

**EFFECT OF SOME INDIGENOUS PLANT EXTRACTS
AGAINST LESSER GRAIN BORER, *RHYZOPERTHA DOMINICA*
(COLEOPTERA : BOSTRICHIDAE)**

**A THESIS
BY**

Md. Arifuzzaman
Examination Roll No. 1105057
Registration No. 1105057
Semester: July – December, 2013



**MASTER OF SCIENCE
IN
ENTOMOLOGY**



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

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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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The author

ABSTRACT

The study was carried out in the laboratory of the Department of Entomology, Hajee Mohammad Danesh Science and Technology University, Dinajpur during the period from April to December 2012 to evaluate the efficacy of different plant extract viz. Ata (*Annona reticulata*), Karabi (*Nerium oleander*), Neem (*Azadirachta indica*), Biskatali (*Polygonum hydropiper*) and Akanda (*Calotropis gigantea*) in petroleum ether for the toxicity, residual and repellent effects against lesser grain borer, *Rhyzopertha dominica*. The leaf extracts of above mentioned plants were applied at three doses viz. 4.0, 6.0 and 8.0% concentration. The result showed that extracts of five tested plants had toxic and repellent effects on lesser grain borer. Neem leaf extract showed the highest toxicity and repellent efficacy on lesser grain borer. Even though all the doses had toxicity, residual and repellency effects but the highest efficacy showed at 8% concentration (8% dose showed the highest response). On the basis of the LD_{50} values, it was observed that the neem extract showed the highest performance. Considering all the concentrations, the order of toxicity levels of five plant extracts on lesser grain borer was found as neem > biskatali > karabi > akanda > ata. Mortality percentages were directly proportional to the time after treatment. In case of repellency, it was found that all the tested extracts were in the same repellency class i.e. class II. As the progress of time mortality percentage increased (with the progress of time) meanwhile the repellency effect decreased.

CONTENTS

Chapter		PAGE
	Acknowledgement	iii
	Abstract	iv
	List of plates	v
	List of tables	vi
	List of figures	vii
	List of Appendices	viii
I	INTRODUCTION	1
	1.1 General background	1
	1.2 Research objectives	4
II	REVIEW OF LITERATURES	5
	2.1 Toxic effects	5
	2.2 Repellent effects	7
	2.3 Residual effects	10
	2.4 The test plants	11
	2.4.1 Ata	11
	2.4.2 Karabi	13
	2.4.3 Neem	15
	2.4.4 Biskatali	16
	2.4.5 Akanda	17
	2.5 The Test Insect	18
III	MATERIALS AND METHODS	23
	3.1 Collection of test plant	23
	3.2 Preparation of plant dust and extracts	23
	3.2.1. Processing of plant materials	23
	3.2.2. Preparation of plant dust	23
	3.2.3. Preparation of plant extracts	24
	3.2.4. Preparation of doses	25
	3.3 Collection and rearing of test insect	26
	3.3.1. Collection of test insect	26

Chapter		PAGE
	3.3.2. Disinfestations of wheat	26
	3.3.3. Rearing of test insect	26
3.4	Insect Bioassays	27
	3.4.1. Toxicity test (Topical application method)	27
	3.4.2. Residual toxicity test	28
	3.4.3. Repellency test	28
3.5	Statistical analysis	29
IV	RESULT AND DISCUSSION	30
	4.1. Effects of direct toxicity against lesser grain borer	30
	4.2. Probit Analysis for Direct Toxicity	34
	4.3. Probit regression lines	36
	4.4. Residual effects against lesser grain borer	40
	4.5. Probit analysis for residual toxicity	43
	4.6. Probit regression lines	45
	4.7. Repellent effect on lesser grain borer	49
V	SUMMARY AND CONCLUSION	53
	REFERENCES	55
	APPENDICES	64

LIST OF PLATES

Plate No.	Titles	Page No.
1.	Twigs, fruits and seeds of <i>Annona reticulata</i>	13
2.	Plant parts of <i>Nerium oleander</i>	14
3.	Twig of <i>Azadirachta indica</i> .	16
4.	Plant parts of <i>Polygonum hydropiper</i>	17
5.	Plant parts of <i>Calotropis gigantea</i>	18
6.	Adult <i>Rhyzopertha dominica</i>	21
7.	Food habit of <i>Rhyzopertha dominica</i>	21
8.	Larva of <i>Rhyzopertha dominica</i>	22
9.	Dust of test plants	24
10.	Preparation of plant extract	25
11.	Preparation of doses	25
12.	Repellency test of <i>Rhyzopertha dominica</i>	29

LIST OF TABLES

Table No.	Titles	Page No.
1.	Toxicity of different plant extracts on lesser grain borer at different HAT (Hours after treatment)	32
2.	Toxic effect of different concentrations of different plant extracts on lesser grain borer at different HAT (Hours after treatment)	32
3.	Interaction effects of plant extract and concentration on lesser grain borer at different HAT (Hours after treatment)	33
4.	Relative toxicity (by probit analysis) of different plant extracts treated against lesser grain borer at 24, 48 and 72 HAT	35
5.	Toxicity of different plant extracts on the lesser grain borer at different DAT (Days after treatment).	41
6.	Toxic effect of different concentration of different plant extracts on the lesser grain borer of different DAT (Days after treatment).	41
7.	Interaction effects of plant extract and concentration on the lesser grain borer at different DAT (Days after treatment).	42
8.	Relative toxicity (by probit analysis) of different plant extracts treated against lesser grain borer at 1, 2, 7, 15 and 21 DAT	44
9.	Repellency effects of different plant extracts on lesser grain borer at different HATs	51
10	Repellency effects of different plant extracts at different concentration on lesser grain borer at different HATs	51
11	Interaction effect of different plant extract and their doses on lesser grain borer at different HATs	52

LIST OF FIGURES

Figure No.	Titles	Page No.
1	Relationship between probit mortality and log doses of different plant extracts on lesser grain borer, <i>R. dominica</i> at 24 HAT	37
2	Relationship between probit mortality and log doses of different plant extracts on lesser grain borer, <i>R. dominica</i> at 48 HAT	38
3	Relationship between probit mortality and log doses of different plant extracts on lesser grain borer, <i>R. dominica</i> at 72 HAT	39
4	Relationship between probit mortality and log doses for residual effect of different plant extracts on lesser grain borer, <i>R. dominica</i> at 1 day	46
5	Relationship between probit mortality and log doses for residual effect of different plant extracts on lesser grain borer, <i>R. dominica</i> at 2 days	47
6	Relationship between probit mortality and log doses for residual effect of different plant extracts on lesser grain borer, <i>R. dominica</i> at 7 days	48
7	Relationship between probit mortality and log doses for residual effect of different plant extracts on lesser grain borer, <i>R. dominica</i> at 15 days	48
8	Relationship between probit mortality and log doses for residual effect of different plant extracts on lesser grain borer, <i>R. dominica</i> at 21 days	49

LIST OF APPENDICES

Table No.	Titles	Page No.
1	Doses mortality data of <i>Rhyzopertha dominica</i> adult treated with neem leaf extract in petroleum ether at 24 HAT	64
2	Doses mortality data of <i>Rhyzopertha dominica</i> adult treated with neem leaf extract in petroleum ether at 48 HAT.	64
3	Doses mortality data of <i>Rhyzopertha dominica</i> adult treated with neem leaf extract in petroleum ether at 72 HAT.	65
4	Doses mortality data of <i>Rhyzopertha dominica</i> adult treated with Karabi leaf extract in petroleum ether at 24 HAT.	65
5	Doses mortality data of <i>Rhyzopertha dominica</i> adult treated with Karabi leaf extract in petroleum ether at 48 HAT.	66
6	Doses mortality data of <i>Rhyzopertha dominica</i> adult treated with Karabi leaf extract in petroleum ether at 72 HAT.	66
7	Doses mortality data of <i>Rhyzopertha dominica</i> adult treated with bishkatali leaf extract in petroleum ether at 24 HAT.	67
8	Doses mortality data of <i>Rhyzopertha dominica</i> adult treated with bishkatali leaf extract in petroleum ether at 48 HAT.	67
9	Doses mortality data of <i>Rhyzopertha dominica</i> adult treated with bishkatali leaf extract in petroleum ether at 72 HAT.	68
10	Doses mortality data of <i>Rhyzopertha dominica</i> adult treated with ata leaf extract in petroleum ether at 24 HAT	68
11	Doses mortality data of <i>Rhyzopertha dominica</i> adult treated with ata leaf extract in petroleum ether at 48 HAT	69
12	Doses mortality data of <i>Rhyzopertha dominica</i> adult treated with ata leaf extract in petroleum ether at 72 HAT	69
13	Doses mortality data of <i>Rhyzopertha dominica</i> adult treated with akanda leaf extract in petroleum ether at 24 HAT	70
14	Doses mortality data of <i>Rhyzopertha dominica</i> adult treated with akanda	70

leaf extract in petroleum ether at 48 HAT

Table No.	Titles	Page No.
15	Doses mortality data of <i>Rhyzopertha dominica</i> adult treated with akanda leaf extract in petroleum ether at 72 HAT	71
16	Doses mortality data of <i>Rhyzopertha dominica</i> adult treated with neem leaf extract in petroleum ether at 1 day	71
17	Doses mortality data of <i>Rhyzopertha dominica</i> adult treated with neem leaf extract for residual effect in petroleum ether at 2 days	72
18	Doses mortality data of <i>Rhyzopertha dominica</i> adult treated with neem leaf extract for residual effect in petroleum ether at 7 days	72
19	Doses mortality data of <i>Rhyzopertha dominica</i> adult treated with neem leaf extract for residual effect in petroleum ether at 15 days	73
20	Doses mortality data of <i>Rhyzopertha dominica</i> adult treated with neem leaf extract for residual effect in petroleum ether at 21 days	73
21	Doses mortality data of <i>Rhyzopertha dominica</i> adult treated with biskatali leaf extract for residual effect in petroleum ether at 1 day	74
22	Doses mortality data of <i>Rhyzopertha dominica</i> adult treated with biskatali leaf extract for residual effect in petroleum ether at 2 days	74
23	Doses mortality data of <i>Rhyzopertha dominica</i> adult treated with biskatali leaf extract for residual effect in petroleum ether at 7 days	75
24	Doses mortality data of <i>Rhyzopertha dominica</i> adult treated with biskatali leaf extract for residual effect in petroleum ether at 15 days	75
25	Doses mortality data of <i>Rhyzopertha dominica</i> adult treated with biskatali leaf extract for residual effect in petroleum ether at 21 days	76
26	Doses mortality data of <i>Rhyzopertha dominica</i> adult treated with Karabi leaf extract for residual effect in petroleum ether at 1 day	76
27	Doses mortality data of <i>Rhyzopertha dominica</i> adult treated with Karabi leaf extract for residual effect in petroleum ether at 2 days	77

Table No.	Titles	Page No.
28	Doses mortality data of <i>Rhyzopertha dominica</i> adult treated with Karabi leaf extract for residual effect in petroleum ether at 7 days	77
29	Doses mortality data of <i>Rhyzopertha dominica</i> adult treated with Karabi leaf extract for residual effect in petroleum ether at 15 days	78
30	Doses mortality data of <i>Rhyzopertha dominica</i> adult treated with Karabi leaf extract for residual effect in petroleum ether at 21 days	78
31	Doses mortality data of <i>Rhyzopertha dominica</i> adult treated with ata leaf extract for residual effect in petroleum ether at 1 day	79
32	Doses mortality data of <i>Rhyzopertha dominica</i> adult treated with ata leaf extract for residual effect in petroleum ether at 2 days	79
33	Doses mortality data of <i>Rhyzopertha dominica</i> adult treated with ata leaf extract for residual effect in petroleum ether at 7 days	80
34	Doses mortality data of <i>Rhyzopertha dominica</i> adult treated with ata leaf extract for residual effect in petroleum ether at 15 days	80
35	Doses mortality data of <i>Rhyzopertha dominica</i> adult treated with ata leaf extract for residual effect in petroleum ether at 21 days	81
36	Doses mortality data of <i>Rhyzopertha dominica</i> adult treated with akanda leaf extract for residual effect in petroleum ether at 1 day	81
37	Doses mortality data of <i>Rhyzopertha dominica</i> adult treated with akanda leaf extract for residual effect in petroleum ether at 2 days	82
38	Doses mortality data of <i>Rhyzopertha dominica</i> adult treated with akanda leaf extract for residual effect in petroleum ether at 7 days	82
39	Doses mortality data of <i>Rhyzopertha dominica</i> adult treated with akanda leaf extract for residual effect in petroleum ether at 15 days	83
40	Doses mortality data of <i>Rhyzopertha dominica</i> adult treated with akanda leaf extract for residual effect in petroleum ether at 21 days	83

CHAPTER 1
INTRODUCTION

1. INTRODUCTION

1.1 General Background

Losses due to insect infestation is one of the most serious problem in storage, particularly in villages and towns of developing countries like Bangladesh. Storage loss may also be significant in developing countries where more than 70% of cereal production is stored in farms (Kavita, 2004). It has been estimated that about 15-20% of the world agricultural production is lost every year due to insect infestation (Wright, 1985). In Bangladesh, the annual grain losses cost over taka 100 cores (Alam, 1971) whereas in India losses caused by insects accounted for 6.5% of stored grain (Kumar, 2009). The climate and storage conditions, especially in the tropics, are often highly favorable for insect growth and development (Jacobson, 2004).

Stored food grain of Bangladesh suffers seriously from the attack of a number of insect pests. Insect pests become important in stored grains soon after man first learned to keep grain for seed and other purposes. Their attacks reduce both the quantity and quality of stored seed (Salim, 1978). The stored product insects not only feed on the stored food but also cause organoleptic change in food grains as a result of which the nutritional value of the food grains is reduced. In addition, they also cause fermentation and acidification of food grains and many other changes. Sometimes moulds grow in the insect infested food grain and these moulds produce a chemical substance called aflatoxin which is reported to be associated with the liver cancer of human being (Singh, 1983). Stored grain at high temperature and relative humidity provides an ideal condition for a rapid buildup of the population of many stored insects (Wallbank and Greening, 1976) and the grains become unfit for human consumption and sowing in the field (Husain and Roy, 1997).

Insects belonging to different families have been identified from stored product in various part of the world (Hinton, 1945). About 600 species of insects have been identified from stored products in various parts of the world (Imamura, 2004). Among these species lesser grain borer, *Rhyzopertha dominica* (Coleptera: Bostrichidae) is common and most destructive pest. This insect is originally an inhabitant of India (Atwal, 1976). Now it occurs all over the world and specially encountered in warm situation in temperate zones. Both the adults and grubs cause serious damage to stored grains and stored products such as rice, wheat, sorghum, barley, lentil, corn flour, beans, millets etc. They feed inside the grains and cause damage reducing them to more shells, having many irregular holes. In severe infestation, the damage is very high where a considerable amount of loss is caused by spoiling more than what they eat.

A good number of devices and principles and practices have been reported for the management of grain borer and other stored grain pests (Das *et al.* 1999). Many investigators suggested chemical insecticides for their control. Chemical control of insects in storage has been used with serious drawbacks (Sharaby, 1988). Indiscriminate use of chemical pesticides has given rise too many serious problems, including genetic resistance of pest species, toxic residues, increasing costs of application, environmental pollution, hazards from handling etc. (Ahmed *et al.*, 1981; Khanam *et al.*, 1990). Developing of cross and multiple resistant strains in many important insect species resulting from the continuous use of chemical insecticides has been reported from all over the world (Dyte, 1970; Pasalu and Bhatia, 1983; Zettler and Cuperus, 1990; Zettler, 1991). Fumigation of stored food grains with toxic gases is effective but not applicable at the farm level because the storage structures are not airtight. This situation indicates the need for safe but effective, biodegradable pesticides with no toxic effects on non-target organisms, locally available and less expensive materials for pest control in storage. This has created a worldwide interest in the development of alternative

1.2 Research Objectives

The present study was carried out with five locally grown plants namely neem, biskatali, ata, karabi, and akanda to investigate their compatibility in the IPM programme by determining their toxicity, repellency and residual effects against a major stored product insect, namely lesser grain borer, *R. dominica*. Therefore the specific objectives of the present research work are as follows:

1. To determine the direct toxic effect of plant extracts against lesser grain borer.
2. To evaluate the repellent properties of test plant extracts under simple laboratory technique.
3. To determine the residual effect of five test plant extracts against *R. dominica*.

