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Combined Action of Piperonyl Butoxide with Some Insecticides, Against the Lesser Grain Borer Rhylopertha Dominica (Coleoptera: Bostrichidae)

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COMBINED ACTION OF PIPERONYL BUTOXIDE WITH SOME INSECTICIDES, AGAINST THE LESSER GRAIN BORER *RHYZOPERTHA DOMINICA* (COLEOPTERA: BOSTRICHIDAE)

Ph.D. Thesis

A Thesis Submitted to the faculty of Life and Earth Sciences
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Doctor of Philosophy

SUBMITTED BY

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Dedicated
To
My Parents
and Husband

DECLARATION

I do hereby declare that the thesis entitled" Combined action of piperonyl butoxide with some insecticides against the lesser grain borer, *Rhyzopertha dominica* (Coleoptera: Bostrichidae)" submitted to the Department of Zoology, University of Rajshahi for the award of Doctor of Philosophy is the result of my own investigation and the research conducted under the supervision of Dr. A. S. M. Shafiqur Rahman, Professor, Department of Zoology, University of Rajshahi, Bangladesh.

I further declare that this thesis or any part of it has not been submitted to any other University for any degree or diploma or for other similar purposes.

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CERTIFICATE

I have the pleasure to certify that the work presented in this dissertation entitled **"Combined action of piperonyl butoxide with some insecticides against the lesser grain borer, *Rhyzopertha dominica* (Coleoptera: Bostrichidae)"** is an original research work carried out by Mst. Rezwana Ferdoush, Registration No. 07628 Session 2007-2008 in partial fulfillment for requirements for the degree of Doctor of Philosophy (Ph.D) in the Department of Zoology, Rajshahi University under my supervision.

This thesis has been found satisfactory. To the best of my knowledge this is the researcher's own achievement and I am forwarding this thesis to be examined for the award of the degree.

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Mst. Rezwana Ferdoush

CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATE

ABSTRACT

The toxicity of four (4) commercially formulated insecticides *viz.* organophosphate, pyrethroid, neonicotinoid and organo carbamate was investigated following residual-film method. 7 days old adults was used in this experiment. The data was recorded after 24 hours of treatment. All tested insecticides showed satisfactory killing ability towards the beetle tested.

The insecticides were highly or moderately toxic to adult with LD_{50} values of 0.0745 (chloropyrifos), 0.7182 (cypermethrin), 1.9997 (imidacloprid) and 3.6513 (carbaryl) respectively. Chloropyrifos was found most toxic and carbaryl was less toxic to adult. The order of toxicity was chloropyrifos > cypermethrin> imidacloprid > carbaryl.

Synergistic effect of piperonyl butoxide (PBO) in combination with all the insecticides tested was also investigated. The synergistic effects were calculated by using co- toxicity coefficient (>100) values. piperonyl butoxide considerably increased the toxicity of all the insecticides except of chloropyrifos. PBO did not produce synergistic effect against the adult.

The interaction between insecticides and synergist was analyzed by co-toxicity coefficients and through plotting isoboles of the LD_{50} values.

Chapter One

Introduction

CHAPTER-1

GENERAL INTRODUCTION

1.1 Introduction

The struggle between man and insects began long before the down of civilization, has continued without cessation to the present time, and will continued, no doubt, as long as the human race endures. It is due to the fact that men and certain insects constantly want the same things at the same times. With passes of time, change of system of living and culture man has developed new measures for their survival. Considering the future demand, man began to store food (Metcalf &Flint, 1962).

Food clothes and shelter are the basic human needs of which food mostly comes from the crops and fibers from the plants. The world food demand is increasing day by day as population is growing up. The growing demand for food has lead to a substantial increase in the production (Heijnen, 2001). It has been possible with the help of technology driven agricultural practice (Morris *et al.*, 2005). But modern agriculture has selected genetically engineered high yielding varieties of crops which are mostly vulnerable to crop pest attacks and diseases. So, successful production of food and fiber globally is dependent on the effective control of pests and diseases. Hense pesticides have become very important especially to the developing nations in their efforts to produce adequate food and fibers. Now it is obvious that modern agricultural practices have led to sharp increase in pesticide use (Dasgupta *et al.*, 2005; Altieri, 2003; Rahman, 2003). The indiscriminate use of pesticides constitutes one of the main public health problems in developing countries (Waichman *et al.*, 2007; Piperakis *et al.*, 2006; Sorensen *et.al.*, 2003; He *et al.*, 2002).

