pulses are grown in Bangladesh. Pulse is very popular dish used as main diet for most of the Bangladeshi people. It is a vital source of protein (20-40%) and considered as “poor men or vegetarians meat” (Bhalla *et al.*, 2008). Additionally, pulses contain many other essential nutrients like carbohydrates (50-60%) as well fairly good sources of thiamin, niacin, calcium and iron and hence they are called vegetarian’s meat (Darmadi-Blackberry *et al.*, 2004).

The cultivated area under pulse crops in Bangladesh coverage 1011000 acres with annual production of 726000 metric tons (BBS, 2016). This amount is not sufficient to meet up the demand. Recently, pulse productions is gradually decreasing day by day. One of the major hindrances to increase the pulse production is the damage of pulse grain from insect infestation in storage. Among the stored bruchids, pulse beetle is globally distributed as major threats of stored pulses (Atwal and Dhaliwal, 2005). The larvae destroy seeds by feeding inside partially or completely and make them unfit for human consumption as well as propagating materials. It attacks all kinds of pulses (Ahmed *et al.*, 2003). Pulse seeds can be damaged up to 100% due to pulse beetle infestation at 3 months of storage (Yamane, 2013).

At present, control of this insect mostly relied on synthetic insecticides and fumigants (Atwal and Dhaliwal, 2005). But chemical protection measures suffer so many serious drawbacks (Lee *et al.*, 2001, Hossain *et al.*, 2014). Moreover, continuous uses of insecticides lead to hazardous on beneficial organisms on both field and store, and also

create environmental pollution (Saxena, 1992; Nagarare and More, 1998 and Hossain, 2001). Hence, scientists are globally now trying to adopt alternative methods to protect grains from insect infestation.

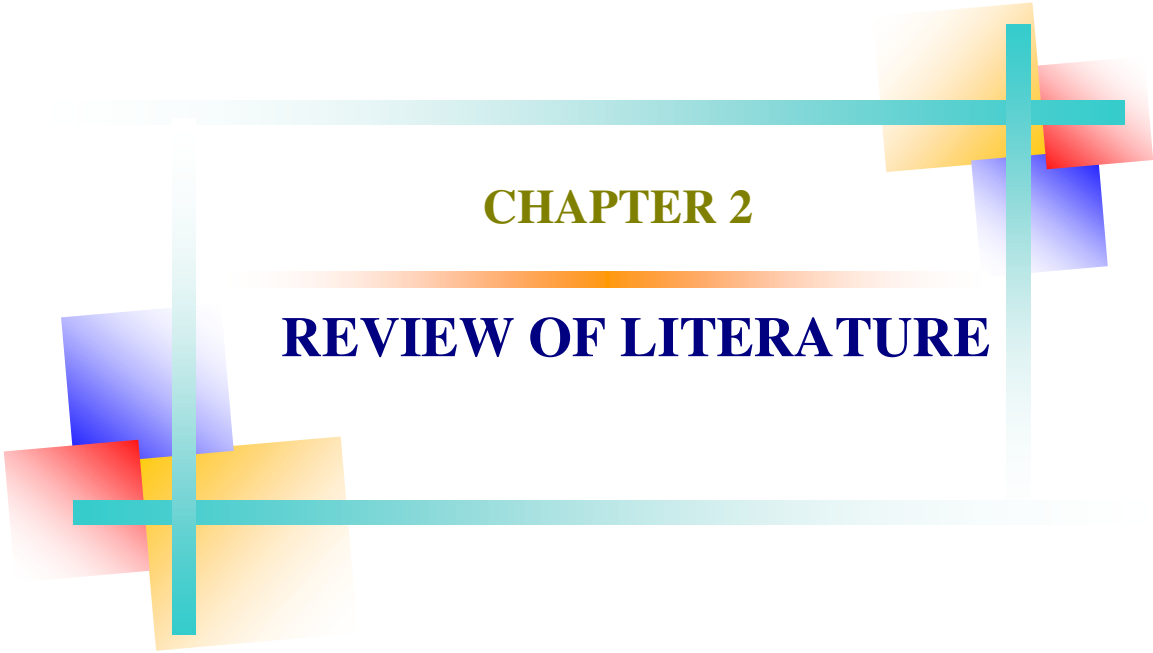
More current research illustrated that botanical oils and their constituents may have potentials as alternative of fumigants (Tunc *et al.*, 2000; Lee *et al.*, 2001 and Jat *et al.*, 2013). They become promising grain protectants by offering many advantages in comparing to insecticides, such as, host specific, non-toxic to mammals and beneficial organisms, less prone to insect resistance, readily biodegradable and less expensive (Saxena, 1992 and Wink, 1993). They also having less environmental impact and could benefit our agricultural sector. Beside, botanicals are cheaper, easy to process and raw materials are available at farmer's level.

The use of locally indigenous plant materials in the control of pests are an ancient technology and used in many parts of the world (Roy *et al.*, 2005). Plants contain a large number of secondary metabolites and those categorized under terpenoids, alkaloids, glycosides, phenols, tannins etc. play a major role in plant defense and cause behavioral and physiological effects on insect indigenous botanical oils reported to be effective in managing storage insects (Yadav *et al.*, 2004; Ghosal *et al.*, 2005 and Upadhyay *et al.*, 2007).

In Bangladesh, limited work has been done in recognizing the insecticidal potency of edible oils against pulse beetle (Rahman and Rahman, 2004; Sarker *et al.*, 2015). It is, therefore, necessary to identify locally available indigenous botanicals as natural insecticide for grain protection by resource limited farmers of developing countries like

Bangladesh. Keeping these in views, the present research work was therefore planned and designed with the following objectives:

1. To explore the appropriate indigenous edible oils for the management of *Callosobruchus chinensis* in stored chick pea seed.
2. To examine the suitable doses of indigenous edible oil's for the management of *Callosobruchus chinensis* in stored chick pea seed.

An abstract geometric design featuring two large, light blue squares that overlap each other. Overlaid on these squares are several smaller, semi-transparent squares in red, yellow, and blue. Two thin, light blue lines, one horizontal and one vertical, intersect to form a cross that spans the width and height of the central design area.