CHAPTER 2
REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

Stored grain suffers seriously from the attack of a number of insect pests. Control of insect pest by botanicals has drawn special attention all over the world. The use of botanical as stored grain protectant is known since long. There were many reports published on the use of plant materials against stored pests but the information against lesser grain borer *R. dominica* Herbs. are scanty. Relevant information of the research works in home and abroad on stored product insect pests management by botanical insecticides has been reviewed below.

2.1 Toxic effects

Jagadiswari, R. and Anand, P. (1999) studied the insecticidal potentialities of goat weed on rice grain protectant against storage insects. The leaf powder of goat weed controlled the multiplication of *R. dominica* up to 6 months at the rate of 1 and 2 % and showed no grain damage on rice

Tripathi *et al.* (2005) studied on bioactivities of l-carvone, d-carvone, and dihydrocarvone toward three stored product insects. It observed that *R. dominica* adults were more susceptible than the other species to fumigant vapours of l-carvone, d-carvone, and dihydrocarvone. The comparison of contact and fumigant toxicity of the test compounds indicates that l-carvone and d-carvone possess 24 times more fumigant toxicity toward *R. dominica* adults than its contact toxicity. The order of toxicity was l-carvone > d-carvone > dihydrocarvone.

Roy *et al.* (2005) studied on leaf extracts of shiyalmutra as botanical insecticides against lesser grain borer and rice weevil. The average mortality percentage showed that the extract at 3% was also the most toxic to both species (57.41 and 56.71% mortality in lesser grain

borer and rice weevil, respectively). The percentage of insect mortality increased with the increase in the time interval after treatment.

Salema *et al.* (2007) studied on entomocidal effect of *Brassica napus* extracts on two store pests, *Sitophilus oryzae* (L.) and *Rhyzopertha dominica* (Fab.) They conducted that surface treatment of wheat seeds with acetone or petroleum ether extracts of *B. napus* showed various toxicities to *R. dominica* adults, acetone extract was found to be the highest effective extract as it resulted 92.4% mortality at 4.0% conc. level, followed by diethyl ether extract (90%).

Djelatnost and Borah (2009) conducted an experiment on food production, enhanced pest resistance, as well as environment pollution exact researching for new reduced risk insecticides in stored pest's control. They showed that the application of botanical pesticides azadiractin, a seed isolate from the plant *Azadirachta indica* are highly toxic for lesser grain borer.

Kavallieratos *et al* (2009) studied the pyrole chlorfenapyr as a potential grain protectant against adults of *R. dominica*. They showed that mortality was low at 0.1 and 1 ppm for paddy rice but reached 74.4% in barley after 14 days of exposure and adult emergence for *S. oryzae* or *R. dominica*, was higher in paddy rice than in the other three commodities.

Kathirvelu *et al.* (2009) studied on botanical control of pongam and neem against lesser grain borer in stored paddy. The results revealed that mixing neem oil at 30 ml per kg of grains was effective against the pest, with 57.95% mortality of the borer, followed by neem leaf dust at 30% (50.90% borer mortality) and pongam oil at 30 ml per kg of grains (46.74% borer mortality).

Li-GuanHua and Batra (2011) tested to insecticidal activities of the essential oils from *Tephrosia purpurea* leaves, *T. purpurea* barks and *T. purpurea* seeds against *R. dominica* for toxicity test. The results showed all the three kinds of essential oils had exhibited contact toxicity against the 5-day old nymph of *R. dominica*.

Rani and Datta (2011) assessed the leaf extracts of *Cocos nucifera* L. and *Terminalia catappa* L. as potential grain protectants against four major pests of stored grains, *Sitophilus oryzae* (L.), *R. dominica* (F.), *Tribolium castaneum* (Herbst), and *Callosobruchus chinensis* (L.). Results showed that adults of *R. dominica* showed tolerance to all the extracts tested in both fumigation and contact mode bioassays.

2.2 Repellent effects

Bailey and Bailey (1976) studied on effectiveness of spinosad against major stored-grain insects on corn. Insect species tested were the red flour beetle, lesser grain borer, rice weevil and maize weevil. Corn kernels were treated with spinosad at 0, 0.1, 0.5, 1, and 2 mg/kg for controlling the above insect species. Their results indicated that spinosad at the rate of 1 mg/kg was effective against the above stored-grain insect pests on corn.

Stemley and Wilbur (1996) conducted an experiment on susceptibility of maize grains to storage insects. Grains of seven elite maize genotypes were evaluated for their comparative resistance to lesser grain borer on free choice basis under laboratory conditions. The results indicated that adult progeny of both species and weight loss of grains were significantly low. The results suggested that resistant varieties could be incorporated in maize breeding.

Jagadiswari and Anand (1999) tested 20 new botanicals doses (11 oils and 9 leaf powder formulations) for their efficacy as paddy grain protectants against *R. dominica*. The botanicals were applied as repellent treatment under controlled conditions of infestation on

rice. Among the oils, cotton-seed and eucalyptus oils indicated absolutely highest repellent effect (75.6%) of the pest after 5 hours treatment.

Jagadiswari-Rao and Ezhilkumar (2001) studied on prophylactic treatment against lesser grain borer, *Rhyzopertha dominica* Fab. Result indicated that 0.5 and 1.0% doses showed maximum repellency (80.2%).

Tripathi *et al.* (2003) studied of l-carvone, d-carvone, and dihydrocarvone toward three stored product insects. After treatment to l-carvone, d-carvone, and dihydrocarvone on lesser grain borer they observed that l-carvone showed highest repellent effect after 4 hours treatment. The order of repellent effect was l-carvone > d-carvone > dihydrocarvone.

Sikha (2003) presented an extensive survey conducted to record the indigenous technological knowledge of rice crop in Assam, India, during 2002-03. Farmers have been using neem, Siam weed and chick weed in stores as well as in standing crops to prevent insect infestation since long back. Sweet flag *Acorus calamus*, Sanhund, Lantana, Sukhadarshan and Aloe are also grown along with rice as these indigenous plants are repellant to some insect pests.

Roy *et al.* (2005) conducted an experiment on the leaf extracts of shiyalmutra as botanical insecticides against lesser grain borer and rice weevil. The repellent activity increased with the increase of extract concentration. The extract of 3% concentration resulted highest repellency against lesser grain borer (55.71%).

Borah *et al.* (2005) studied on insecticidal activity of the petroleum ether extract of *Annona reticulata* Linn. leaves were evaluated against lesser grain borer (*R. dominica*) and rice weevil (*Sitophilus oryzae*). The repellent activity of the extract (.5, 1 and 1.5%) increased with the increase in extract concentration. The extract at 1.5% resulted in the greatest repellency against lesser grain borer (63.33%).

Kholy *et al.* (2007) showed that the tested *Brassica napus* extracts at different concentrations exhibited different repellency on adults of *R. dominica*. Chloroform extract was found to be the highest repellent agent (78.3%) repellency on the 2nd day against adults of *R. dominica*.

Kys (2007) studied on the influence of the herbs sage and wormwood on the migration of *R. dominica* (F.) populations. Under conditions permitting the migration of adult *R. dominica*, the addition of powdered sage to wheat grain resulted in a lower colonization of this substrate. It was also found that emigration from grain containing sage was very high, especially in the initial period of grain infestation. This leads to the conclusion that sage is a strong repellent effect.

Salema *et al.* (2007) tested the repellency effect on *Brassica napus* in laboratory against adults of *Sitophilus oryzae* and *R. dominica* at different concentrations. The obtained data showed that 4.0% conc. level of chloroform extract was found to be the highest repellent (78.3%) effect on the 2nd day against adults of *R. dominica*.

Li *et al.* (2009) studied on toxic action of essential oils from *Tephrosia purpurea* on *Rhyzopertha dominica*. They showed that repellent rate reached to the maximum value (94.3%) after 48 hour treatment against adults of *R. dominica*.

Kumar (2009) studied on the effect of pea on repellent nature against stored product insects. Rice grains treated with protein-rich fractions obtained from wrinkled-seeded pea cultivars and a smooth-seeded pea cultivar at 1.0% concentration was effective in repelling the red flour beetle, rice weevil and lesser grain borer from treated grains compared to other treatment. Bonneville was highly effective in its repellent action against *T. castaneum*, *S. oryzae* and *R. dominica*.

2.3 Residual effects

Pasalu and Bhatia (1983) evaluated the toxic effects of *Brassica napus* against adults of *Sitophilus oryzae* and *R. dominica* at different concentrations. All tested extracts of *B. napus* showed various toxicities to *R. dominica* adults, acetone extract was found to be the highest effective extract as it gave 92.4% mortality at 4.0% conc. level, followed by diethyl ether extract (90%) after 7 days of treatment.

Wright (1985) studied the leaf powder of goat weed to control the multiplication of *R. dominica* up to 6 months (dose 1.0 and 2.0% w/w) and showed no grain damage on rice. Grain damage caused by *R. dominica* was also nil at both the higher doses of 1.0 and 2.0% w/w but at lower doses this borer caused grain damage.

Saraby (1988) studied the leaf extract of *Calotropis gigantean* against adults of *R. dominica*. They showed that the mortality was low at 0.5 and 1 ppm for rice but 74.4% in barley after 14 days of exposure.

Zettler (1991) studied on insecticidal activity of the petroleum ether extract of *B. lacera* leaves against *R. dominica* and *Sitophilus oryzae*. The extract at 4% resulted in the greatest toxicity against lesser grain borer (60.33%) and rice weevil (58.58%).

Kys (2007) studied on toxic action of essential oils from *Nerium oleander* on *Rhyzopertha dominica*. The results showed that highest toxicity was found (94.3% mortality) after 14 days of treatment.

Athanassiou *et al* (2007) studied the plant extract of neem against adults of *R. dominica*. The doses rates were 50, 100 and 150 ppm and mortality was measured after 7 and 14 days of exposure, at three temperatures (20⁰, 25⁰ and 30⁰ C) and 65% R.H. All adults were dead after 14 days of exposure at 55% R.H. at 100 ppm.

Rajendran *et al* (2008) studied on plant essential oils and their constituents as fumigants against stored-product insects. Fumigant toxicity tests conducted with essential oils of plants and their components have largely focused on beetle pests such as *R. dominica*, and *Sitophilus oryzae*. Adults were generally susceptible whereas, eggs were either tolerant or highly susceptible depending on insect species and the type of essential oil or component.

Kathirvelu *et al.* (2009) studied to assess the effect of botanicals *Azadirachta indica* and *Pongamia glabra* on *R. dominica* in stored rice, using leaf dusts and oils at different concentrations. Adult mortality was recorded 7, 15 and 21 days after treatment. The results revealed that mixing neem oil at 30 ml per kg of grains was effective against the pest, recording 57.95% mortality of the borer, followed by neem leaf dust at 30% (50.90% mortality) and pongam oil at 30 ml per kg of grains (46.74% mortality).

2.4 Test Plant

The test plant materials of five indigenous plants were collected from around the HSTU campus, Dinajpur. The descriptions of the plants are given below.

2.4.1 Ata

Scientific name: *Annona reticulata* Linn.

Family: Annonaceac

Morphology

A. reticulata tree is erect, with a rounded or spreading crown and trunk 10 to 14 inch thick. Height ranges from 15 to 35 ft. Leaves are deciduous, alternate, oblong or narrow-lanceolate, 4 to 8 inch long, 3/4 to 2 inch wide, with conspicuous veins (Plate 1). Flowers, in drooping clusters, are fragrant, slender, with 3 outer fleshy, narrow petals 3/4 to 1 1/4 in long; light-

green externally and pale-yellow with a dark-red or purple spot on the inside at the base. The compound fruit are heart-shaped, lopsided, or irregular; or nearly round, or oblate, with a deep or shallow depression at the base. Single, hard, dark-brown or black, glossy seed (Plate 1); oblong, smooth, less than ½ inch long (Hooker, 1980).

Origin and Distribution

The *A. reticulata* is believed to be a native of the West Indies but it was carried in early times through Central America to southern Mexico. It is commonly grown in the Bahamas and occasionally in Bermuda and southern Florida (Athanassiou and Korunic, 2007).

Toxicity and uses

The seeds are so hard that they may be swallowed whole with no ill effects but the kernels are very toxic. The seeds, leaves and young fruits have insecticidal value. The leaf juice kills lice Ref. The bark contains 0.12% anonaine Ref. Injection of an extract from the bark caused paralysis in a rear limb of an experimental toad. Sap from cut branches is acrid and irritant and can severely injure the eyes. The root bark has yielded 3 alkaloids: anonaine, liriodenine and reticuline (muricinine) (Hertlein *et al.*, 2011)

The leaves have been employed in tanning and they yield a blue or black dye. A fiber derived from the young twigs is superior to the bark fiber from *Annona reticulata*. Custard apple wood is yellow, rather soft, fibrous but durable, moderately close-grained, with a specific gravity of 0.65. It has been used to make yokes for oxen (Nathan *et al.*, 2007). Morphology of Karabi is too detailed but in case of Neem, Biskatali are not as Karabi, so try to describe the main & same characters of plants for 5 tested plants.



Plate 1. Twigs, fruits and seeds of *Annona reticulata*

2.4.2 Karabi

Scientific name: *Nerium oleander* Linn.

Family : Apocynaceae

Morphology

Erect glabrous shrubs, leaves usually in whorls of three, narrow coriaceous; nerves slender, very close. Flowers are large, in terminal racemose cymes. Calyx 5-partite, glandular within, segments narrow. Corolla funnel shaped lobes overlapping to the right. Stamens inserted near the mouth of the tube, filaments short; anthers conniving around and adhering to the stigma, tipped with long hairy appendages, each cell produced downwards into a rigid spur-like appendage. Carpels 2, distinct, many ovuled; style filiform or dilated upwards; stigma with a reflected lobed membrane surmounted by 5 tubercles, tip sub-globose. Follicles cylindric, straight adpressed. Seeds oblong, villous; coma terminal, caducous; albumen fleshy, cotyledons flat (Kirtikar and Basu, 1980) (Plate 2).

Distribution

Upper Gangetic Plain, Himalaya from Nepal westwards to Kashmir up to 6,500 ft., salt Range, Waziristan, Baluchistan, Central and South India. Extensively cultivated throughout the greater part of India as well China and Japan. (Yamasaki *et al.*, 2007).

Toxicity and Uses

All parts of the *N. oleander* contain cardiac glycosides, including the roots and the smoke produced from burning, as heat does not inactivate the glucocides. The toxic components are the two cardiac glycosides, glucocides, nerrin and olenderin which can be isolated from all parts of the plant. *N. Oleander* poisons are cardiac and gastrointestinal in nature to insects (Yamasaki *et al.*, 2007). *N. oleandar* has been known to be poisonous. The leaves are highly toxic and root is a cardiac poison. The bark of the root and the sweet-smelling leaves of this shrub are considered by the vydians as powerful repellents.



Plate 2. Plant parts of *Nerium oleander*

2.4.3 Neem

Scientific name : *Azadirachta indica*.

Family : Meliaceae.

Morphology

Neem is a perennial tree, 15-20m in height with a straight trunk; leaves are simple, number of leaflets 9-15, opposite, sub-opposite or alternate, lanceolate, acuminate or subfalcate. Flowers are white, Fruit (Drupe) oblong. (Rice *et al.*, 2007) (Plate 3).