CHAPTER 2

REVIEW OF LITERATURE

CHAPTER 2

REVIEW OF LITERATURE

Different types of stored grains and their products are widely infested by the pulse beetle (*Callosobruchus chinensis*) and considered as a major stored product pest. Numerous research works have been performed in the world on the effect of the plant oils namely Kalozira, mustard, tishi, soybean, ground nut, and palm oils against the pulse beetle as well as other stored product pests are reviewed below:

2.1 The test insect

Pulse beetle, *Callosobruchus chinensis* (L.) was used as the test insect in the present studies. It is originated in Asia, but is now cosmopolitan in the tropics and subtropics. It is notorious pest of pulses.

2.2 Systematic position

Phylum-Arthropoda

Class-Insecta

Sub-class-Pterygota

Division –Endopterygota

Order-Coleoptera

Family-Bruchidae

Genus- *Callosobruchus*

Species- *C. chinensis*

2.3 Life cycle

Just after emergence copulation takes place between male and female. After copulation and fertilization, the eggs are laid singly but several of them can be seen on one grain. In the field the eggs are laid on green pods. The egg when freshly laid is translucent, smooth and shining but later on turns pale yellowish or greyish white. It is elongated and oval in shape. The hatching takes place in 4-5 days. The young grub burrows into the pod or grain. The larva feeds and develops inside the grain and is fully grown within 2-3 weeks. A fully developed larva is about 5.5-6.5 mm in length, cylindrical in shape, fleshly and strongly wrinkled. It is perfectly white except in the region of its mouth where the mouth parts are brown. The pupal stage lasts for about 4 days during summer season but in winter season this stage lasts for a considerably longer period. The adult emerges after cutting a circular hole in the seed coat. The time taken to complete the life cycle varies according to temperature and the nature of grains on which the pest breed. In soya-beans it is completed in 41 days, in lentil it takes 34-41 days, while in arhar it is completed in 25-34 days (Mahbub, 2010).

2.4 Origin and distribution of pulse beetle

C. chinensis was first reported and described from China in 1958 (Alam, 1971). Southgate (1979) has mentioned that the species of bruchidae have their origin in Afro Asian region. According to him, the species of bruchidae in every continent except Antarctica have their cosmopolitan distribution. Most of the species lived in the tropical regions of Asia, Africa, Central and South America. *C. chinensis* is of Asian origin, where it is still the dominant species (Dennis, 1990). He mentioned that *C. maculatus* thought to be African. However, both the species are now widely distributed throughout the warmer parts of the world.

2.5 Nature of damage

It has been reported that pulse beetle, *C. chinensis* is a major pest of all pulses and causes 40-50% in losses of pulse in storage (Gosh and Durbey, 2003). Among the species of pulse beetle, *Callosobruchus chinensis* L. (Coleoptera: Bruchidae) is one of the most destructive pests of cowpeas, lentils, black gram and other stored legumes in Bangladesh, India and other tropical and subtropical countries (Park *et al.*, 2003; Mummigatti and Krishnaiah, 2007; Roy *et al.*, 2012). Gujar and Yadav (1978) recorded 55-60% loss by seed weight and 45-66% loss in protein content by the pulse beetle. Results revealed that 50.37-57.58% (Ali *et al.*, 1999) and 37.30-55.30% (Ali and Rahman, 2006) grain content loss of mungbean seed occurred by *C. chinensis* and *C. maculatus*, respectively.

Khalequzzaman *et al.* (2007) and Srinivasan (2008) they reported that stored grains treated with different oils provided lowest seed weight loss over the control.

Biswas and Biswas (2005) cited that edible oils comprising mustard, kalozira, and sesame at 8.0 ml/kg seeds gave best protection of pigeon pea seeds against pulse beetle.

The biological activities of tested oils can be ascribed to several alkaloid contents as insecticidal potency (Ghosal *et al.*, 2005). Thus trialed oils of kalozira and mustard had optimistic effect to check the ravages of black gram seed against.

Singh (2003) reported that botanical oils treated grain impaired oviposition of pulse beetle.

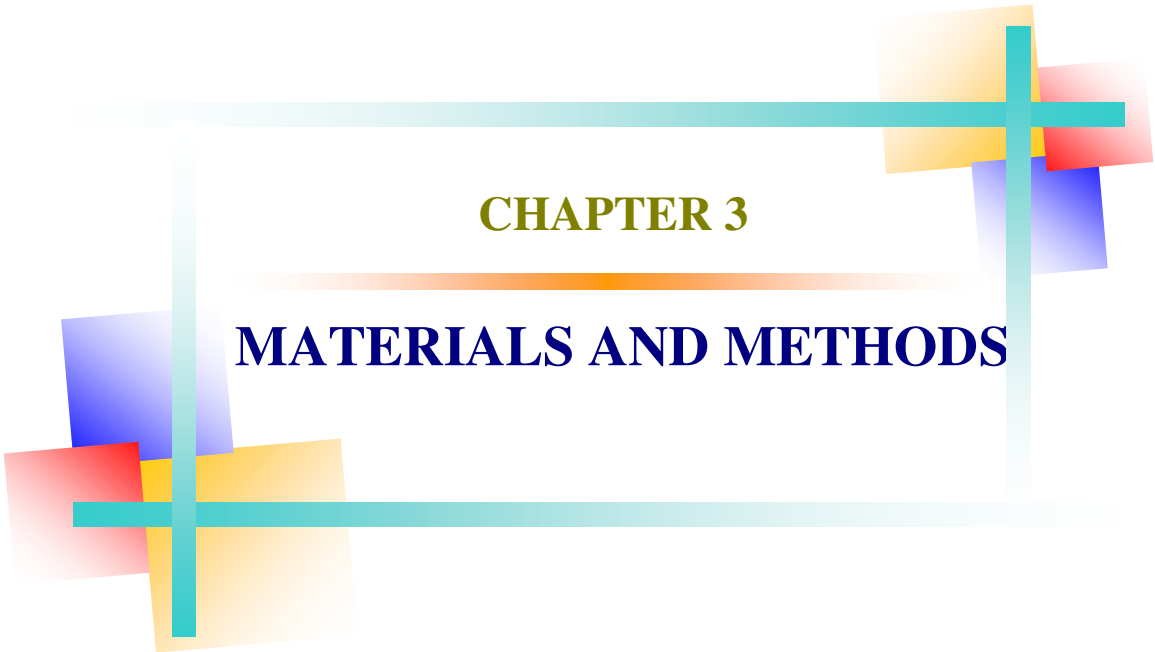
Ahmed *et al.* (2003) used neem and sesame oil and found both oils can control the larvae of *C. chinensis* inside the cotyledons of azuki beans.