Distribution

A common tree of Bangladesh and throughout the greater part of India, planted in the hot climate (Hooker, 1978).

Toxicity and uses

Neem is famous for its medicinal properties. The major active constituent is azadirachtin, which is well known for its antifeedant, toxic and growth regulating effects on insects (Saxena *et al.*, 2004).

Neem oil are contained three bitter compounds i.e. nimbin, nimbinin and nimbidin. It has been an aged practice in rural areas of Bangladesh to mix dried Neem leaves with stored grain to control stored grain insects. A variety of preparations based on Neem extracts have been tested against stored product insects. Azadirachtin-rich commercial "Margosan-O" is already produced in developing countries for controlling pests. (Jacobson, 2004).



Plate 3. Twig of *Azadirachta indica*.

2.4.4 Biskatali

Scientific name : *Polygonum hydropiper*

Family : Polygonaceae

Morphology

Annual herb, reproducing by seeds stems are jointed, somewhat flattened ascending or erect.

Leaves are alternate, simple, with waxy margins, dark green to brown (Rahman *et al.*, 2006) (Plate 4).

Distribution

Damp soils, low meadows, pastures, cultivated ground, and waste places of Bangladesh, India, North America, Europe and many other countries of the world. (Rahman *et al.*, 2006)

Toxicity and uses

It is a medicinal plant and also used to control insect pests as repellence, antifeedant and toxic materials. In rural areas of Bangladesh farmers are used to mix dried biskatali with stored

grain to control stored grain insects. The toxic components are the two potent cardiac glycosides and i.e. are highly toxic for any kind of insect (Rahman *et al.*, 2006).



Plate 4. Plant parts of *Polygonum hydropiper*

2.4.5. Akanda

Scientific name : *Calotropis gigantea*

Family : Asclepiadaceae

Morphology

It is shrub or small tree to 4 m, exuding copious milky sap when cut or broken; leaves opposite, grey-green, large up to 15 cm long and 10 cm broad, with a pointed tip, two rounded basal lobes and no leaf stalk; flowers waxy white, 5 petals, purple-tipped inside and with a central purplish crown, carried in stalked clusters at the ends of the branches; fruit grey green, inflated, 8 to 12 cm long (Kleinschmidt and Johnson, 1977) (Plate 5).

It is originated from Iran to China; India to Sri Lanka; Indo-China, Indonesia and Malaysia. The *C. gigantea*, is native to the Old World tropics but has been widely naturalized throughout the New World tropics including the Caribbean and on the continent from Mexico to Brazil (Pedriniet *al.*, 2011).

Toxicity and uses

The worms in the intestines get killed (Bailey and Bailey, 1976).



Plant 5. Plant parts of *Calotropis gigantea*

2.5 The Test Insect

The lesser grain borer, *Rhyzopertha dominica*, a major pest of stored products, was used in this study.

Systematic position

Systematic position

Phylum : Arthropoda

Class : Insecta

Subclass : Pterygota

Division : Endopterygota

Order : Coleoptera

Family : Bostrichidae

Genus : *Rhyzopertha*

Species : *R. dominica*

Morphological feature

The adult is 2-3 mm long with rounded pronotum bent downward at the front where there are transverse rows of teeth centrally with posteriorly flattened tubercles. Between the pronotum and elytra lies the scutellum which is almost square. The elytra have regular rows of punctures and short curved setae posteriorly. Apically the elytra are gently convex rather than flattened. Each antenna has 10 segments; and a loose three segmental club. The sexes may be distinguished by the features of abdominal sternite (Stemley and Wilbur, 1996; Ghorpade and Thyagarajan, 1980) (Plate 6).

Distribution

It occurs mostly in tropical and subtropical regions of the world and causes damage to stored grains in many countries of Asia, Africa, North America and Europe (Stemley and Wilbur, 1996).

Biology

The females prefer laying their eggs in crevices or on the rough surfaces of the grain. After hatching, the larvae use even minute husk cracks to bore into the grain (Potter, 1935). The larvae are white and they do not taper. The legs are rather short and the head, capsule is small relative to the size of the body. The first instar larva is distinctive in having a median posterior spine. Larval development occurs more rapidly on whole grains than on flours at the optimum temperature of 34°C and at 40% R.H. Larvae undergo 3-5 moults and develop to the pupal stage in about 17 days (Howse, 1950) (Plate 8). The pupal stage lasts approximately for three days at the temperature of 34°C and 70% R.H. The adult mates soon after emergence and under favorable condition oviposition may continue for up to four months. There is no definite peak in egg laying. The average numbers of eggs laid per female are 244 at 25°C rising to an average of 418 at 34°C temperature. (Kavallieratos *et al.*, 2009).

Food habit

The larvae and adults of *R. dominica* are capable of boring and feeding on a wide range of commodities. Undamaged grains, whole cereals, dried cassava, pulses, lentils, soybeans can be severely damaged by *R. dominica*. They also develop on milled rice and cereal flour, but not highly polished rice (Plate 7).



Plate 6. Adult *Rhyzopertha dominica*



Plate 7. Feeding nature of *Rhyzopertha dominica*



Plant 8. Larva of *Rhizopertha dominica*

CHAPTER 3

MATERIALS AND METHODS

3. MATERIALS AND METHODS

The present study was conducted to evaluate some indigenous plants viz Ata (*Annona reticulata*), Karabi (*Nerium oleander*), Neem (*Azadirachta indica*), Biskatali (*Polygonum hydropiper*) and Akanda (*Caloropis gigantea*) leaves extracts against lesser grain borer, *R. dominica* Fab. in the laboratory of the Department of Entomology, Hajee Mohammad Danesh Science and Technology University, Dinajpur during the period from April to December 2012 for their toxic, residual and repellent effects.

3.1 Collection of test plant

The test plant materials of five indigenous plants were collected from the HSTU campus, Dinajpur. The descriptions of the plants are given below.

3.2 Preparation of plant dust and extracts

3.2.1 Processing of plant materials

After collection, they were washed in running water. First of all, the plant materials were kept in laboratory for 7 days for the purpose of air drying followed by one day sun drying before making powder.

3.2.2 Preparation of plant dust

Dust was prepared by pulverizing the dried leaves with the help of a grinder. Then the dust was passed through a 25-mesh diameter sieve to obtain fine and uniform dust. These were preserved separately at room temperature in airtight condition in plastic containers till their use in extract preparation (Plate 9).



Plate 9. Dust of test plants

3.2.3 Preparation of plant extracts

The prepared leaf dusts were used for preparation of plant extract. The extracts were prepared according to Chitra *et al.*, (1993). Ten grams of each category of dust were taken in a 600 ml beaker and separately mixed with 100 ml of petroleum ether solvent. Then the mixture was stirred for 30 minutes by a magnetic stirrer and left stand for next 72 hours (Plate 10). The mixture was then filtered through a filter paper (Whatman No.1). The filtered materials were taken into a round bottom flask and then allowed to evaporate the solvent. After the evaporation of solvent from filtrate, the condensed extracts were preserved in tightly corked-labeled bottles and stored in a refrigerator until their use for insect bioassays.



Plate 10. Preparation of plant extracts

3.2.4 Preparation of doses

Different doses of plant extracts were prepared separately by diluting the condensed extracts with petroleum ether. Three concentrations (4.0%, 6.0% and 8.0%) along with control of each category of plant extracts were prepared in the petroleum ether solvent prior to insect bioassay (Plate 11). Pilot experiments were done to obtain the appropriate dose.



Plate 11. Preparation of doses

3.3 Collection and rearing of test insect

3.3.1 Collection of test insect

Collection of lesser grain borer was necessary for rearing to ensure continuous supply of the test insect during the study. Initially, the test insect species, *R. dominica* with infested wheat grains were collected from the godown of pulse seller of Dinajpur town and were maintained in the laboratory of the Department of Entomology, HSTU, Dinajpur, at 27-30°C temperature and 70-75% relative humidity.

3.3.2 Disinfestations of wheat

The wheat grains were spread on black polythene sheet and sun-dried from 9 a.m. to 2 p.m. with air temperature ranging from 32-42°C for five consecutive days. Sun-dried grains were kept in the oven at 45° C for few hours, packed in polythene bag and sealed to avoid future infestation.

3.3.3 Rearing of test insect

The insects were reared on sterilized wheat grains in rectangular jars (14 x 10.5 x30 cm). Each jar was set up with 10 pairs of adult insects. The mouth of the jar was covered with a piece of cloth fastened with rubber band to prevent contamination and insect escape. After allowing them for free oviposition for a period of 7 days. The adults were removed from each jar and jars were left undisturbed for completing the life cycle of the insect. After adult emerged, one day old adults were sorted from the wheat grain by sieving and transferred regularly into separate jars with wheat grains. Jars along with insects were then kept in the same place, temperature and humidity. Three to seven days old insects were used for study.

3.4 Insect bioassays

Different types of insect bioassay were conducted at 27°C to 30°C temperatures and 70-75% relative humidity to determine the toxicity, residual toxicity and repellency effect. Plant extracts of ata, akanda, karabi, neem, and biskatali in petroleum ether were used with 4.0, 6.0 and 8.0% concentrations.

3.4.1 Toxicity test (Topical application method)

Toxicity test of five indigenous plant extracts against *R. dominica* were conducted according to the method described by Talukder and Howse (1993) with minor modification. Before applying extracts to the thorax of the insect, 10 minutes chilling were done with 4°C in refrigerator. Then 1 µl of prepared solution was applied to the dorsal surface of each insect using a micropipette. Ten insects per replication were treated and each treatment was replicated 3 times. In addition, the same numbers of insects were treated with petroleum ether solvent only for control. After treatment, the insects were transferred into 9 cm diameter petridishes (10 insects / petridish). Insect mortality was recorded at 24, 48 and 72 hours after treatment (HAT).

Original data of the insects were corrected by Abbott's (1987) formula:

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

Where,

P= Percentage of corrected mortality

P'= Observed mortality (%)

C= Control mortality (%)

3.4.2 Residual toxicity test

Residual toxicity test was conducted according to the method of Talukder and Howse (1993) with some modifications. Three different concentrations of each plant extracts were prepared with the petroleum ether solvent. Then 1 ml. of prepared solution was applied to 50g wheat and mixed properly. Then it was air-dried about 10 minutes. Five pairs of one day old adults were released into the pot containing treated with plant extracts and pot was covered with perforated lid. Three replications were maintained for each of the concentration of the individual plant extracts along with control. All treated pots were kept at ambient temperature ($32 \pm 3^{\circ}\text{C}$). Insect mortality was recorded at 1, 2, 7, 15 and 21 DAT (Days after treatment).

3.4.3 Repellency test

The repellent activities of the five indigenous plant extracts against *R. dominica* were evaluated using the filter paper impregnation method followed by Talukdar and Howse (1994) with minor modifications. First of all, the Whatman No. 40 filter papers were cut into two halves. With the help of a pipette 1ml solution of each plant extract was applied to one half of the filter paper. The treated half and control half was then air-dried. Ten insects were released at the centre of each Petridish and a cover was placed on the Petridish (Plate 12). Three replication were used for each plant extract and each dose. Then the insect present on each portion were counted at hourly intervals up to 6th hour. The data were expressed as percentage repulsion (%PR) by the following formula:

$$\%PR = (N_c - 50) \times 2$$

Where,

%PR = percentage repulsion.

N_c = The percentage of insects present in the control half.

Positive (+) values expressed repellency and negative (-) values attractency. The average values were then categorized according to the following classes (McDonald *et al.*, 1970):

Class	Repellency rate(%)
0	>0.01 to 0.1
I	0.1 to 20
II	20.1 to 40
III	40.1 to 60
IV	60.1 to 80
V	80.1 to 100

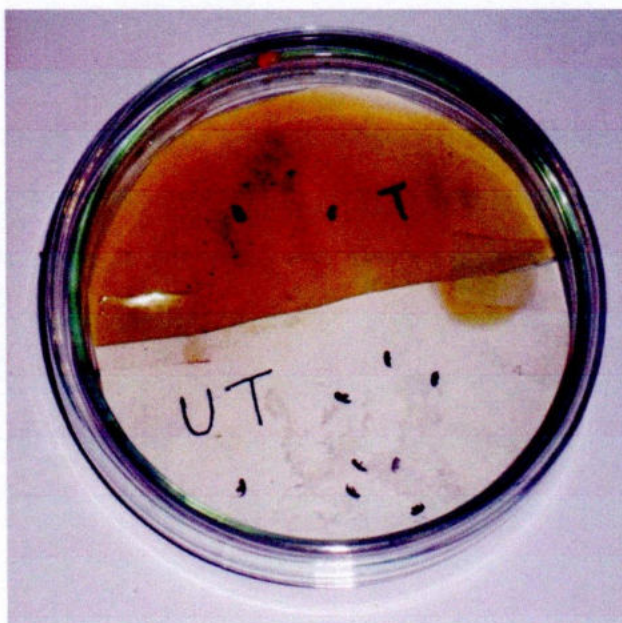


Plate 12.Repellency test of *Rhizopertha dominica*

3.5 Statistical analysis

The collected data were statistically analyzed by completely randomized design (CRD) using MSTAT statistical software. The treatment mean values were adjusted by Duncun's New Multiple Range Test (DMRT). The observed mortality data was also subjected to probit analysis.

CHAPTER 4

RESULTS AND DISCUSSION

4. RESULT AND DISCUSSION



The result of various experiments conducted during the study period are presented and discussed under the following subheadings.

4.1 Effects of direct toxicity against lesser grain borer

The results of the direct toxicity effects of neem, biskatali, karabi, ata and akanda leaf extracts against lesser grain borer, *R. dominica* are presented in Tables 1 to 3.

Average mortality percentage of lesser grain borer at 24, 48 and 72 hours after treatment (HAT) indicated that neem leaf extract (56.33%) possessed the highest toxic effect which was statistically different from all other plant extract (Table 1). The order of toxicity of five plant extracts against lesser grain borer were found as neem > biskatali > karabi > akanda > ata. Mortality percentages were directly proportional to the time after treatment.

Mortality of lesser grain borer was also differed significantly among all the concentration level at different time interval of different plant extracts. The highest mortality (84.65%) was observed at the maximum concentration of plant extracts while the lowest mortality (30.0%) was found in minimum concentration. No mortality was observed in control treatment (Table 2).

All plant extracts achieved hundred percent mortality at 72 HAT in 8.0% concentrations except karabi extract (Table 3). Same trend was also observed at 24 and 48 HAT. Average mortality showed that neem leaf extract possessed highest toxicity (mortality, 98.90 %) at maximum concentration whereas lowest (mortality 8.90 %) in karabi extract in minimum concentration

From the above findings it was found that all five plants had toxic effects against lesser grain borer but neem plant extracts showed the highest toxic effect. This findings agreed with Djelatnost and Borah (2009) who found that neem plant extracts are highly toxic than biskatali. The present study also coincides with the earlier findings of Kathirvelu *et al.*, (2009). Therefore, this experiment also confirms the validity of traditional use of leaf of neem, biskatali, karabi, akanda and ata against stored grain pest.

Table 1: Toxicity of different plant extracts on lesser grain borer at different HAT
(Hours after treatment)

plant extracts used	% Insect mortality(HAT)			Average mortality
	24 HAT	48 HAT	72 HAT	
Neem	32.33a	66.67a	70.00a	56.33a
Biskatali	35.00b	50.00b	57.50b	47.507b
Karabi	36.17b	45.83b	50.00c	44.667bc
Akanda	27.50c	38.33c	47.50c	37.777cd
Ata	13.33d	25.00d	35.83d	24.72bc
P- value	0.0089	0.0180	0.0180	0.3333
LSD	1.176	1.419	1.060	1.221
CV (%)	12.29	13.55	13.55	49.41

Mean followed by row the same letter(s) did not differ significantly at 5% level by DMRT.

HAT = Hours after treatment.

Table 2: Toxic effect of different concentrations of different plant extracts on lesser grain borer at different HAT (Hours after treatment)

Dose (%)	% Insect mortality(HAT)			Average mortality
	24 HAT	48 HAT	72 HAT	
4.0	20.00c	30.00c	40.00c	30.00c
6.0	36.67b	62.00b	73.33b	57.35b
8.0	70.00a	88.67a	95.33a	84.65a
Control	0.00d	0.00d	0.00d	0.00d
P- value	0.0005	0.0047	0.0376	0.3689
LSD	5.91	6.69	5.35	5.07
CV (%)	4.29	4.06	3.90	3.98

Mean followed by row the same letter(s) did not differ significantly at 5% level by DMRT.

HAT = Hours after treatment.

Table 3: Interaction effects of plant extract and concentration on lesser grain borer at different HAT (Hours after treatment)

plant extracts used	Concentrations (%)	% Insect mortality(HAT)			
		24 HAT	48 HAT	72 HAT	Average
Neem	0.0	0.00h	0.00g	0.00i	0.00f
	4.0	40.00d	76.67bc	80.00bc	65.57c
	6.0	56.67c	90.00ab	100.0a	82.23b
	8.0	96.67a	100.0a	100.0a	98.90a
Biskatali	0.0	0.00h	0.00g	0.00i	0.00f
	4.0	16.67fg	23.33f	40.00ef	26.67e
	6.0	40.00d	76.67bc	90.00ab	68.90c
	8.0	83.33b	100.0a	100.0a	94.43a
Karabi	0.0	0.00h	0.00g	0.00i	0.00f
	4.0	3.33gh	3.33g	20.00h	8.90f
	6.0	16.67fg	36.67ef	46.67de	33.37de
	8.0	33.33de	60.00d	76.67c	56.63c
Akanda	0.0	0.00h	0.00g	0.00i	0.00f
	4.0	23.33ef	23.33f	26.67gh	24.43e
	6.0	36.67de	63.33cd	73.33c	57.77c
	8.0	76.67b	96.67a	100.0a	91.10ab
Ata	0.0	0.00h	0.00g	0.00i	0.00f
	4.0	16.67fg	23.33f	33.33fg	24.43e
	6.0	33.33de	43.33e	56.67d	44.47d
	8.0	60.00c	86.67ab	100.0a	82.20b
P- value		0.0001	0.0001	0.0001	0.0001
LSD		13.24	14.98	11.98	11.36
CV (%)		4.29	4.06	3.90	3.98

Mean followed by column the same letter(s) did not differ significantly at 5% level by MRT.

HAT = Hours after treatment.

4.2 Probit analysis for direct toxicity

The results of the probit analysis for the estimation of LD₅₀ values and their 95% fiducial limits at 24, 48 and 72 HAT for the mortality of lesser grain borer are shown in Table 4.

The LD₅₀ values of neem (6.958 µg), biskatali (8.444 µg), karabi (9.191 µg), akanda (10.589 µg) and ata (14.522 µg) at 24 HAT (Table 4) indicated that neem leaf extract was found to be highly toxic. Similarly, neem leaf extract maintain its highest toxicity at 48 HAT while at 72 HAT neem and biskatali extracts showed similar toxicity. The chi-square (χ^2) values of different plant extracts at different HAT were insignificant at 5% level of probability and did not show any heterogeneity of the mortality data.

From the above probit results it is clear that all the tested plants would be more or less effective for controlling lesser grain borer but neem and biskatali will be the most effective. The lowest LD₅₀ values of neem plant extract indicated that the highest toxic effects against lesser grain borer and agreed with the earlier finding of Kathirvelu *et al.*, (2009). From the above probit results it is concluded that neem will be effective for controlling the lesser grain borer.

Table 4: Relative toxicity (by probit analysis) of different plant extracts treated against lesser grain borer at 24, 48 and 72 HAT

Name of the plant extracts	No. of insect used	LD 50 values (μg)	95 % fiducially limits	χ^2 values
24 HAT				
Neem	90	6.958	3.646 - 13.278	.0055
Biskatali	90	8.444	4.251 - 16.770	.0514
Karabi	90	9.191	4.262 - 19.822	.0943
Akanda	90	10.589	4.827 - 23.226	.1735
Ata	90	14.522	3.644 - 57.870	.3752
48 HAT				
Neem	90	1.448	0.021 - 97.257	.0439
Biskatali	90	3.709	1.716 - 8.013	.0015
Karabi	90	5.771	4.4933 - 7.412	.2081
Akanda	90	6.349	4.947 - 8.148	.0476E-04
Ata	90	9.342	5.466 - 15.966	.1611
72 HAT				
Neem	90	2.744	1.630 - 4.755	.3102
Biskatali	90	2.784	1.449 - 5.195	.0730
Karabi	90	3.432	2.131 - 5.528	.0339
Akanda	90	4.127	2.940 - 5.794	.1356
Ata	90	5.924	4.989 - 7.034	.0116

HAT= Hour after treatment

Values were based on three concentrations, three replications of 10 insects each.

χ^2 = Goodness of fit

The tabulated value of χ^2 is 5.99 (d. f=2 at 5% level)

4.3 Probit regression lines for direct toxicity

The probit regression lines for the effect of five plants extracts (neem, biskatali, karabi, akanda and ata) are presented in figure 1, 2 and 3. The insect mortality rate showed positive correlation with doses in all cases. The probit regression lines for the effects of five plant extracts on lesser grain borer showed a clear linear relationship between probit mortality and their log doses. The calculated probit regression equations of five plant extracts are $Y = 1.087x + 4.088$, $Y = 1.436x + 3.676$, $Y = 1.436x + 3.596$, $Y = 1.820x + 3.134$ and $Y = 1.620x + 3.13$ for neem, biskatali, karabi, ata and akanda respectively at 24 HAT (Fig. 1). Comparing among these lines, neem showed the higher probit mortality.

The calculated probit regression equations of five plant leaf extracts at 48 HAT are $Y = 0.875x + 4.767$, $Y = 1.493x + 4.149$, $Y = 2.436x + 3.156$, $Y = 2.433x + 3.041$ and $Y = 2.226x + 2.825$ for neem, biskatali, karabi, ata and akanda respectively (Fig. 2).

At 72 HAT the probit regression equations were calculated as $Y = 3.699x + 3.318$, $Y = 2.966x + 3.672$, $Y = 2.760x + 3.515$, $Y = 2.877x + 3.233$ and $Y = 3.673x + 2.168$ for neem, biskatali, karabi, ata and akanda respectively (Fig. 3). Among the five lines, the regression line for neem plant extract also showed the highest probit mortality.

The probit regression lines became steeper as doses increased. Because the adult insects were treated with more toxins for the same period at higher doses.

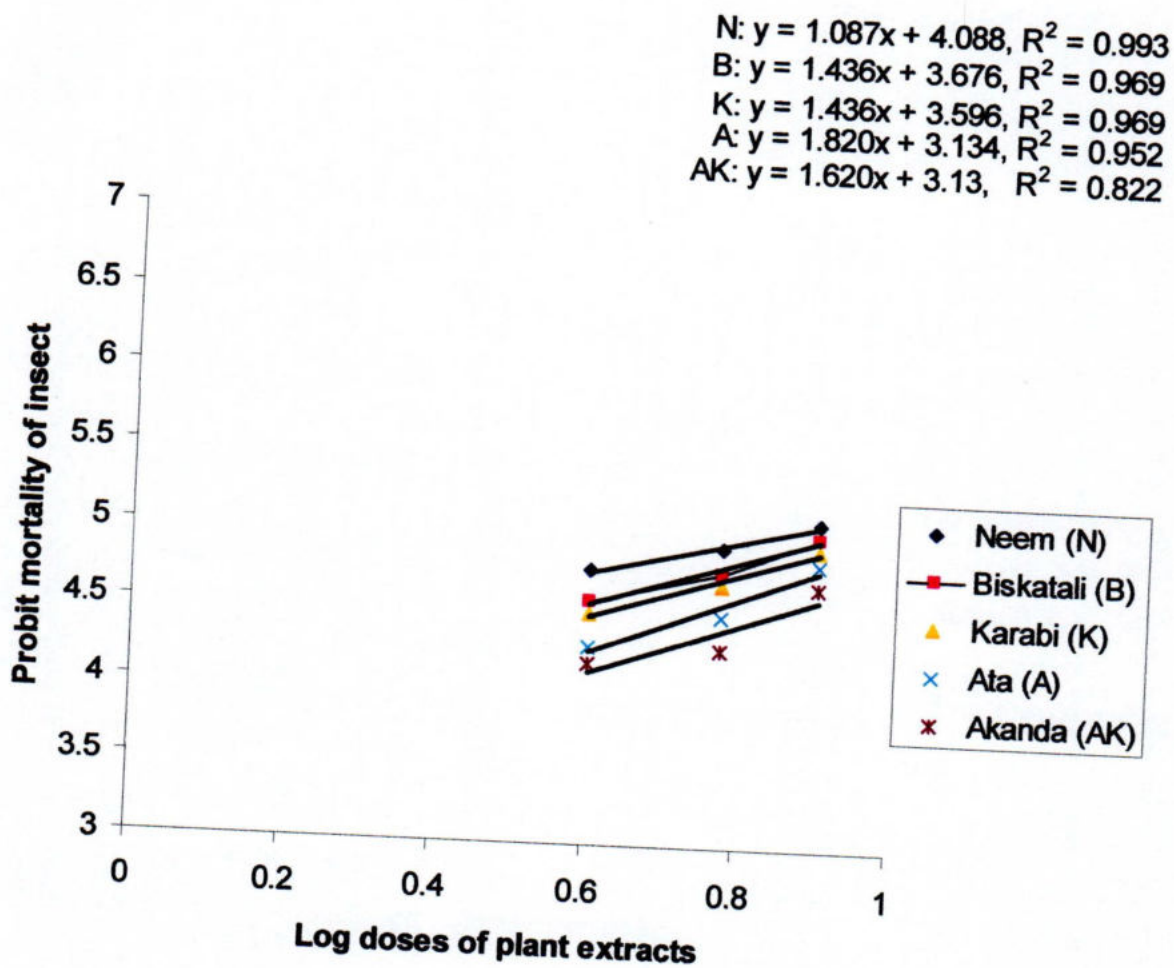


Figure 1. Relationship between probit mortality and log doses of different plant extracts on lesser grain borer, *R. dominica* at 24 HAT.

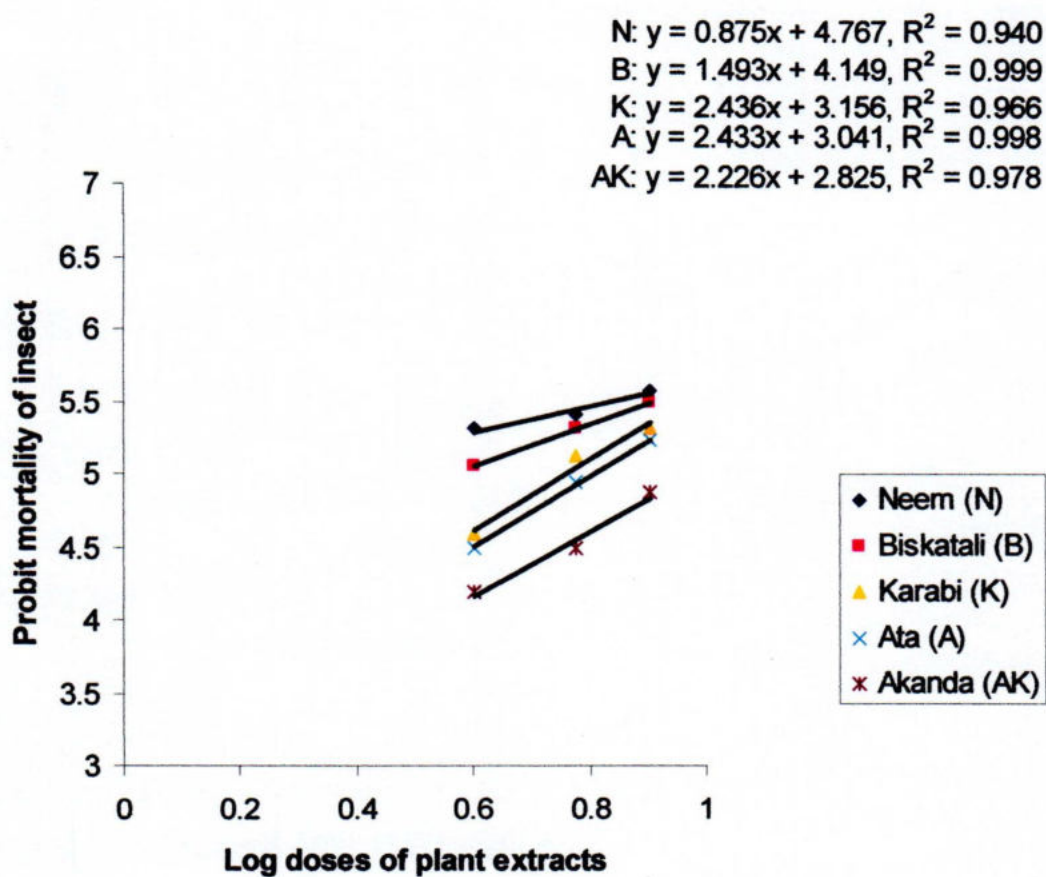


Figure 2. Relationship between probit mortality and log doses of different plant extracts on lesser grain borer, *R. dominica* at 48 HAT

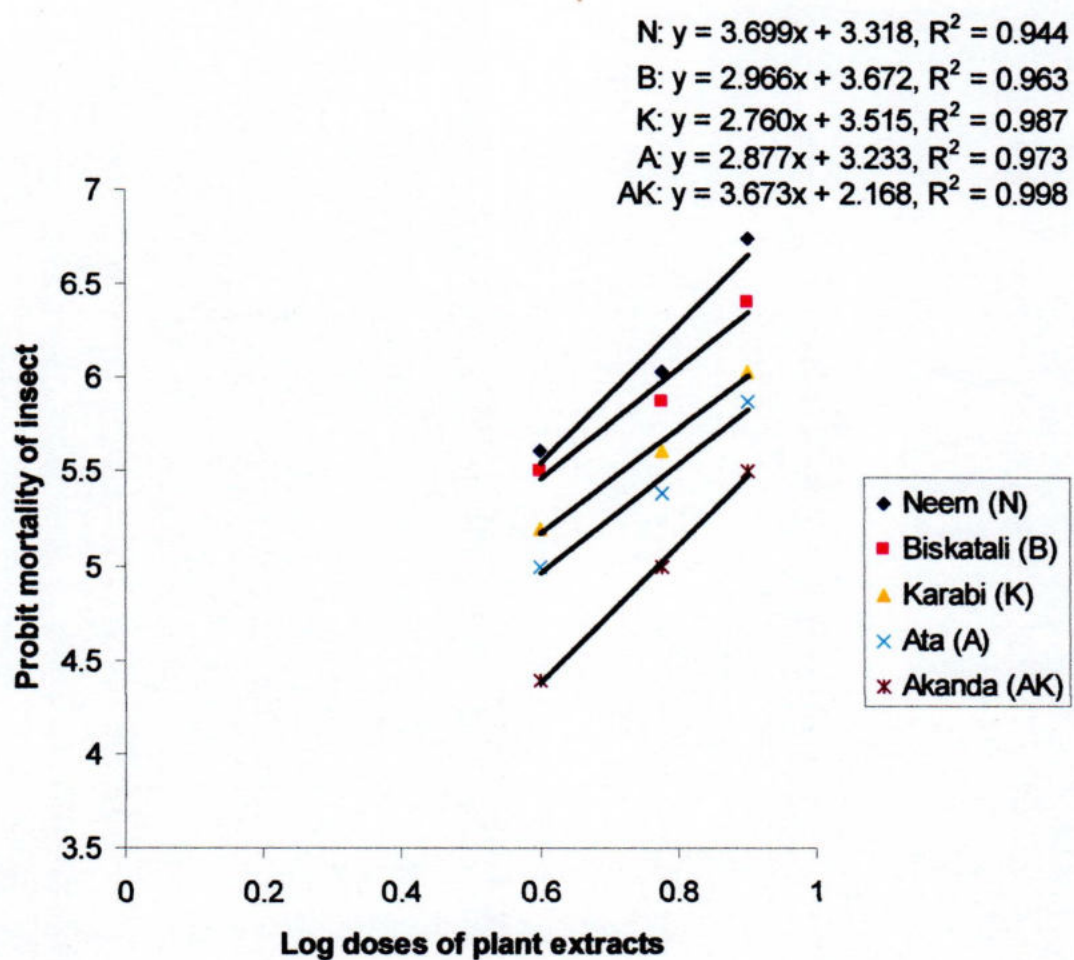


Figure 3. Relationship between probit mortality and log doses of different plant extracts on lesser grain borer, *R. dominica* at 72 HAT

4.4 Residual effects against lesser grain borer

The result of residual toxic effects of neem, biskatali, karabi, akanda and ata plant extracts on lesser grain borer, *R. dominica* are presented in Tables 5 to 7.