Bhatnagar *et al.* (2001), Abulude *et al.* (2007), Bamaiyai *et al.* (2007) and Haghtalab *et al.* (2009) cited that botanical oils completely reduced the adult emergence of pulse beetle when if it mixed with pulse seeds.

Isman (2000), Raja and Ignacimuthu (2001), Singh and Sharma (2003), Yadav and Bhargava (2005), and Upadhyay *et al.* (2007) used various plant oils including Kalozira, mustard, and sesame with various doses against the bruchids hosted on pulse seeds.

Bhatnagar *et al.* (2001) and Bhargava and Meena (2002) opined that those oils showed ovicidal and larvicidal properties that suppressed the emergence of pulse beetle.

Khaire *et al.* (1992) studied efficacy of ten vegetable oils viz. sunflower, castor, mustard, safflower, palm, groundnut, sesame, neem, karanj and maize each applied at rates of 5, 7.5 and 10 ml.kg⁻¹ of grains (0.5, 0.75, and 1% v/w concentration) as grain protectants of pigeon pea against *C. chinensis*.



CHAPTER 3

MATERIALS AND METHODS

CHAPTER 3

MATERIALS AND METHODS

The experiment was conducted from May to November 2017 at the laboratory of the Department of Entomology, Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur, Bangladesh. The ambient temperature and relative humidity during the experimental period were $30 \pm 3^{\circ}\text{C}$ and 87-99% RH, respectively. Details methodologies of the experiment are described below.

3.1 Sources and collection of chickpea seeds

Healthy chickpea (*Cicer atietinum* L.) seeds was purchased from the local market of Dinajpur town. The seeds were thoroughly cleaned, sun dried, cooled, and stored with $12 \pm 1\%$ moisture content. The seeds were kept in air tight plastic container (25H x 15D cm) and preserved at room temperature for study.

3.2 Source and collection of oils

Commercially available plant oils of tishi, mustard, palm, soybean, groundnut, and kalozira (Plate 1) were collected from the market of Maldaho Patty of Dinajpur town in separate glass bottles. Collected oils were preserved.



Plate 1: Six different indigenous plant oils used in the experiment against *C. chinensis*

3.3 Location of the study

To evaluate the effects of 6 indigenous plant oils against pulse beetle (*Callosobruchus chinensis*) the experiments were conducted in the laboratory conditions of the Department of Entomology, Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur during the from May to November 2017.

3.4 Source and collection of Pulse beetle

Pulse beetle, *Callosobruchus chinensis* (L.) (Plate 2) a major stored grain insect was used in this study. The insect was collected from the godown of ‘Chak Bazar’ of Dinajpur town.

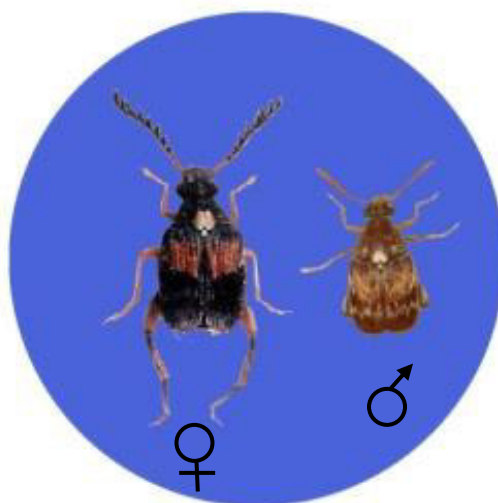


Plate 2: Adult female and male of *C. chinensis*

3.5 Collection of test materials

Test materials of this study were used fresh chickpea seed and healthy adult pulse beetle. They were collected from the local market of Dinajpur town. Chickpea seeds were thoroughly cleaned, sterilized and cooled at 8-10% moisture level. Before setting up the experiment, the chickpea seeds were stored at room temperature in big air tight plastic containers for further use.

Table 1: List of indigenous plant oils against *C. chinensis* in chickpea seeds

Serial no.	Common name	Scientific name	Family
1	Mustard	<i>Brassica campestris</i>	Cruciferae
2	Kalozira	<i>Nigella sativa</i>	Ranunculaceae
3	Palm	<i>Elaeis guinensis</i>	Palmae
4	Soybean	<i>Glycine max</i>	Leguminosae
5	Tishi	<i>Sesamum indicum</i>	Pedaliaceae
6	Ground nut	<i>Arachis hypogaea</i>	Fabaceae

3.6 Collection and rearing of test insects

Adults beetle of *Callosobruchus chinensis* (Plate 3) were collected from naturally infested gram seeds of the local market of Dinajpur town. By maintaining ambient laboratory temperature of 28 ± 5 °C, and $75 \pm 5\%$ relative humidity adult beetle were cultured in separate glass jar (500 ml) with the food medium.

About 200 adults in each jar were released containing 0.5 kg of chickpea grains. To avoid get off of the beetle, the jars were closed with pieces of white muslin cloth tightly fixed with the help of rubber bands. The jars with gram grains were left undisturbed for a maximum period of 7 days for oviposition. After oviposition, the adult beetles were separated from the seeds by sieving and seeds along with eggs were left in the jar for emergence of adult. After emergence, the newly emerged adults were collected and again released in new seeds allowed for oviposition in different jar for the continuation of the culture. The stock culture of the test insect was continued during the experimental period. Only 1 to 5 days old adult of *C. chinensis* were used for the whole experiments purposes.



Plate 3: Mass culture of pulse beetle, *C. chinensis*

3.7 Data recording procedure

Fifty gram of healthy chickpea seeds were taken in a plastic container (300 ml) and mixed with 5, 7.5 and 10% concentrations of each tested oils (v/w) and then air dried for 30 minutes. Five pairs of newly emerged one day old adult beetles were released in each plastic container and the mouth was closed with its lid. Each treatment was replicated thrice including control. All treated containers were kept at ambient room temperature (27-33°C) in the laboratory for oviposition. After 7 days, dead and alive beetles were removed from each container and seeds along with eggs were kept in the laboratory for further development of the insect. The efficacy of plant materials as protectant of chickpea seeds against *C. chinensis* was assessed considering oviposition, adult's emergence, seed infestation and seed weight loss done. For the determination of oviposition of the beetle seeds were collected from each plastic container of each treatment and examined under 10 x magnifying glasses and the number of seeds along with eggs (egg bearing seeds) and the number of eggs deposited were counted. After each observation, the grains were returned to the respective containers for the further

development of the beetle. The adults were removed daily from each plastic container and the data were recorded. Percent seed infestation and weight loss were determined at the completion of adult emergence. The sample of each replicate were examined carefully and damaged and healthy seeds were separated, cleaned, counted and weighed. Percent seed infestation and weight loss were calculated by using the following formulae.

$$\text{Infestation (\%)} = \frac{N_b}{T_n} \times 100 \text{ (Enbakhare and Law-Ogbomo, 2002)}$$

Where N_b = Number of bored seed and T_n = Total number of seeds

$$\text{Weight loss (\%)} = \frac{IW - FW}{IW} \times 100 \text{ (Keita et al., 2001)}$$

Where, IW = Initial Weight of seed, FW = Final Weight of seed.