Average mortality percentage of lesser grain borer at 1, 2, 7, 15 and 21 days after treatment (DAT) indicated that neem plant extract (61.67%) possessed highest toxic effect which was statistically different from all other plant extracts (Table 5). The orders of toxic effect of five plant extracts against lesser grain borer was: neem > biskatali > karabi > akanda > ata. Mortality percentages were also directly proportional to the time after treatment.

Mortality of lesser grain borer was also differed significantly among all the concentration level of different plant extracts. The lowest average mortality (30.80%) was found in minimum concentration (4%) but the highest (85.15%) was observed at the maximum concentration (8%) of plant extracts and no mortality was observed in control treatment (Table 6).

Considering the interaction of plant extracts, doses and time, neem plant extract possessed the highest residual effect (average mortality, 98.97%) followed by biskatali (average mortality 95.20 %) against lesser grain borer at maximum dose. Same trend was also observed at 1, 2, 7 15 and 21 DAT. Mortality percentages were differed significantly between plant extracts and doses (Table 7).

The study of residual effect of plant extracts was agreed by the findings of Rajendran *et al* (2008), who found that neem plant extracts possessed highly toxic substances than biskatali.

Table 5: Toxicity of different plant extracts on the lesser grain borer at different DAT
(Days after treatment).

Name of the plant extracts	% Insect mortality(DAT)					
	1 DAT	2 DAT	7 DAT	15 DAT	21 DAT	Average mortality
Neem	48.33a	52.35a	63.67a	70.00a	74.00a	61.67a
Biskatali	35.00b	50.00b	53.00b	57.50b	65.00b	52.10b
Karabi	34.17b	45.83b	47.00b	50.00c	55.33c	46.47bc
Akanda	27.50c	38.33c	42.00c	47.50c	51.00c	41.27c
Ata	20.33d	25.00d	30.33d	35.83d	45.33d	31.37d
P- value	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
LSD	6.61	7.48	8.33	5.99	4.83	5.67
CV (%)	4.29	4.06	4.33	3.90	3.33	3.98

Mean followed by the same letter(s) did not differ significantly at 5% level by DMRT.

DAT = Days after treatment.

Table 6: Toxic effect of different concentration of different plant extracts on the lesser grain borer of different DAT (Days after treatment).

Dose (%)	Mortality percentage at different time intervals					
	1 DAT	2 DAT	7 DAT	15 DAT	21 DAT	Average mortality
4.0	20.00c	26.00c	30.00c	37.00c	40.00c	30.80c
6.0	36.67b	48.00b	62.00b	69.67b	75.33b	58.33b
8.0	70.00a	77.00a	89.67a	93.69a	95.33a	85.15a
Control	0.00d	0.00d	0.00d	0.00d	0.00d	0.00d
P- value	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
LSD	5.91	6.33	6.69	5.87	5.35	6.03
CV (%)	4.29	4.10	4.06	4.00	3.90	4.07

Mean followed by the same letter(s) did not differ significantly at 5% level by DMRT. DAT = Days after treatment.

Table 7: Interaction effects of plant extract and concentration on the lesser grain borer at different DAT (Days after treatment).

plant extracts used	Concentrations (%)	% Insect mortality(HAT)					
		1 DAT	2 DAT	7 DAT	15 DAT	21 DAT	Average
Neem	0.0	0.00h	0.00f	0.00g	0.00i	0.00i	0.00f
	4.0	40.00d	56.67d	76.67bc	78.33bc	80.00bc	66.33c
	6.0	55.67c	75.33c	90.00ab	96.00ab	100.0a	83.40b
	8.0	94.33a	95.67a	100.0a	100.0a	100.0a	98.97a
Biskatali	0.0	0.00h	0.00f	0.00g	0.00f	0.00i	0.00f
	4.0	15.67fg	20.33e	23.33f	29.67g	55.00ef	28.80e
	6.0	38.00d	46.33c	76.67bc	85.67a	93.00ab	67.93c
	8.0	83.33b	92.67b	100.0a	100.0a	100.0a	95.20a
Karabi	0.0	0.00h	0.00f	0.00g	0.00f	0.00i	0.00f
	4.0	20.33ef	22.33e	23.33f	25.33ef	36.67gh	25.60e
	6.0	35.33de	46.67cd	63.33cd	69.67c	79.33c	58.87c
	8.0	74.67b	88.67b	96.67a	100.0a	100.0a	92.00ab
Akanda	0.0	0.00h	0.00f	0.00g	0.00f	0.00i	0.00f
	4.0	13.67fg	21.67e	22.33f	34.67e	35.33fg	25.53e
	6.0	30.33de	40.67c	48.33e	54.67c	59.67d	46.73d
	8.0	58.33c	76.67b	89.67ab	95.33bc	100.0a	84.00b
Ata	0.0	0.00h	0.00f	0.00g	0.00f	0.00i	0.00f
	4.0	7.33gh	9.33f	12.33g	16.67f	20.00h	13.13f
	6.0	16.33fg	25.67de	39.67ef	45.33de	49.67de	35.33de
	8.0	35.67de	49.67c	66.00d	70.00c	80.67c	60.40c
P- value		0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
LSD		14.24	14.98	15.98	13.98	12.98	14.43
CV (%)		5.29	6.20	7.06	4.65	3.90	5.42

Mean followed by the same letter(s) did not differ significantly at 5% level by DMRT

DAT = Days after treatment.

4.5 Probit analysis for residual toxicity

The results of the probit analysis for the estimation of LD₅₀ values and their 95% fiducially limits at 1, 2, 7, 15 and 21 DAT for the mortality of lesser grain borer are shown in Table 8.

The LD₅₀ values of neem (18.543 µg), biskatali (29.817 µg), karabi (30.937 µg), akanda (34.883 µg) and ata (31.204 µg) at 1 DAT (Table 8) indicated that neem plant extracts possessed the highest toxicity while lowest in akanda against lesser grain borer. Similarly, neem plant extract maintained its highest toxicity at 2, 7, 15 and 21 DAT. The chi-square (χ^2) values of different plant extracts at different hours after treatment were insignificant at 5% level of probability and insignificant χ^2 value did not show any heterogeneity of the mortality data.

Athanassiou *et al.*, (2008) studied the plant extract of neem against adults of *R. dominica* and found that at 100 ppm neem oil showed 100 % adult mortality after 14 days exposure. The present study also indicated that neem plant extract has lowest LD₅₀ values and highest residual effects on lesser grain borer and agreed with the earlier findings of Kathirvelu *et al.*, (2009). From the probit results it is clear that neem will be effective for controlling the lesser grain borer.

Table 8: Relative toxicity (by probit analysis) of different plant extracts treated against Lesser grain borer at 1, 2, 7, 15 and 21 DAT

Name of the plant extracts	No. of insect used	LC 50 values (ug)	95 % fiducially limits	χ^2 values
1 DAT				
Neem	90	18.543	2.721 – 126.347	0.019
Biskatali	90	29.817	1.071 – 830.053	0.051
Karabi	90	30.937	1.388 – 689.277	0.095
Akanda	90	34.883	1.432 – 849.918	0.023
Ata	90	31.204	1.648 – 590.492	0.082
2 DAT				
Neem	90	11.446	3.591 – 36.476	0.097
Biskatali	90	12.573	3.974 – 39.774	0.241
Karabi	90	19.754	2.579 – 151.281	0.214
Akanda	90	31.204	1.648 – 590.492	0.082
Ata	90	15.259	4.986 – 246.693	0.052
7 DAT				
Neem	90	5.941	4.833 – 7.302	0.378
Biskatali	90	6.312	5.131 – 7.765	0.405
Karabi	90	8.267	5.983 – 11.421	0.103
Akanda	90	11.057	7.065 – 17.303	0.238
Ata	90	10.243	6.164 – 17.023	0.339
15 DAT				
Neem	90	4.431	3.580 – 5.482	0.240
Biskatali	90	4.508	3.500 – 5.803	0.139
Karabi	90	5.412	4.609 – 6.354	0.559
Akanda	90	8.224	6.663 – 10.149	0.001
Ata	90	6.240	5.325 – 7.312	0.002
21 DAT				
Neem	90	3.765	2.829 – 5.010	0.341
Biskatali	90	3.803	2.849 – 5.076	0.217
Karabi	90	5.259	3.268 – 5.549	0.629
Akanda	90	6.429	5.381 – 7.679	0.973
Ata	90	4.849	3.879 – 6.062	0.005

DAT= Day after treatment, Values were based on three concentrations, three replications of 10 insects each, χ^2 = Goodness of fit, The tabulated value of χ^2 is 5.99 (d. f=2 at 5% level)

4.6 Probit regression lines for residual toxicity

The probit regression lines for the effect of five plants extracts (neem, biskatali, karabi, akanda and ata) are presented in figure 4 to 8 against lesser grain borer. The insect mortality rate showed positive correlation with doses in all cases. The probit regression lines for the residual effects of five plant leaf extracts on lesser grain borer also showed a clear linear relationship between probit mortality and their log doses. The probit regression lines became steeper as doses increased.

The calculated probit regression equations line of five plant extracts at 1 DAT were: $Y = 1.413x + 3.187$, $Y = 1.318x + 3.093$, $Y = 1.421x + 2.834$, $Y = 1.719x + 2.454$ and $Y = 2.011x + 1.922$ for neem, biskatali, karabi, ata and akanda respectively (Fig. 4). Comparing among these lines, neem showed the higher probit mortality.

The calculated probit regression equations line of five plant extracts at 2 DAT were: $Y = 1.398x + 3.525$, $Y = 1.569x + 3.272$, $Y = 1.376x + 3.182$, $Y = 2.203x + 2.376$ and $Y = 1.719x + 2.454$ for neem, biskatali, karabi, ata and akanda respectively (Fig. 5). Neem also showed the highest probit mortality at 2 DAT.

The calculated probit regression equations line of five plant extracts at 7 DAT were: $Y = 3.056x + 2.640$, $Y = 6.067x + 0.592$, $Y = 3.023x + 2.235$, $Y = 2.954x + 2.0.8$ and $Y = 3.919x + 0.840$ for neem, biskatali, karabi, ata and akanda respectively (Fig. 6). Comparing among these lines, neem also maintained its highest probit mortality. Similar trend of probit mortality lines were found at 15 and 21 days after treatments.

Kathirvelu *et al.*, (2009) studied to assess the effect of botanicals *Azadirachta indica* and *Pongamia glabra* on *R. dominica* in stored rice and the results revealed that mixing neem oil at 30 ml per kg of grains was effective against the pest, recording 57.95% mortality. and agreed with the present study.

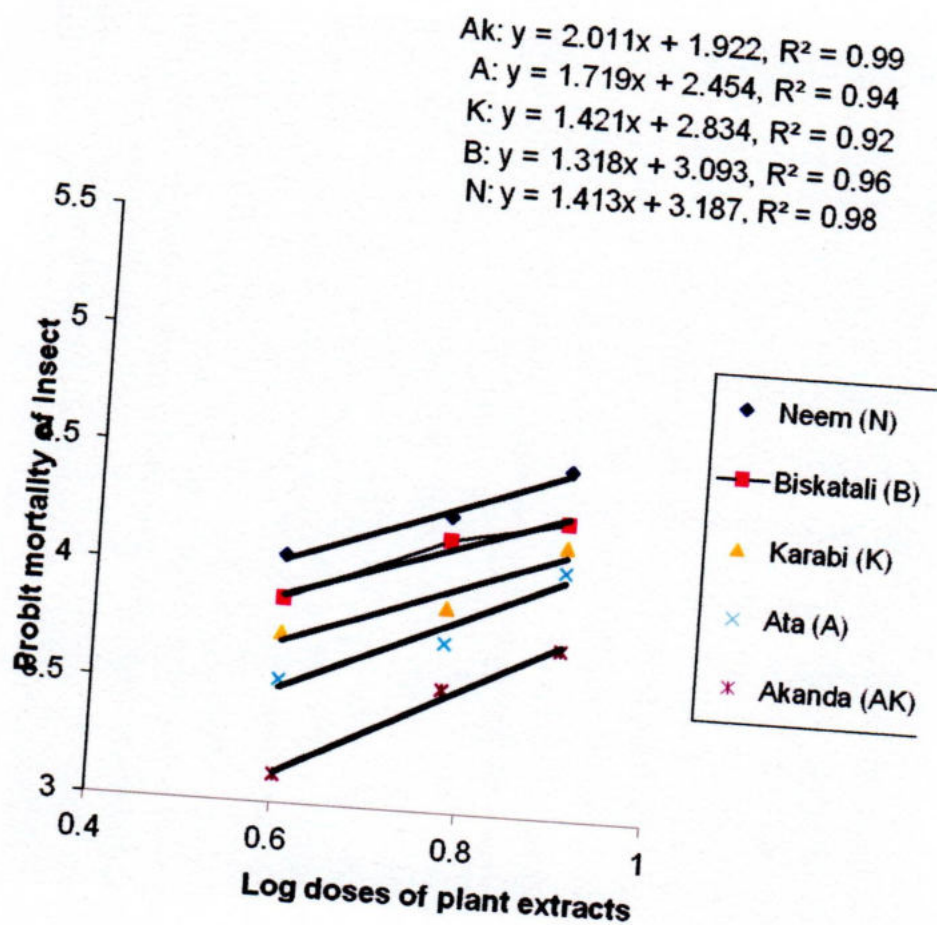


Figure 4. Relationship between probit mortality and log doses for residual effect of different plant extracts on lesser grain borer, *R. dominica* at 1 day

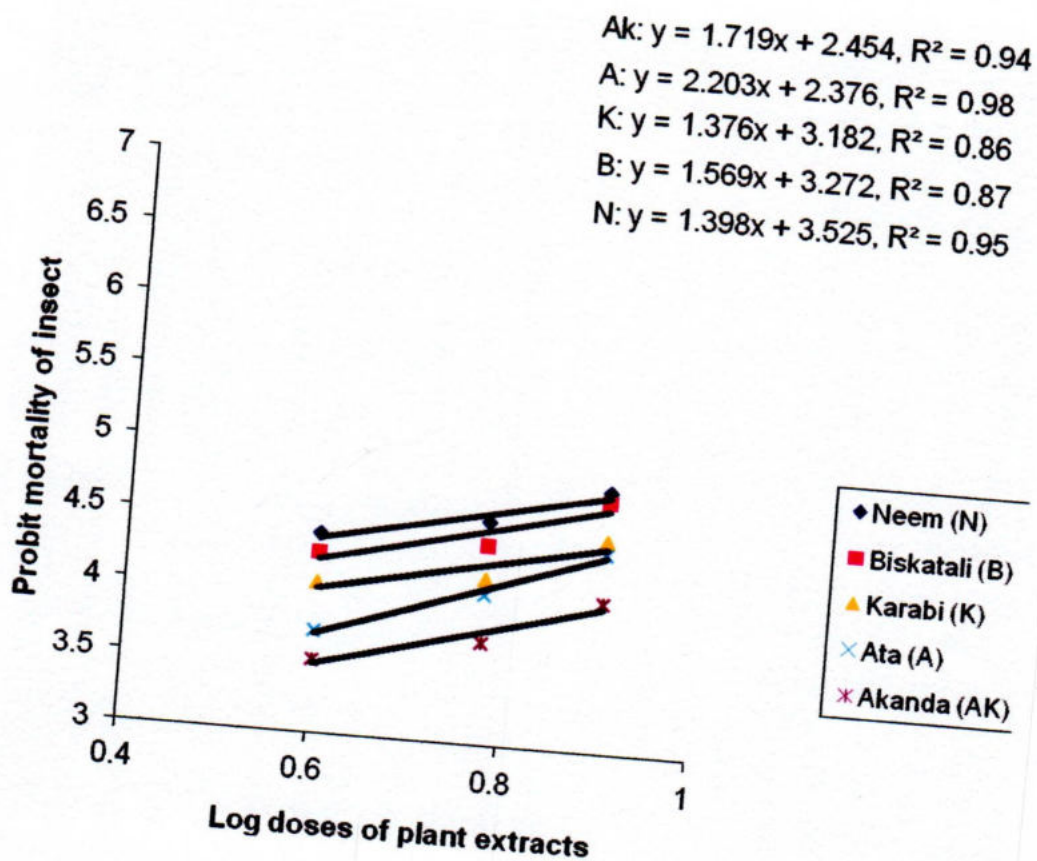


Figure 5. Relationship between probit mortality and log doses for residual effect of different plant extracts on lesser grain borer, *R. dominica* at 2 days

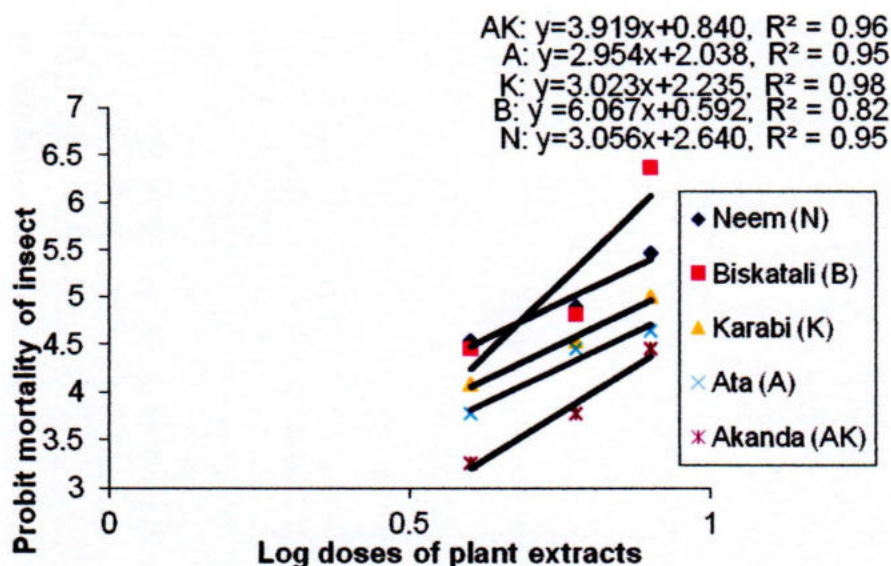


Figure 6. Relationship between probit mortality and log doses for residual effect of different plant extracts on lesser grain borer, *R. dominica* at 7 days

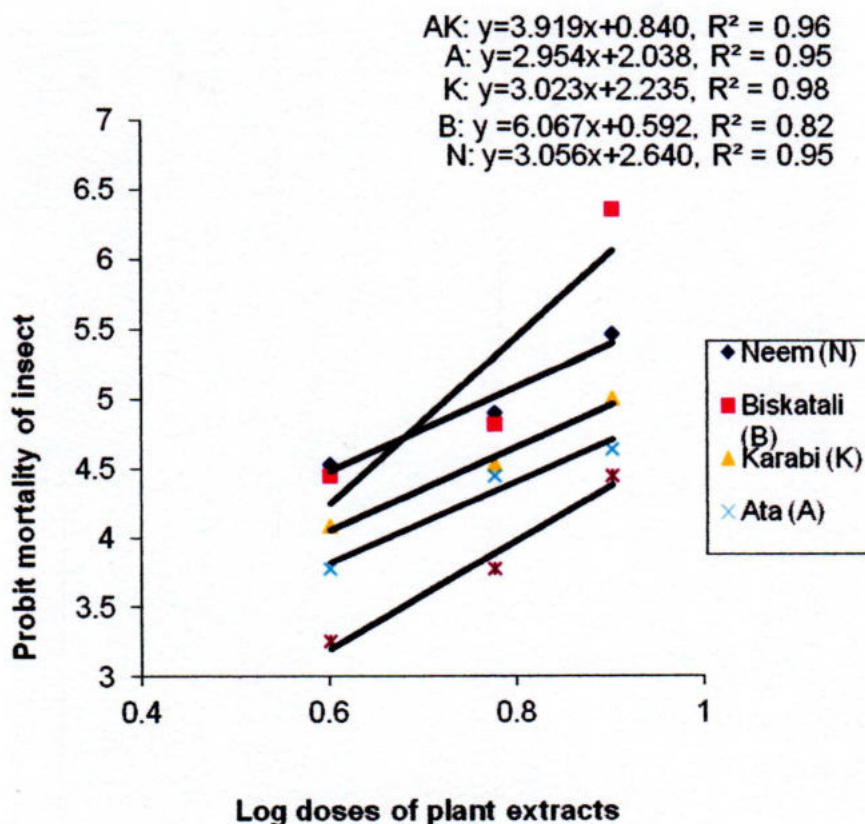


Figure 7. Relationship between probit mortality and log doses for residual effect of different plant extracts on lesser grain borer, *R. dominica* at 15 days

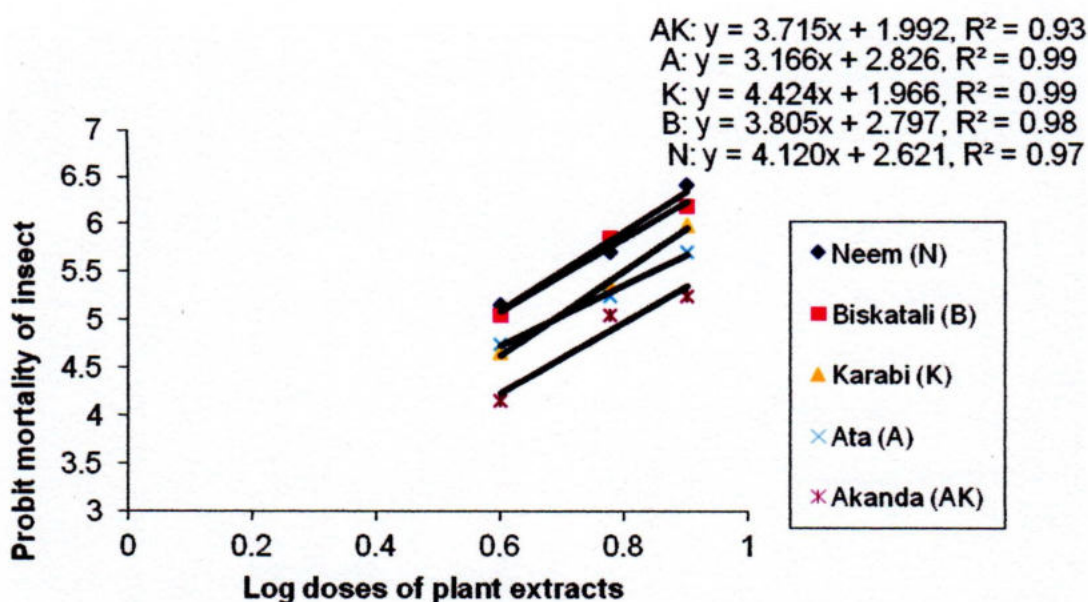


Figure 8. Relationship between probit mortality and log doses for residual effect of different plant extracts on lesser grain borer, *R. dominica* at 21 days

4.7 Repellent effect on lesser grain borer

The repellency effect of five plant extracts is presented in Table 10. Neem, biskatali, karabi, ata and akanda plant extracts showed medium repellent effect against lesser grain borer. Among the five plant extracts tested, neem showed the highest mean repellent effect (37.79%) followed by biskatali (36.12%), karabi (35.55%), akanda (34.17%) and ata (22.51%). However, these differences were not statistically significant. In some cases, repellency rate decreased with the progress of time. The rate of repellency differed with the extract type. All plant extracts were in the same repellency class i.e. class II.

Mean repellent effect of different plant extracts in different dose level on lesser grain borer is presented in Table 10. The repellency was influenced by the concentration of extracts. Repellency rate did not increase proportionally with the doses. The highest mean repellency effect was found with 8% dose extract (46.21%) (Table 10).

Interaction repellent effect of different plant extract and their doses on lesser grain borer at different HATs is presented in (Table 11). The highest mean repellency effect was found on 8% dose of neem extract (82.20%).

Jilani and Saxena (1990) observed that the repellency of compounds with low molecular weights and high volatility decreased rapidly over time. The present study has shown that neem and biskatali leaf extract have moderate repellent effects on lesser grain borer and agreed with the earlier findings of Bowery *et al.*, (1984). This study is proved our traditional use of leaf of neem, biskatali, karabi, ata and akanda to protect stored grain pests.

Table 9: Repellency effects of different plant extracts on lesser grain borer at different HATs

Plant extracts used	Repellency rate (%) at six different HATs							Repel. class
	1 st hour	2 nd hour	3 rd hour	4 th hour	5 th hour	6 th hour	Average	
Neem	38.33ns	40.00ab	50.00a	30.00ns	30.00ns	38.33ns	37.79a	II
Biskatali	35.00ns	31.67ab	30.00ab	36.67ns	38.33ns	45.00ns	36.12a	II
Karabi	32.30ns	25.23b	43.33a	35.00ns	36.67ns	32.30ns	35.55a	II
Akanda	18.33ns	43.33a	36.67a	38.33ns	33.33ns	35.00ns	34.17a	II
Ata	25.00ns	10.00c	13.33b	23.33ns	20.00ns	43.33ns	22.51b	II
P- value	0.1161	0.0003	0.0033	-	0.2033	-	0.0118	
LSD	18.17	14.35	18.47	19.88	16.62	19.38	9.09	
CV (%)	4.91	3.23	4.48	3.63	3.52	3.12	2.11	
SE	6.34	5.01	6.45	6.94	5.80	6.76	3.17	

Mean followed by the same letter(s) did not differ significantly at 5% level and ns indicate no significant by DMRT.

ns means non significance

Table 10: Repellency effects of different plant extracts at different concentration on lesser grain borer at different HATs

Dose (%)	Repellency rate (%) at six different HATs							Repel. class
	1 st hour	2 nd hour	3 rd hour	4 th hour	5 th hour	6 th hour	Average	
0.0	4.00c	4.00b	24.00a	12.00b	0.00b	4.00b	8.02b	I
4	29.33b	44.00a	38.67a	36.00a	45.33a	45.33a	39.78a	II
6	42.67ab	33.33a	37.33a	36.00a	36.00a	48.00a	38.90a	II
8	48.00a	40.00a	38.67a	46.67a	45.33a	58.67a	46.21a	III
P- value	0.0001	0.0001	0.2224	0.0029	0.0001	0.0001	0.0001	
LSD	16.25	12.83	16.52	17.78	14.87	17.33	8.13	
CV (%)	4.91	3.23	4.48	3.63	3.52	3.12	2.11	
SE	5.67	4.48	5.77	6.21	5.19	6.05	2.84	

Mean followed by the same letter(s) did not differ significantly at 5% level by DMRT.

Table 11: Interaction effect of different plant extract and their doses on lesser grain borer at different HATs

Plant extracts used	Repellency rate (%) at six different HATs								Repel. class
	Dose (%)	1 st hour	2 nd hour	3 rd hour	4 th hour	5 th hour	6 th hour	Average	
Neem	0.0	0.00f	0.00e	20.00de	0.00c	0.00f	0.00g	3.30h	I
	4	33.33a-f	26.67b-e	20.00de	26.67bc	26.67c-f	26.67d-g	26.67c-g	II
	6	53.33a-d	26.67b-e	33.33c-e	20.00c	33.33b-f	13.33fg	30.03d-f	II
	8	66.67ab	53.33ab	100.0a	93.33a	86.67a	93.33a	82.20a	V
Biskatali	0.0	0.00f	20.00b-e	20.00de	0.00c	0.00f	0.00g	6.70gh	I
	4	26.67b-f	40.00a-d	40.00b-e	26.67bc	53.33a-d	66.67a-d	42.20c-e	III
	6	60.00a-c	40.00a-d	46.67b-d	73.33ab	80.00a	80.00ab	63.33b	IV
	8	53.33a-d	26.67b-c	13.33de	46.67bc	20.00d-f	33.33c-g	32.23d-f	II
Karabi	0.0	0.00f	0.00e	0.00e	40.00bc	0.00f	0.00g	6.70gh	I
	4	26.67b-f	66.67a	73.33a-c	46.67bc	60.00a-c	66.67a-d	56.67bc	III
	6	6.66ef	60.00a	46.67bcd	40.00bc	13.33ef	20.00e-g	31.13d-f	II
	8	40.00a-f	46.67a-c	26.67de	26.67bc	60.00a-c	53.33a-f	42.20c-e	III
Akanda	0.0	0.00f	0.00e	80.00ab	0.00c	0.00f	20.00efg	16.70fgh	I
	4	46.67a-c	66.67a	46.67b-d	46.67bc	66.67ab	33.33c-g	51.13b-d	III
	6	73.33a	33.33a-e	40.00b-e	40.00bc	13.33ef	60.00a-e	43.33c-e	III
	8	33.33a-f	60.00a	33.33c-e	33.33bc	40.00b-e	40.00b-g	40.00c-e	III
Ata	0.0	20.00c-f	0.00e	0.00e	20.00c	0.00f	0.00g	6.700gh	I
	4	13.33d-f	20.00b-e	13.33de	33.33bc	20.00d-f	33.33c-g	22.23e-h	II
	6	20.00c-f	6.66de	20.00de	6.66c	40.00b-e	66.67a-d	26.67e-g	II
	8	46.67a-e	13.33c-e	20.00de	33.33bc	20.00d-f	73.33a-c	34.43d-f	II
P- value		0.0867	0.0439	0.0002	0.0071	0.0002	0.0021	0.0001	
Lsd		36.33	28.70	36.95	39.76	33.25	38.76	18.19	
CV (%)		4.91	3.23	4.48	3.63	3.52	3.12	2.11	
SE		12.69	10.02	12.91	13.89	11.61	13.54	6.35	

Mean followed by the same letter(s) did not differ significantly at 5% level by DMRT.

CHAPTER 5

SUMMARY AND CONCLUSION

5. SUMMARY AND CONCLUSION

The present experiment was undertaken to study the effectiveness of neem, biskatali, karabi, ata and akanda plant extracts against lesser grain borer in the laboratory of the Department of Entomology, HSTU, Dinajpur during the period from April to December 2012. The petroleum ether extracts of these plants were evaluated for toxic, residual and repellent effect against lesser grain borer, *R. dominica*.

Mortality percentage of lesser grain borer at 24, 48 and 72 HAT indicated that neem plant extracts possessed the highest toxic effects among all the plant extracts. Mortality percentage was also found directly proportional to the level of concentrations of plant extracts and to the exposure period.