3.8 Ovicidal and larvicidal efficacies of oils

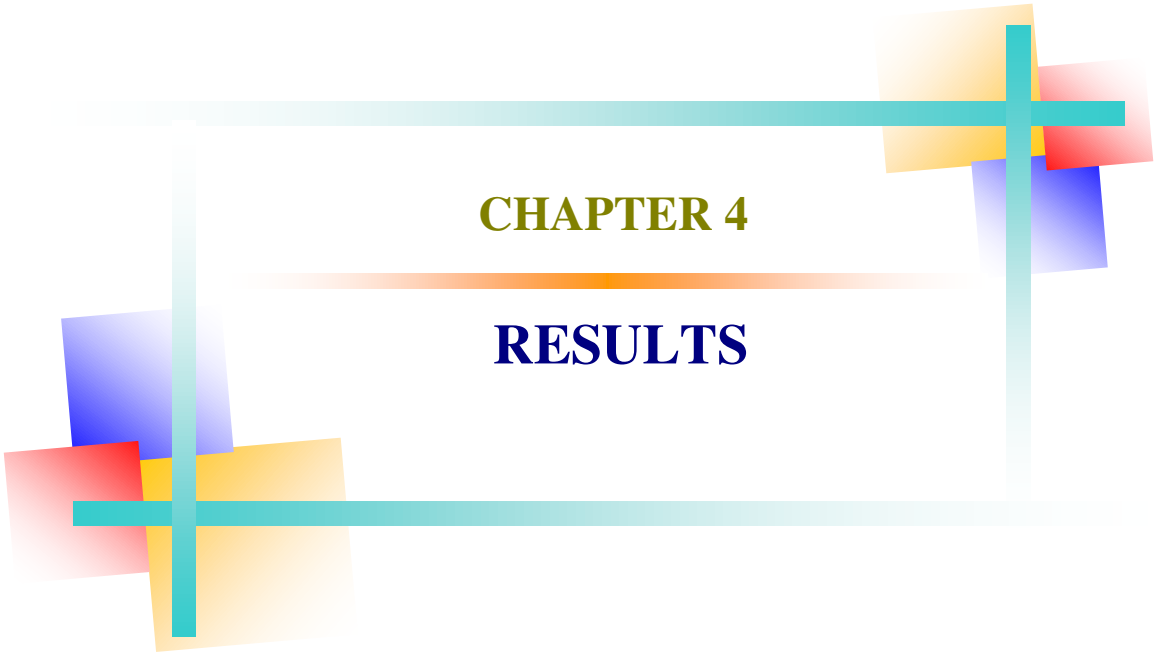
This experiment it was conducted to investigate whether kalozira, mustard, tishi, soybean, groundnut, and palm oils have ovicidal and larvicidal efficacy. For this, 100 number of chickpea seeds along with one day old egg bearing seeds for (ovicidal experiment) and 1-2 days old larvae bearing seeds for (larvicidal experiment) containing one egg or larvae per seed were separately placed in each container and mixed properly with those oils at 5, 7.5, 10% concentration including control treatment. Here each treatment was replicated thrice. Until adult emergence the mouth of the container was closed with its cap and left undisturbed in the laboratory after proper mixing. Counted and recorded the number of adult beetles daily from the first to last emergence and

removed from the containers. After completion of adult emergence, the inhibition was computed by using the following formula as stated by Shukla *et al.* (2007).

$$\text{Inhibition (\%)} = \frac{\text{Control mean} - \text{Treatment mean}}{\text{Control mean}} \times 100 \text{ (Shukla } et al., 2007).$$

3.9 Statistical analyses

The collected data were statistically analyzed in accordance with Completely Randomized Design (CRD) along with analysis of variance ANOVA) through MSTAT program. Mean values were separated with DMRT at 5% level of probability.



CHAPTER 4

RESULTS

CHAPTER 4

RESULTS

The efficacy of six different indigenous plant oils on the protection of chickpea seed against the attack of *C. chinensis* was assessed under laboratory conditions. All data were recorded on oviposition, adult emergence, seed infestation and seed weight loss done by *C. chinensis*.

4.1 Effect of plant oils on oviposition

It was observed from the results that the effects of different plant oils, doses and their interactions differed significantly ($P < 0.05$) among the treatments on oviposition of *C. chinensis* (Tables 2-4). Contrary, the lowest number of eggs were recorded in kalozira (9.0) followed by mustard (15.67) oil treated seeds. Among the doses, significantly ($P < 0.05$, $F = 115.12$, $df = 2$) the highest (49.05) and the lowest (38.24) number of eggs were found at 5% and 10% concentrations, respectively (Table 3). In the interaction effects of oils and doses, significantly ($P < 0.05$, $F = 6.67$, $df = 12$) the highest (42.33) in palm at 5% concentration but the lowest (5.0) number of eggs was found in kalozira oil at 10% concentration (Table 4). On the contrary, the highest number of eggs was recorded in the untreated control (181.0).

The number of egg bearing seeds significantly different among the treatments. Among the oils, significantly ($P < 0.05$, $F = 3080.86$, $df = 6$) the highest number of egg bearing seeds was recorded in the untreated control (146.7). Significantly the lowest number of egg bearing seeds was (Table 2) recorded from kalozira (7.67) followed by mustard (13.56) oil treated seeds. Among the doses, significantly ($P < 0.05$, $F = 88.82$, $df = 2$) (Table 3) the highest (42.38) and the lowest (31.52) number of egg bearing seeds was found at 5% and 10% concentrations, respectively (Table 3). Owing to interaction effects

of oils and doses, significantly ($P < 0.05$, $F = 5.13$, $df = 12$) the highest (40.67) and the lowest (3.33) number of eggs was found in palm and kalozira oils at 5% and 10% concentration, respectively (Table 4).

4.2 Effect of oils on adult emergence

Treatments with all oils significantly ($P < 0.05$) reduced the number of adult emergence as compared to control (Table 2). Among the oils, significantly ($P < 0.05$, $F = 3004.03$, $df = 6$) the highest number of adult emergence was recorded in the control (165.7). On the other hand, the lowest number of adult emergence was recorded in kalozira (6.22) oil treated seeds. Among the doses, significantly ($P < 0.05$, $F = 188.07$, $df = 2$) the highest (43.29) and the lowest (24.52) number of adult emergence was found at 5% and 10% concentrations (Table 3). Due to the interaction effects of oils and their doses, significantly ($P < 0.05$, $F = 12.32$, $df = 12$) the highest (37.67) number of eggs were experienced in palm oil but the lowest (7.67) in kalozira oil at 5% and 7.5% concentrations, respectively (Table 4).

4.3 Effect of oils on seed infestation

All the treatments significantly ($P < 0.05$) reduced the seed infestation percentage compared to control (Table 2). Among the oils, significantly ($P < 0.05$, $F = 3299.96$, $df = 6$) the highest seed infestation percent was recorded in the control (42.35%) while, the lowest number of seed infestation was recorded in kalozira (2.09%), mustard (3.11%) and tishi (3.31%) oils treated seeds. Among the doses, among the oils, significantly ($P < 0.05$, $F = 426.84$, $df = 2$) the highest (13.11%) and the lowest (6.42%) number of seed infestation was found at 5% and 10% concentrations, respectively (Table 3). In the interaction effects of oils and doses, significantly ($P < 0.05$, $F = 31.03$, $df = 12$) the

highest (14.17%) number of eggs was found in palm oil at 5% concentration but the lowest (0.42%) in kalozira oil at 10% concentration (Table 4).

4.4 Effect of oils on seed weight loss

The weight losses caused by *C. chinensis* on chickpea seeds varied among the treatments (Table 2). Among the oils, significantly ($P < 0.05$, $F = 2740.60$, $df = 6$) the control treatment showed the highest (7.20 %) seed weight loss while the lowest (0.45%) number of seed weight loss was recorded in the kalozira, mustard (0.70%) and tishi oil (0.72%) oils treated seeds. Among the doses, significantly ($P < 0.05$, $F = 325.59$, $df = 2$) the highest (2.14%) and the lowest (1.09%) number of seed weight loss was found at 5% and 10% concentrations respectively (Table 3). Owing to interaction effects of oils and doses, significantly ($P < 0.05$, $F = 13.62$, $df = 12$) the highest (1.78%) and lowest (0.05%) number of eggs was found in palm and kalozira oils at 5% and 10% concentration, respectively (Table 4).

4.5 Ovicidal efficacy of oils

To evaluate the ovicidal activity of oils, chickpea seeds along with one old eggs of *C. chinensis* were treated with kalozira, mustard, tishi, soybean, groundnut and palm oils and their activity was assessed based on the number of adult emerged. The effects of treatments on eggs were differed significantly ($P < 0.05$) among the treatments (Table 5).

Among the oils, significantly ($P < 0.05$, $F = 901.27$, $df = 6$) the highest number of adult emergence was recorded in control (97.0). Contrary, the lowest number of adult emergence was recorded in kalozira (12.89) oil treated seeds. Similarly, among the oils, significantly ($P < 0.05$, $F = 58.17$, $df = 5$) the highest (86.59%) and lowest (65.15%) inhibition was recorded in kalozira and palm oil treated seeds. Among the doses,

significantly ($P < 0.05$, $F = 317.23$, $df = 2$) (Table 6) the highest number of adult emergence (45.67) and the lowest (24.24) number of adult emergence was found at 5% and 10% concentrations, respectively (Table 6). Likewise Among the doses, significantly ($P < 0.05$, $F = 310.72$, $df = 2$) the highest (87.50%) and lowest (61.67%) inhibition was found at 10% and 5% concentrations, respectively (Table 6). Owing to interaction effects of oils and doses significantly ($P < 0.05$, $F = 14.10$, $df = 2$) the lowest (4.0) and highest (50.0) number of adult emergence was found in kalozira oil and palm oil at 10% and 5% concentrations, respectively (Table 7). Significantly ($P < 0.05$, $F = 5.23$, $df = 10$) the highest (95.86%) and lowest (48.43%) inhibition over control was found in kalozira and palm oil at 10% and 5% concentrations, respectively.

4.6 Larvicidal activity of oils

To evaluate the larvicidal activity of oils, chickpea seeds along with newly hatched larvae were treated with kalozira, mustard, tishi, soybean, ground nut and palm oils and their activity was assessed based on the number of adult emerged. The effects of treatments on eggs were differed significantly ($P < 0.05$) among the treatments (Table 8).

Among the oils, significantly ($P < 0.05$, $F = 614.20$, $df = 6$) the highest number of adult emergence was recorded in control (96.0). Contrary, the lowest number of adult emergence recorded in kalozira (17.78) oil treated seeds. Significantly ($P < 0.05$, $F = 76.70$, $df = 5$) the highest (81.48%) and lowest (58.59%) inhibition was observed in kalozira and palm oil treated seeds. Among the doses significantly ($P < 0.05$, $F = 272.85$, $df = 2$) the highest number of adult emergence (52.67) and the lowest (30.48) number of adult emergence was found at 5% and 10% concentrations, respectively (Table 9). Significantly ($P < 0.05$, $F = 336.14$, $df = 2$) the highest (79.67%) and lowest (52.67%) inhibition was found at 10% and 5% concentrations, respectively. Owing to interaction

effects of oils and doses significantly ($P < 0.05$, $F = 10.36$, $df = 12$) the lowest (5.33) and highest (54.67) number of adult emergence was found in kalozira oil and palm oil at 10% and 5% concentrations, respectively (Table 10). Significantly ($P < 0.05$, $F = 3.54$, $df = 10$) the highest (94.47%) and lowest (43.08%) inhibition was found as compared to control from kalozira and palm oils at 10% and 5% concentrations, respectively.