The lowest LD₅₀ values of neem plant extract at 24, 48 and 72 HAT indicate that neem plant extract possessed highest toxic effects. The Chi-square values of different plant extracts at different hours after treatment are insignificant at 5% level of probability and did not show any heterogeneity of the mortality data.

Mortality percentage for residual toxicity of lesser grain borer at 1, 2, 7, 15 and 21 DAT indicated that neem extracts also possessed the highest toxic effects comparing to biskatali, karabi, ata and akanda. Mortality percentage was also found directly proportional to the level of concentrations of plant extracts and to the exposure period. The lowest LD₅₀ values of neem plant extract at different DAT also indicate that neem plant extract possessed highest toxic effects. The Chi-square values of different plant extracts at different days after treatment are insignificant at 5% level of probability and did not show any heterogeneity of the mortality data.

The repellent effect of neem, biskatali, karabi, ata and akanda extracts showed that the five plant extracts had moderate repellent effect on lesser grain borer. The highest mean repellency was observed in neem extract for lesser grain borer, *R. dominica* comparing to bskatali, karabi, ata and akanda. In maximum cases the repellency rate decrease for increasing of exposure period.

Our country imports a large amount of insecticides to minimize the severe damage caused by insect pests every year. As a result production costs are increased for buying insecticides. Botanical materials as insecticides can be used as substitute as these substances are not only of low cost, but also have less environmental impact in terms of insecticidal hazards. Finally, it is concluded that the present study will be very useful in controlling our storage pest successfully and will help us to save precious foreign currency from importing synthetic insecticides.

Recommendations:

1. It is recommended that farmers can use leaf extract of neem, biskatali, karabi, ata and akanda as botanical insecticide against lesser grain borer as they showed toxic effect.
2. Farmers may use leaf extract of neem, biskatali, karabi, akanda and ata in their storage as repellent against lesser grain borer because they have moderate repellent effect.

CHAPTER 6
REFERENCES

6. REFERENCES

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CHAPTER 7

APPENDICES

APPENDICES

Appendix table 1: Doses mortality data of *Rhyzopertha dominica* adult treated with neem leaf extract in petroleum ether at 24 HAT.

dose	logdose	number	kill	% kill	corr%	emp	expt	wrk	weight	final
							probit	probit	probit	probit
4	0.6020	30	12	40.00	40	4.75	4.744	4.74	18.48	4.734
6	0.7781	30	14	46.66	47	4.92	4.935	4.92	19.02	4.928
8	0.9030	30	16	53.33	53	5.08	5.071	5.08	19.11	5.066

$Y = 4.068378 + 1.105752 X$; χ^2 is 5.559922E-03 with 1 degrees of freedom; No sig. heterogeneity, LD-50 is 6.958637; 95% Confidence limits are 3.646632 to 13.27873

Appendix table 2: Doses mortality data of *Rhyzopertha dominica* adult treated with neem leaf extract in petroleum ether at 48 HAT.

dose	logdose	number	kill	% kill	corr%	emp	expt	wrk	weight	final
							probit	probit	probit	probit
4	0.6020	30	19	63.33	62	5.31	5.294	5.332	18.81	5.316
6	0.7781	30	20	66.66	66	5.41	5.448	5.402	18.03	5.442
8	0.9030	30	22	73.33	72	5.58	5.557	5.556	17.43	5.531

$Y = 4.884684 + .7164404 X$; χ^2 is 4.397384E-02 with 1 degrees of freedom; No sig. heterogeneity; LD-50 is 1.448629; 95% Confidence limits are 2.157704E-02 to 97.25739

Appendix table 3: Doses mortality data of *Rhyzopertha dominica* adult treated with neem leaf extract in petroleum ether at 72 HAT.

dose	logdose	number	kill	% kill	corr%	emp probit	expt probit	wrk probit	weight	final probit
4	0.6020	30	23	76.66	73	5.61	5.54	5.584	17.43	5.54
6	0.7781	30	26	86.66	85	6.04	6.19	6.040	12.15	6.16
8	0.9030	30	29	96.66	96	6.75	6.65	6.720	7.14	6.59

$Y = 3.449528 + 3.486454 X$; χ^2 is 0.3102975 with 1 degrees of freedom; no sig heterogeneity; LD-50 is 2.744281; 95% Confidence limits are 1.630264 to 4.755194

Appendix table 4: Doses mortality data of *Rhyzopertha dominica* adult treated with Karabi leaf extract in petroleum ether at 24 HAT.

dos e	logdos e	numbe r	kil l	% kill	corr %	emp probi t	expt probit	wrk probi t	weigh t	final probit
4	0.6020	30	9	30.0 0	30	4.48	4.46153 2	4.48	16.74	4.45351 1
6	0.7781	30	11	36.6 6	37	4.67	4.71449 4	4.662	18.48	4.71982
8	0.9030	30	14	46.6 6	47	4.92	4.89397 5	4.942	18.81	4.90877

$Y = 3.542992 + 1.512354 X$; $9.434509E-02 \chi^2$ is with 1 degrees of freedom; no sig heterogeneity; LD-50 is 9.191874; 95% Confidence limits are 4.262275 to 19.82288

Appendix table 5: Doses mortality data of *Rhyzopertha dominica* adult treated with Karabi leaf extract in petroleum ether at 48 HAT.

dose	logdose	number	kill	% kill	corr%	emp probit	expt probit	wrk probit	weight	final probit
4	0.6020	30	11	36.66	34	4.59	4.622	4.578	18.03	4.615
6	0.7781	30	17	56.66	55	5.13	5.051	5.125	19.11	5.040
8	0.9030	30	19	63.33	62	5.31	5.355	5.292	18.48	5.342

$Y = 3.159251 + 2.418024 X$; χ^2 is .208108 with 1 degrees of freedom; no sig heterogeneity; LD-50 is 5.771143; 95% Confidence limits are 4.493326 to 7.412345

Appendix table 6: Doses mortality data of *Rhyzopertha dominica* adult treated with Karabi leaf extract in petroleum ether at 72 HAT.

dose	logdose	number	kill	% kill	corr%	emp probit	expt probit	wrk probit	weight	final probit
4	0.6020	30	19	63.33	58	5.2	5.177	5.19	19.02	5.176
6	0.7781	30	23	76.66	73	5.61	5.663	5.61	16.74	5.645
8	0.9030	30	26	86.66	85	6.04	6.008	6.005	13.17	5.978

$Y = 3.573983 + 2.662472 X$; χ^2 is 3.393602E-02 with 1 degrees of freedom; no sig heterogeneity; LD-50 is 3.432409; 95% Confidence limits are 2.131109 to 5.528312

Appendix table 7: Doses mortality data of *Rhyzopertha dominica* adult treated with Bishkatali leaf extract in petroleum ether at 24 HAT.

dose	logdose	number	kill	% kill	corr%	emp probit	expt probit	wrk probit	weight	final probit
4	0.6020	30	10	33.33	33	4.56	4.541	4.544	17.43	4.525
6	0.7781	30	12	40	40	4.75	4.794	4.74	18.48	4.782
8	0.9030	30	15	50	50	5.00	4.973	4.99	19.02	4.965

$Y = 3.64414 + 1.463343 X$; χ^2 is 5.144453E-02 with 1 degrees of freedom; No sig heterogeneity; LD-50 is 8.444025; 95% 95% Confidence limits are 4.251723 to 16.77004

Appendix table 8: Doses mortality data of *Rhyzopertha dominica* adult treated with Bishkatali leaf extract in petroleum ether at 48 HAT.

dose	logdose	number	kill	% kill	corr%	emp probit	expt probit	wrk probit	weight	final probit
4	0.6020	30	16	53.33	52	5.05	5.049	5.05	19.11	5.047
6	0.7781	30	19	63.33	62	5.31	5.312	5.292	18.48	5.299
8	0.9030	30	21	70	69	5.5	5.498	5.483	18.03	5.478

$Y = 4.183964 + 1.43351 X$; χ^2 is 1.546979E-03 with 1 degrees of freedom; no sig heterogeneity; LD-50 is 3.709004; 95% Confidence limits are 1.716787 to 8.013053

Appendix table 9: Doses mortality data of *Rhyzopertha dominica* adult treated with

Bishkatali leaf extract in petroleum ether at 72 HAT.

dose	logdose	number	kill	% kill	corr%	emp probit	expt probit	wrk probit	weight	final probit
4	0.6020	30	22	73.33	69	5.5	5.458	5.483	18.03	5.464
6	0.7781	30	25	83.33	81	5.88	5.980	5.908	14.13	5.964
8	0.9030	30	28	93.33	92	6.41	6.351	6.366	10.08	6.319

$Y = 3.754648 + 2.840138 X$; χ^2 is 7.301521E-02 with 1 degrees of freedom; no sig heterogeneity; LD-50 is 2.784625; 95% Confidence limits are 1.44994 to 5.19536

Appendix table 10: Doses mortality data of *Rhyzopertha dominica* adult treated with

Ata leaf extract in petroleum ether at 24 HAT.

dose	logdose	number	kill	% kill	corr%	emp probit	expt probit	wrk probit	weight	final probit
4	0.6020	30	7	23.33	23	4.26	4.230	4.252	15.09	4.213
6	0.7781	30	9	30	30	4.48	4.551	4.46	17.43	4.540
8	0.9030	30	13	43.33	43	4.82	4.778	4.818	18.48	4.773

$Y = 3.092919 + 1.860801 X$; χ^2 is .1735697 with 1 degrees of freedom; no sig heterogeneity; LD-50 is 10.5894; 95% Confidence limits are 4.827913 to 23.22645

Appendix table 11: Doses mortality data of *Rhyzopertha dominica* adult treated with ata leaf extract in petroleum ether at 48 HAT.

dose	logdose	number	kill	% kill	corr%	emp probit	expt probit	wrk probit	weight	final probit
4	0.6020	30	10	33.33	31	4.5	4.506	4.488	17.43	4.489
6	0.7781	30	15	50	48	4.95	4.934	4.94	19.02	4.937
8	0.9030	30	18	60	59	5.23	5.238	5.25	18.81	5.255

$Y = 2.956455 + 2.545793 X$; χ^2 is -4.768372E-06 with 1 degrees of freedom; no sig heterogeneity; LD-50 is 6.349138; 95% Confidence limits are 4.947153 to 8.148436

Appendix table 12: Doses mortality data of *Rhyzopertha dominica* adult treated with ata leaf extract in petroleum ether at 72 HAT.

dose	logdose	number	kill	% kill	corr%	emp probit	expt probit	wrk probit	weight	final probit
4	0.6020	30	17	56.66	50	5.0	4.965	4.99	19.02	4.962
6	0.7781	30	21	70	65	5.39	5.472	5.37	18.03	5.444
8	0.9030	30	25	83.33	81	5.88	5.831	5.83	15.09	5.785

$Y = 3.31703 + 2.733581 X$; χ^2 is .1356492 with 1 degrees of freedom; no sig heterogeneity; LD-50 is 4.127291; 95% Confidence limits are 2.940012 to 5.794036

Appendix table 13: Doses mortality data of *Rhyzopertha dominica* adult treated with akanda leaf extract in petroleum ether at 24 HAT.

dose	logdose	number	kill	% kill	corr%	emp probit	expt probit	wrk probit	weight	final probit
4	0.6020	30	6	20	20	4.16	4.105	4.17	14.13	4.11
6	0.7781	30	7	23.33	23	4.26	4.390	4.26	15.96	4.39
8	0.9030	30	11	36.66	37	4.67	4.593	4.65	17.43	4.58

$Y = 3.15615 + 1.586753 X$; χ^2 is .375225 with 1 degrees of freedom; no sig heterogeneity; LD-50 is 14.52202; 95% Confidence limits are 3.644176 to 57.87014

Appendix table 14: Doses mortality data of *Rhyzopertha dominica* adult treated with akanda leaf extract in petroleum ether at 48 HAT.

dose	logdose	number	kill	% kill	corr%	emp probit	expt probit	wrk probit	weight	final probit
4	0.6020	30	7	23.33	21	4.19	4.165	4.208	14.13	4.168
6	0.7781	30	10	33.33	31	4.5	4.557	4.488	17.43	4.565
8	0.9030	30	14	46.66	45	4.87	4.836	4.890	18.81	4.847

$Y = 2.808878 + 2.257793 X$; χ^2 is .1611655 with 1 degrees of freedom; no sig heterogeneity; LD-50 is 9.342666; 95% Confidence limits are 5.466707 to 15.96672

Appendix table 15: Doses mortality data of *Rhyzopertha dominica* adult treated with akanda leaf extract in petroleum ether at 72 HAT.

dose	logdose	number	kill	% kill	corr%	emp probit	expt probit	wrk probit	weight	final probit
4	0.6020	30	11	36.66	27	4.39	4.379	4.39	15.96	4.384
6	0.7781	30	17	56.66	50	5.0	5.025	5.00	19.11	5.019
8	0.9030	30	22	73.33	69	5.5	5.484	5.48	18.03	5.470

$Y = 2.211045 + 3.609528 X$; χ^2 is 1.161957E-02 with 1 degrees of freedom; no sig heterogeneity; LD-50 is 5.924679; 95% Confidence limits are 4.989793 to 7.034726

Appendix table 16: Doses mortality data of *Rhyzopertha dominica* adult treated with neem leaf extract in petroleum ether at 1 day.

dose	logdose	number	kill	% kill	corr%	emp probit	expt probit	wrk probit	weight	final probit
4	0.6020	30	5	16.66	17	4.05	4.038	4.037	13.17	4.023
6	0.7781	30	7	23.33	23	4.26	4.287	4.252	15.09	4.281
8	0.9030	30	9	30	30	4.48	4.463	4.48	16.74	4.464

$Y = 3.140078 + 1.466612 X$; χ^2 is 1.957512E-02 with 1 degrees of freedom; no sig heterogeneity; LD-50 is 18.54284; 95% Confidence limits are 2.721362 to 126.3474

Appendix table 17: Doses mortality data of *Rhyzopertha dominica* adult treated with neem leaf extract for residual effect in petroleum ether at 2 days

dose	logdose	number	kill	% kill	corr%	emp probit	expt probit	wrk probit	weight	final probit
4	0.6020	30	8	26.66	27	4.39	4.367	4.394	15.96	4.366
6	0.7781	30	10	33.33	33	4.56	4.613	4.551	18.03	4.610
8	0.9030	30	13	43.33	43	4.82	4.788	4.818	18.48	4.784

$Y = 3.530215 + 1.388354 X$; χ^2 is 9.783566E-02 with 1 degrees of freedom; No sig. heterogeneity, LD-50 is 11.44597; 95% Confidence limits are 3.591616 to 36.47671

Appendix table18: Doses mortality data of *Rhyzopertha dominica* adult treated with neem leaf extract for residual effect in petroleum ether at 7 days

dose	logdose	number	kill	% kill	corr%	emp probit	expt probit	wrk probit	weight	final probit
4	0.6020	30	11	36.66	32	4.53	4.480	4.54	16.74	4.486
6	0.7781	30	25	50	46	4.9	5.018	4.9	19.11	5.012
8	0.9030	30	21	70	68	5.47	5.400	5.456	18.03	5.386

$Y = 2.687561 + 2.988135 X$; χ^2 is .3788939 with 1 degrees of freedom; No sig. heterogeneity, LD-50 is 5.941194; 95% Confidence limits are 4.833789 to 7.302303

Appendix table 19: Doses mortality data of *Rhyzopertha dominica* adult treated with neem leaf extract for residual effect in petroleum ether at 15 days

dose	logdose	number	kill	% kill	corr%	emp probit	expt probit	wrk probit	weight	final probit
4	0.6020	30	15	50	44	4.85	4.820	4.864	18.81	4.828
6	0.7781	30	21	70	67	5.44	5.510	5.416	17.43	5.509
8	0.9030	30	26	86.66	85	6.04	5.999	6.06	14.13	5.992