Table 2. Effect of plant oils on oviposition, adult emergence, seed infestation, and weight loss done by *C. chinensis*

Treatments (oils)	Number of eggs	Number of egg bearing seeds	Number of adult emergence	Seed infestation (%)	Seed weight loss (%)
Kalozira	9.0 f	7.67 f	6.22 e	2.09 f	0.45 e
Mustard	15.67 e	13.56 e	9.11 de	3.11 e	0.70 d
Tishi	16.56 e	14.33 e	9.67 d	3.31 e	0.72 d
Soybean	22.11 d	20.00 d	15.0 c	5.29 d	0.86 c
Groundnut	27.44 c	24.89 c	16.11 c	6.42 c	0.95 bc
Palm	31.56 b	29.44 b	21.44 b	7.85 b	1.08 b
Control	181.0 a	146.7 a	165.7 a	42.35 a	7.20 a
LSD	2.21	2.52	3.02	0.71	0.13
CV (%)	5.35	7.24	9.13	7.47	8.15
P-value	0.00	0.00	0.00	0.00	0.00

Means in a column having the same letters are not significantly differed among the treatments at 5% level of probability.

Table 3. Effect of different concentrations on oviposition, adult emergence, seed infestation, and weight loss done by *C. chinensis*.

Doses (%)	Number of eggs	Number of egg bearing seeds	Number of adult emergence	Seed infestation (%)	Seed weight loss (%)
5.0	49.05 a	42.38 a	43.29 a	13.11 a	2.14 a
7.5	42.71 b	36.05 b	36.43 b	10.65 b	1.90 b
10.0	38.24 c	31.52 c	24.52 c	6.42 c	1.09 c
LSD	1.45	1.65	1.98	0.47	0.08
CV (%)	5.35	7.24	9.13	7.47	8.15
P-value	0.00	0.00	0.00	0.00	0.00

Means in a column having the same letters are not significantly differed among the treatments at 5% level of probability.

Table 4. Effects of plant oil concentrations on the oviposition, adult emergence, seed infestation, and weight loss done by *C. chinensis*.

Treatments (oils)	Doses (%)	Number of eggs	Number of egg bearing seeds	Number of adult emergence	Seed infestation (%)	Seed weight loss (%)
Kaloziira	5.0	12.0 gh	11.0 h	10.0 ef	3.41 gh	0.78 f
	7.5	10.0 h	8.67 h	7.67 f	2.44 h	0.51 g
	10.0	5.0 i	3.33 i	1.0 g	0.417 i	0.0467 h
Mustard	5.0	19.33 f	17.33 fg	15.33 de	5.11 f	1.09 de
	7.5	15.33 g	13.0 gh	11.0 ef	3.80 g	0.95 ef
	10.0	12.33 gh	10.33 h	1.0 g	0.42 i	0.06 h
Tishi	5.0	23.0 ef	20.33 ef	17.33 d	5.89 f	1.13 cde
	7.5	15.0 g	13.0 gh	10.67 ef	3.63 gh	0.98 ef
	10.0	11.67 gh	9.67 h	1.0 g	0.43 i	0.07 h
Soybean	5.0	30.0 d	28.0 d	26.33 c	9.46 d	1.38 c
	7.5	21.33 f	19.33 ef	17.67 d	5.97 f	1.13 cde
	10.0	15.0 g	12.67 gh	1.0 g	0.437 i	0.087 h
Groundnut	5.0	35.67 c	32.67 c	30.67 c	11.43 c	1.61 b
	7.5	26.0 e	24.0 de	16.67 d	7.41 e	1.18 cde
	10.0	20.67 f	18.0 f	1.0 g	0.42 i	0.05 h
Palm	5.0	42.33 b	40.67 b	37.67 b	14.17 b	1.79 b
	7.5	30.33 d	27.67 d	25.67 c	8.95 d	1.33 cd
	10.0	22.0 ef	20.0 ef	1.0 g	0.45 i	0.11 h
Control		181.0 a	146.7 a	165.7 a	42.35 a	7.20 a
LSD		3.82	4.37	5.23	1.24	0.23
CV (%)		5.35	7.24	9.13	7.47	8.15
P-value		0.00	0.00	0.00	0.00	0.00

Means in a column having the same letters are not significantly differed among the treatments at 5% level of probability.

Table 5. Effect of plant oils on number of adults of *C. chinensis* emerged from egg bearing chickpea seeds.

Treatments (oils)	Number of adult emerged	Inhibition (%)
Kalozira	12.89 f	86.59 a
Mustard	20.44 e	78.92 b
Tishi	26.22 d	72.97 c
Soybean	29.33 c	69.74 d
Groundnut	31.33 bc	67.68 de
Palm	33.78 b	65.15 e
Control	97.00 a	-
LSD	2.65	3.00
CV (%)	7.77	4.27
P-value	0.00	0.00

Means in a column having the same letters are not significantly differed among the treatments at 5% level of probability.

Table 6. Effect of different doses on number of adults of *C. chinensis* emerged from egg bearing chickpea seeds.

Doses (%)	Number of adult emerged	Inhibition (%)
5.0	45.67 a	61.67 c
7.5	37.67 b	71.35 b
10.0	24.24 c	87.50 a
LSD	1.73	2.12
CV (%)	7.77	4.27
P-value	0.00	0.00

Means in a column having the same letters are not significantly differed among the treatments at 5% level of probability.

Table 7. Effect of different indigenous oils with 3 concentrations on number of adults of *C. chinensis* emerged from egg bearing chickpea seeds.

Treatments (oils)	Doses (%)	Number of adult emerged	Inhibition (%)
Kaloziira	5.0	20.67 g	78.33 de
	7.5	14.00 hi	85.57 bc
	10.0	4.000 k	95.86 a
Mustard	5.0	26.67 f	72.50 f
	7.5	26.00 f	73.20 ef
	10.0	8.667 j	91.05 ab
Tishi	5.0	37.33 cd	61.53 hi
	7.5	30.67 ef	68.40 fg
	10.0	10.67 ij	88.98 b
Soybean	5.0	41.33 c	57.40 i
	7.5	33.33 de	65.61 gh
	10.0	13.33 hij	86.23 bc
Groundnut	5.0	46.67 b	51.87 j
	7.5	29.33 ef	69.74 fg
	10.0	18.00 gh	81.43 cd
Palm	5.0	50.00 b	48.43 j
	7.5	33.33 de	65.61 gh
	10.0	18.00 gh	81.42 cd
Control	0.0	97.00 a	-
LSD	-	4.59	5.20
CV (%)	-	7.77	4.27
P-value	-	0.00	0.00

Means in a column having the same letters are not significantly differed among the treatments at 5% level of probability.

Table 8. Effect of plant oils on number of adults of *C. chinensis* emerged from larvae bearing chickpea seeds.

Treatments (oils)	Number of adult emerged	Inhibition (%)
Kalozira	17.78 e	81.48 a
Mustard	27.56 d	71.35 b
Tishi	33.33 c	65.30 c
Soybean	42.00 b	56.23 d
Groundnut	31.33 c	67.36 c
Palm	39.78 b	58.59 d
Control	96.00 a	-
LSD	2.93	2.99
CV (%)	7.51	4.69
P-value	0.00	0.00

Means in a column having the same letters are not significantly differed among the treatments at 5% level of probability.

Table 9. Effect of different concentrations on number of adults of *C. chinensis* emerged from larvae bearing chickpea seeds.

Doses (%)	Number of adult emerged	Inhibition (%)
5.0	52.67 a	52.67 c
7.5	40.19 b	67.82 b
10.0	30.48 c	79.67 a
LSD	1.92	2.18
CV (%)	7.51	4.69
P-value	0.00	0.00

Means in a column having the same letters are not significantly differed among the treatments at 5% level of probability.

Table 10. Effects of plant oil and this concentrations on number of adults of *C. chinensis* emerged from larvae bearing chickpea seeds.

Treatments (oils)	Doses (%)	Number of adult emerged	Inhibition (%)
Kaloziira	5.0	26.67 g	72.21 d
	7.5	21.33 h	77.77 c
	10.0	5.333 j	94.47 a
Mustard	5.0	42.67 c	55.58 h
	7.5	28.67 fg	70.14 de
	10.0	11.33 i	88.33 b
Tishi	5.0	46.00 c	52.12 h
	7.5	33.33 def	65.28 efg
	10.0	20.67 h	78.49 c
Soybean	5.0	58.00 b	39.57 i
	7.5	37.33 d	61.10 g
	10.0	30.67 efg	68.03 def
Groundnut	5.0	44.67 c	53.44 h
	7.5	29.33 fg	69.43 de
	10.0	20.00 h	79.21 c
Palm	5.0	54.67 b	43.08 i
	7.5	35.33 de	63.21 fg
	10.0	29.33 fg	69.48 de
LSD	-	96.00 a	-
CV (%)	-	5.08	5.18
P-value	-	0.00	0.00

Means in a column having the same letters are not significantly differed among the treatments at 5% level of probability.

CHAPTER 5

DISCUSSION

CHAPTER 5

DISCUSSION

Tested oils showed promising results against the invasion of *C. chinensis* on chick pea seeds. Among all the tested oils, kalozira and mustard had proficient effects against the ravages. Activity of these oils inhibits oviposition, adult emergence, seed infestation and weight loss from *C. chinensis*. Among the tested oils, kalozira and mustard oil markedly reduced the oviposition and significantly inhibited the adult emergence of *C. chinensis* on chickpea seeds (Table 2). Also showed that kalozira and mustard oil at 10% concentration distinctly reduced the insect oviposition, number of egg bearing seeds, and adult emergence (Table 4). The present results on the rate of oviposition and number of egg bearing seeds are comparable with the findings of Isman (2000), Raja and Ignacimuthu (2001), Singh and Sharma (2003), Yadav and Bhargava (2005), Upadhyay *et al.* (2007) and Hossain *et al.* (2014). They used various plant oils including kalozira, mustard, tishi, soybean, groundnut and palm with various doses against the bruchids hosted on pulse seeds. Singh (2003) reported that botanical oils treated grain impaired oviposition of pulse beetle. Biswas and Biswas (2005) cited that edible oils comprising mustard, kalozira and sesame at 8.0 ml/kg seeds gave best protection of pea seeds against pulse beetle.

Significant effects in reducing the emergence of adults of *C. chinensis* had found owing to all tested oils as compared to control (Tables 2-4). However, it was experienced from the results that with all the tested oils, kalozira and mustard at 10% concentration inhibited the adult emergence with a good degree of success by reducing oviposition. The present results are in agreement with the results opined by Misra (2000), Chander *et al.* (2007), Haghtalab *et al.* (2009) and Hossain *et al.* (2014). They reported that oils of

neem, castor, karanja, sesame and mustard markedly reduced adult emergence of pulse beetle when admixed with various pulse seeds. Again, Rahman and Rahman (2004) experienced that oils of kalozira, mustard, tishi, soybean, groundnut and palm at 10.0 ml/kg seeds reduced emergence of adult of pulse beetle on chickpea seeds at certain potential level although could not completely inhibited.

The seed infestation was found differed significantly ($P < 0.05$) among all the treatments of plant oils, doses and their interactions (Tables 2-4). The plant oils had profound significant effect on seed infestation. The infestation of seeds due to various plant oils (Table 2) ranged from (2.09 to 7.85%). The highest seed infestation was recorded in control (42.35%) treatment followed by palm (7.86%). On the contrary, the lowest infestation (2.09%) was found in kalozira oil. Among all the doses (Table 3) the highest seed infestation was found at 5% concentration. The interaction effect of different plant oils and doses (Table 4) the highest (42.35%) seed infestation was found in the control (Table 4). While the lowest seed infestation was found in kalozira (0.42%) at 10 % concentration. This result is comparable with those of Bhatnagar *et al.* (2001), Bhargava and Meena (2002), Singh and Yadav (2003), Abulude *et al.* (2007), Bamaiyai *et al.* (2007) and Sarker *et al.* (2015). They cited that oils of neem, castor, sesame, karanja, mustard, groundnut, sunflower, palm and many other oils were also found effective against the pulse beetle in reducing the intensity of infestation on chickpea, mungbean, pigeon pea and black gram seeds at 10.0 and 5.0 ml/kg against the infestation of *C. chinensis*.