$Y = 2.499027 + 3.868567 X$; χ^2 is .24014 with 1 degrees of freedom; No sig.

heterogeneity, LD-50 is 4.430837; 95% Confidence limits are 3.580913 to 5.482488

Appendix table 20: Doses mortality data of *Rhyzopertha dominica* adult treated with neem leaf extract for residual effect in petroleum ether at 21 days.

dose	logdose	number	kill	% kill	corr%	emp probit	expt probit	wrk probit	weight	final probit
4	0.6020	30	19	63.33	56	5.15	5.101	5.14	19.02	5.101
6	0.7781	30	24	80	76	5.71	5.826	5.664	15.09	5.781
8	0.9030	30	28	93.33	92	6.41	6.341	6.366	10.08	6.263

$Y = 2.777434 + 3.860104 X$; χ^2 is .3413468 with 1 degrees of freedom; No sig.

heterogeneity, LD-50 is 3.765121; 95% Confidence limits are 2.829374 to 5.010342

Appendix table 21: Doses mortality data of *Rhyzopertha dominica* adult treated with biskatali leaf extract for residual effect in petroleum ether at 1 days

dose	logdose	number	kill	% kill	corr%	emp probit	expt probit	wrk probit	weight	final probit
4	0.6020	30	4	13.33	13	3.87	3.886	3.873	11.1	3.898
6	0.7781	30	6	20	20	4.16	4.119	4.17	14.13	4.121
8	0.9030	30	7	23.33	23	4.26	4.283	4.252	15.09	4.278

$Y = 3.138922 + 1.262201 X$; χ^2 is 5.199886E-02 with 1 degrees of freedom; No sig. heterogeneity, LD-50 is 29.81748; 95% Confidence limits are 1.071116 to 830.0525

Appendix table 22: Doses mortality data of *Rhyzopertha dominica* adult treated with biskatalileaf extract for residual effect in petroleum ether at 2 days

dose	logdose	number	kill	% kill	corr%	emp probit	expt probit	wrk probit	weight	final probit
4	0.6020	30	7	23.33	23	4.26	4.217	4.252	15.09	4.206
6	0.7781	30	8	26.66	27	4.39	4.493	4.39	16.74	4.487
8	0.9030	30	12	40	40	4.75	4.689	4.74	18.03	4.686

$Y = 3.24714 + 1.594302 X$; χ^2 is .2411891 with 1 degrees of freedom; No sig. heterogeneity, LD-50 is 12.5734; 95% Confidence limits are 3.974652 to 39.77467

Appendix table 23: Doses mortality data of *Rhyzopertha dominica* adult treated with biskatali leaf extract for residual effect in petroleum ether at 7 days

dose	logdose	number	kill	% kill	corr%	emp probit	expt probit	wrk probit	weight	final probit
4	0.6020	30	10	33.33	29	4.45	4.405	4.45	16.74	4.394
6	0.7781	30	14	46.66	43	4.82	4.927	4.815	19.02	4.932
8	0.9030	30	20	66.66	64	5.36	5.297	5.384	18.81	5.314

$Y = 2.554756 + 3.055834 X$; χ^2 is .405457 with 1 degrees of freedom; No sig. heterogeneity, LD-50 is 6.312319; 95% Confidence limits are 5.131212 to 7.765291

Appendix table 24: Doses mortality data of *Rhyzopertha dominica* adult treated with biskatalileaf extract for residual effect in petroleum ether at 15 days

dose	logdose	number	kill	% kill	corr%	emp probit	expt probit	wrk probit	weight	final probit
4	0.6020	30	15	50	44	4.85	4.816	4.864	18.81	4.836
6	0.7781	30	20	66.66	63	5.33	5.410	5.321	18.03	5.391
8	0.9030	30	25	83.33	81	5.88	5.832	5.834	15.09	5.784

$Y = 2.938323 + 3.152156 X$; χ^2 is .139617 with 1 degrees of freedom; No sig. heterogeneity, LD-50 is 4.508719; 95% Confidence limits are 3.500821 to 5.806794

Appendix table 25: Doses mortality data of *Rhyzopertha dominica* adult treated with biskatali leaf extract for residual effect in petroleum ether at 21 days

dose	logdose	number	kill	% kill	corr%	emp probit	expt probit	wrk probit	weight	final probit
4	0.6020	30	18	60	52	5.05	5.088	5.05	19.11	5.081
6	0.7781	30	25	83.33	80	5.85	5.758	5.83	15.96	5.738
8	0.9030	30	27	90	88	6.18	6.233	6.128	11.1	6.204

$Y = 2.835363 + 3.731065 X$; χ^2 is .217679 with 1 degrees of freedom; No sig. heterogeneity, LD-50 is 3.803349; 95% Confidence limits are 2.849404 to 5.076663

Appendix table 26: Doses mortality data of *Rhyzopertha dominica* adult treated with Karabi leaf extract for residual effect in petroleum ether at 1 days.

dose	logdose	number	kill	% kill	corr%	emp probit	expt probit	wrk probit	weight	final probit
4	0.6020	30	3	10	10	3.72	3.690	3.73	9.059	3.690
6	0.7781	30	4	13.33	13	3.87	3.940	3.878	12.15	3.949
8	0.9030	30	6	20	20	4.16	4.118	4.17	14.13	4.133

$Y = 2.802406 + 1.47442 X$; χ^2 is 9.536362E-02 with 1 degrees of freedom; No sig. heterogeneity, LD-50 is 30.93715; 95% Confidence limits are 1.388566 to 689.2774

Appendix table 27: Doses mortality data of *Rhyzopertha dominica* adult treated with Karabi leaf extract for residual effect in petroleum ether at 2 days

dose	logdose	number	kill	% kill	corr%	emp probit	expt probit	wrk probit	weight	final probit
4	0.6020	30	5	16.66	17	4.05	4.011	4.037	13.17	3.990
6	0.7781	30	6	20	20	4.16	4.253	4.15	15.09	4.247
8	0.9030	30	9	30	30	4.48	4.425	4.48	16.74	4.428

$Y = 3.114909 + 1.454925 X$; χ^2 is .2140305 with 1 degrees of freedom; No sig.

heterogeneity, LD-50 is 19.75431; 95% Confidence limits are 2.579518 to 151.2813

Appendix table 28: Doses mortality data of *Rhyzopertha dominica* adult treated with Karabi leaf extract for residual effect in petroleum ether at 7 days

dose	logdose	number	kill	% kill	corr%	emp probit	expt probit	wrk probit	weight	final probit
4	0.6020	30	7	23.33	18	4.08	4.055	4.078	13.17	4.043
6	0.7781	30	11	36.66	32	4.53	4.588	4.516	17.43	4.577
8	0.9030	30	16	53.33	50	5	4.965	4.99	19.02	4.956

$Y = 2.218349 + 3.032338 X$; χ^2 is .103106 with 1 degrees of freedom; No sig.

heterogeneity, LD-50 is 8.266642; 95% Confidence limits are 5.983357 to 11.42124

Appendix table 29: Doses mortality data of *Rhyzopertha dominica* adult treated with Karabi leaf extract for residual effect in petroleum ether at 15 days

dose	logdose	number	kill	% kill	corr%	emp probit	expt probit	wrk probit	weight	final probit
4	0.6020	30	12	40	33	4.56	4.500	4.544	17.43	4.482
6	0.7781	30	17	56.66	52	5.05	5.193	5.04	19.02	5.176
8	0.9030	30	24	80	87	5.77	5.685	5.76	16.74	5.669

$Y = 2.107968 + 3.943475 X$; χ^2 is .5591717 with 1 degrees of freedom; No sig.

heterogeneity, LD-50 is 5.412173; 95% Confidence limits are 4.609252 to 6.354959

Appendix table 30: Doses mortality data of *Rhyzopertha dominica* adult treated with Karabi leaf extract for residual effect in petroleum ether at 21 days

dose	logdose	number	kill	% kill	corr%	emp probit	expt probit	wrk probit	weight	final probit
4	0.6020	30	17	56.66	48	4.95	4.895	4.968	18.81	4.909
6	0.7781	30	21	70	64	5.36	5.490	5.348	18.03	5.495
8	0.9030	30	26	86.66	84	5.99	5.913	6.022	14.13	5.911

$Y = 2.904204 + 3.330338 X$; χ^2 is .6299162 with 1 degrees of freedom; No sig.

heterogeneity, LD-50 is 4.258968; 95% Confidence limits are 3.268312 to 5.549902

Appendix table 31: Doses mortality data of *Rhyzopertha dominica* adult treated with ata leaf extract for residual effect in petroleum ether at 1 days

dose	logdose	number	kill	% kill	corr%	emp probit	expt probit	wrk probit	weight	final probit
4	0.6020	30	2	6.66	7	3.52	3.489	3.54	7.140	3.496
6	0.7781	30	3	10	10	3.72	3.792	3.72	10.08	3.793
8	0.9030	30	5	16.66	17	4.05	4.007	4.037	13.17	4.004

$Y = 2.482547 + 1.684807 X$; χ^2 is .0820905 with 1 degrees of freedom; No sig. heterogeneity, LD-50 is 31.20386; 95% Confidence limits are 1.64893 to 590.4926

Appendix table 32: Doses mortality data of *Rhyzopertha dominica* adult treated with ata leaf extract for residual effect in petroleum ether at 2 days

dose	logdose	number	kill	% kill	corr%	emp probit	expt probit	wrk probit	weight	final probit
4	0.6020	30	3	10	10	3.72	3.703	3.72	10.08	3.692
6	0.7781	30	5	16.66	17	4.05	4.090	4.037	13.17	4.088
8	0.9030	30	8	26.66	27	4.39	4.366	4.394	15.96	4.369

$Y = 2.338107 + 2.249135 X$; χ^2 is .0522573 with 1 degrees of freedom; No sig. heterogeneity, LD-50 is 15.22573 ; 95% Confidence limits are 4.986346 to 46.69333

Appendix table 33: Doses mortality data of *Rhyzopertha dominica* adult treated with ata leaf extract for residual effect in petroleum ether at 7 days

dose	logdose	number	kill	% kill	corr%	emp probit	expt probit	wrk probit	weight	final probit
4	0.6020	30	5	16.66	11	3.77	3.816	3.771	11.1	3.840
6	0.7781	30	10	33.33	29	4.45	4.337	4.458	15.96	4.340
8	0.9030	30	12	40	36	4.64	4.706	4.636	18.48	4.695

$Y = 2.132486 + 2.837806 X$; χ^2 is .3390074 with 1 degrees of freedom; No sig. heterogeneity, LD-50 is 10.24397; 95% Confidence limits are 6.16421 to 17.02392

Appendix table 34: Doses mortality data of *Rhyzopertha dominica* adult treated with ata leaf extract for residual effect in petroleum ether at 15 days

dose	logdose	number	kill	% kill	corr%	emp probit	expt probit	wrk probit	weight	final probit
4	0.6020	30	9	30	22	4.23	4.233	4.218	15.09	4.222
6	0.7781	30	16	53.33	48	4.95	4.941	4.94	19.02	4.931
8	0.9030	30	21	70	67	5.44	5.444	5.429	18.03	5.434

$Y = 1.798905 + 4.025571 X$; χ^2 is 2.228737E-03 with 1 degrees of freedom; No sig. heterogeneity, LD-50 is 6.240082; 95% Confidence limits are 5.325096 to 7.312284

Appendix table 35: Doses mortality data of *Rhyzopertha dominica* adult treated with ata leaf extract for residual effect in petroleum ether at 21 days

dose	logdose	number	kill	% kill	corr%	emp probit	expt probit	wrk probit	weight	final probit
4	0.6020	30	15	50	40	4.74	4.733	4.74	18.48	4.734
6	0.7781	30	20	66.66	60	5.25	5.290	5.28	18.81	5.293
8	0.9030	30	24	80	76	5.71	5.686	5.7	16.74	5.690

$Y = 2.820642 + 3.178314 X$; χ^2 is 5.707741E-03 with 1 degrees of freedom; No sig. heterogeneity, LD-50 is 4.849493; 95% Confidence limits are 3.87909 to 6.062656

Appendix table 36: Doses mortality data of *Rhyzopertha dominica* adult treated with akanda leaf extract for residual effect in petroleum ether at 1 days

dose	logdose	number	kill	% kill	corr%	emp probit	expt probit	wrk probit	weight	final probit
4	0.6020	30	1	3.33	3	3.12	3.133	3.116	4.62	3.146
6	0.7781	30	2	6.66	7	3.52	3.487	3.54	7.140	3.493
8	0.9030	30	3	10	10	3.72	3.738	3.72	10.08	3.739

$Y = 1.959335 + 1.971118 X$; χ^2 is 2.366734E-02 with 1 degrees of freedom; No sig. heterogeneity, LD-50 is 34.88265; 95% Confidence limits are 1.431667 to 849.9182

Appendix table 37: Doses mortality data of *Rhyzopertha dominica* adult treated with akanda leaf extract for residual effect in petroleum ether at 2 days

dose	logdose	number	kill	% kill	corr%	emp probit	expt probit	wrk probit	weight	final probit
4	0.6020	30	2	6.66	7	3.52	3.489	3.54	7.140	3.496
6	0.7781	30	3	10	10	3.72	3.792	3.72	10.08	3.793
8	0.9030	30	5	16.66	17	4.05	4.007	4.037	13.17	4.004

$Y = 2.482547 + 1.684807 X$; χ^2 is .0820905 with 1 degrees of freedom; No sig.

heterogeneity, LD-50 is 31.20386; 95% Confidence limits are 1.64893 to 590.4926

Appendix table 38: Doses mortality data of *Rhyzopertha dominica* adult treated with akanda leaf extract for residual effect in petroleum ether at 7 days

dose	logdose	number	kill	% kill	corr%	emp probit	expt probit	wrk probit	weight	final probit
4	0.6020	30	3	10	4	3.25	3.200	3.248	5.4	3.148
6	0.7781	30	5	16.66	11	3.77	3.890	3.771	11.1	3.887
8	0.9030	30	10	33.33	29	4.45	4.379	4.458	15.96	4.410

$Y = .6253765 + 4.191649 X$; χ^2 is .2381892 with 1 degrees of freedom; No sig.

heterogeneity, LD-50 is 11.05738; 95% Confidence limits are 7.065911 to 17.3036

Appendix table 39: Doses mortality data of *Rhyzopertha dominica* adult treated with akanda leaf extract for residual effect in petroleum ether at 15 days

dose	logdose	number	kill	% kill	corr%	emp probit	expt probit	wrk probit	weight	final probit
4	0.6020	30	5	16.66	7	3.52	3.520	3.519	8.07	3.524
6	0.7781	30	10	33.33	26	4.36	4.357	4.362	15.96	4.354
8	0.9030	30	16	53.33	48	4.95	4.951	4.94	19.02	4.943

$Y = 0.6877336 + 4.712549 X$; χ^2 is 1.436234E-03 with 1 degrees of freedom; No sig. heterogeneity, LD-50 is 8.223568; 95% Confidence limits are 6.663407 to 10.14902

Appendix table 40: Doses mortality data of *Rhyzopertha dominica* adult treated with akanda leaf extract for residual effect in petroleum ether at 21 days

dose	logdose	number	kill	% kill	corr%	emp probit	expt probit	wrk probit	weight	final probit
4	0.6020	30	10	33.33	20	4.16	4.229	4.15	15.09	4.244
6	0.7781	30	18	60	52	5.05	4.883	5.072	18.81	4.890
8	0.9030	30	20	66.66	60	5.25	5.347	5.24	18.48	5.348

$Y = 2.035718 + 3.668132 X$; χ^2 is 0.9734096 with 1 degrees of freedom; No sig. heterogeneity, LD-50 is 6.428619; 95% Confidence limits are 5.381773 to 7.679096