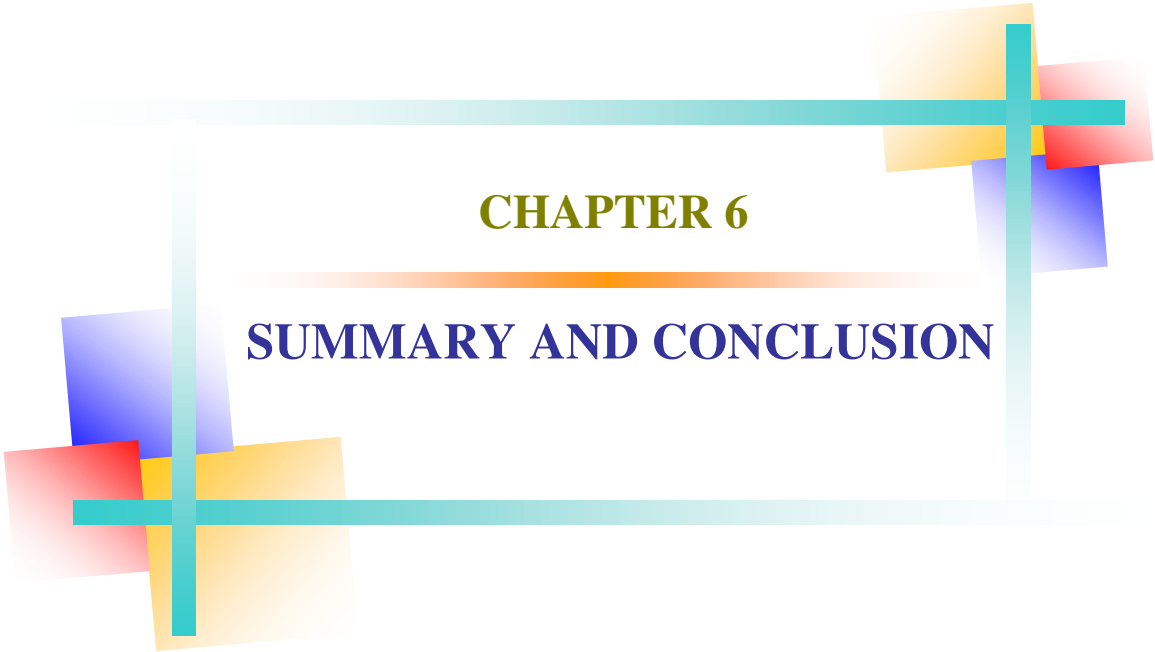
The quantitative losses were assessed in chickpea seeds by the attack of *C. chinensis* in this experiment. The weight loss of seeds due to various plant oils (Table 2) ranged from (0.45 to 1.08%). The highest seed weight loss was recorded in control 7.20% treatment followed by palm 1.08%. On the contrary, the lowest seed weight loss (0.45%) was found

in kalozira oil. Among all the doses (Table 3) the highest seed weight loss was found at 5% concentration. The interaction effect of different plant oils and doses (Table 4) the highest (7.20%) seed weight loss was found in the control. While the lowest seed infestation was found in kalozira oil (0.05%) at 10 % concentration. The findings of present study are also parallel with those of Khaire *et al.* (1992), Ahmed *et al.* (2001), Khalequzzaman *et al.* (2007) and Srinivasan (2008). They experienced that stored grains treated with neem, castor, sesame, karanja and mustard oils provided lowest weight loss over the control.

The ovicidal and larvicidal efficacies of oils was also assessed in laboratory conditions by treating the eggs and larvae of *C. chinensis* it was confirmed from the results that kalozira and mustard oils promisingly inhibited the adult emergence from the eggs when the egg bearing seeds were treated with those oils at 10% concentration. The highest (95.86%) and lowest (48.43%) inhibition was observed while egg bearing seeds was treated with kalozira and palm oils at 10% and 5% concentrations, respectively. The highest (94.47%) and lowest (43.08%) inhibition was observed while larvae bearing seeds was treated with kalozira and palm oils at 10% and 5% concentrations, respectively (Table 10). The results obtained from the present study are in agreement with the observations of Yadav and Bhargava (2005) and Bhatnagar *et al.* (2001). They opined that mustard, and kalozira oils showed ovicidal properties that suppressed the emergence of pulse beetle, *C. chinensis*. Regarding larvicidal efficacy, these oils significantly reduced the F₁ progeny when admixed with 1 to 2 days old larvae by direct coating with oils. It was confirmed from the results that neem, castor and sesame oils only at 8.0 ml/kg seeds reduced the F₁ adult emergence of pulse beetle. These results are at par with those of Ahmed *et al.* (2003) who opined that neem and sesame oils can control the larvae of *C. chinensis* inside the cotyledons of azuki beans. The results, of the

present findings stated that kalozira and mustard oils prevented oviposition, egg from hatching of *C. chinensis* thus reduced the seed infestation and weight loss.

Therefore, kalozira and mustard oils may be used in the eco-friendly management against the notorious pests *C. chinensis* in protecting chickpea seeds in storage.



CHAPTER 6

SUMMARY AND CONCLUSION

CHAPTER 6

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 May to November 2017 to find out the efficacy of kalozira, mustard, tishi, soybean, groundnut, and palm plant oils against *C. chinensis*. The concentrations were applied 10, 7.5 and 5% on pulse beetle.

Pulse beetle, *Callosobruchus chinensis* is a notorious pest of chickpea in storage. The grain protectant efficacy of six indigenous oils in protecting of chickpea seeds from the invasion of *C. chinensis* was assessed. Among all the tested oils, kalozira and mustard oils at 10% concentration showed markedly reduced protection of chickpea seeds by inhibiting oviposition, adult emergence, seed infestation and weight loss against *C. chinensis*. The effects of doses of kalozira and mustard oils differed significantly ($P<0.05$) among the treatments at lower doses 10% concentration. Among the treated oils, the lowest number of eggs, egg bearing seeds, adult emergence, seed infestation and weight loss were found in kalozira and mustard oils. Irrespective of oils among the doses the lowest number of eggs, egg bearing seeds, adult emergence, seed infestation and weight loss were found at the doses of 10% concentration. While the highest number of eggs (181.0), egg bearing seeds (146.7), adult emergence (165.7), seed infestation (42.35%), and weight loss (7.20%) were recorded in the control treatment. To investigate the ovicidal and larvicidal properties lowest number of adult emergence in egg and larva bearing seeds found in kalozira and mustard oils. While the highest was found in control egg bearing seeds (97.00) and larva bearing seeds (96.00). The highest (95.86%) and lowest (48.43%) inhibition was observed while egg bearing seeds were treated with

kalozira and palm at 10% and 5% concentrations, respectively. The highest (94.47%) and lowest (43.08%) inhibition was also recorded at 10% and 5% concentrations as larvae bearing seeds was treated with kalozira and palm oils.

Conclusively, this study indicated that chickpea seeds treated with tested plant oils especially kalozira and mustard oils may be used for sustainable management of chickpea seeds from the invasion of *C. chinensis* in storage.

An abstract geometric design featuring two intersecting teal lines forming a cross. Several semi-transparent squares in yellow, red, and blue are layered over the lines. One yellow square is at the top right, a red one is to its right, and a blue one is below it. Another yellow square is at the bottom left, a red one is to its left, and a blue one is above it. The word "REFERENCES" is centered in the middle of the cross.

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