In tropical countries including Bangladesh, the climate and storage conditions are highly favorable for insect growth and development (Jacobson, 1983). In Bangladesh, 13 species of insects have been recorded on stored rice. Among them lesser grain borer *R. domineca* (F) is one of the most destructive insect pests of stored grain in tropical countries (Alam, 1971).

Estimates of losses to the world supply of stored grain from insect damage range from 10-15% of world's production (Dasgupta and Meisner, 2004) in certain tropical and subtropical countries as well as Bangladesh estimates are much higher (FAO, 1977). Estimation for Bangladesh shows that the annual crop loss due to insect pest alone is 16% for rice, 11% for wheat, 20% for sugarcane, 25% for vegetables, 15% for jute and 25% for pulse (Islam, 2004). However, loss of 20% or more may occur in the tropical countries through insect attack after harvest (Mondal and Port, 1994). Because the climate and storage conditions in the tropical countries are highly favorable for insect growth and development. Both contamination and substantial economic loss due to the pest insects' presence in a stored product sustain to lose the product and a decrease in nutritional value (Burkholder and Faustini, 1991; Wilber and Mills, 1985).

Man has been fighting to protect his food supplies from damage and loss caused by insects. Conservation of the available food from damage, loss and depredation by insects pests is at prime importance from the economic point of view as well as from health reasons and general improvements of living conditions. Grain loses of 20% or more may occur in tropical countries through insects attack after harvest which may lead to considerable loss of life. Thus the need for food conservation in most acute (Hill, 1978).

Wheat crop suffers heavy losses both in quality and quantity every year during its storage due to pest attacks (Aheer and Ahmad, 1993). Losses caused to wheat and other cereals in stores by the pests vary between 5 to 15 percent (Qayyam and Zafar, 1978). Aheer and Ahmad (1993) tested twelve wheat varieties against *T. granarium* and *R. dominica*, and observed significant differences among varieties for number of bored grain and grain weight loss caused by these pests. Twenty five percent losses in wheat grains during storage due to insect pest was reported by Ahmad (1983).

A large number of insects attack stored food. Among them *R. dominica* is major pest of stored products and is worldwide distribution due to the development of world trade (Metcalf and Filant, 1962; Alam, 1971; Cotton, 1947). This is commonly known as Australian wheat beetle.

R. dominica belongs to the family Bostrichideae under the order Coleoptera. This beetle is especially important as a major pest of whole cereals even of rough rice (Metcalf and Flint,1994;Hill,1975). Both larvae and adults are capable of boring into and feeding on a wide range of commodities. Both larvae and adults feed on same food and hollowed out until only a thin porous husk remains. Moreover physiological mycological and biochemical changes in grains occurred to infestation of *R. dominica.* The damage of wheat is caused by spoiling more than they eat. As a result wheat becomes unsuitable for human consumption. It feeds in both Larval and adult stages in the interior of nearly all grains and some other substances, such as seeds drugs, dry roots and cork and eats into wood and paper boxes. It is most common in wheat and one of the most destructive wheat insect. (Metcalf & Flint,1962).

Lists of food substances have been recorded by many workers (Table-2). There seems little doubt that *R. dominica* originally fed on wood, probably living on wood (Lesne, 1911). Its occurrence on wheat in the field may be regarded as a secondary adaptation (Gurney, 1918).

R. dominica was first described by "Fabricius" (1792) from specimens obtained in South America in a shipment of seeds from India (Nayan *et al.*, 1976). He placed *R. dominica* among Lamellicorns under the genus *Sinodendron* of family Lucanidae, composed of wood boring beetles. This association with insects of widely differing genera is undoubtedly due to superficial similarity of appearance and habits (Potter,1935).

Plate 1: Adults have powerful jaws that are used to riddle the grain, creating large, irregular-shaped holes.

The grain mass is also conditioned by a long-standing culture of *R. dominica* due to presence of living or dead insects or insects parts, exuviae, casts, egg shells, faecal matters (Tiwari *et al.*, 1989) and so on. Percentage of fungal flora, total nitrogen, uric acid, free fatty acid of the grain increased considerably due to infestation of *R. dominica* at the end of third or fourth month of storage. Reducing and non-reducing sugar and water-soluble sugar decreased with an increase of infestation (Charjan *et al.*, 1994).

The losses of food to grains during storage due to insect infestations are the most serious problem. Among various staple stored cereals, wheat suffers heavy damage from lesser grain borer *R. dominica* (F.) also called as "Australian weevil" (Metcalf & Flint, 1962, Wilbur & Mills, 1985).

Many plants and animals provide vital solution for eliminating disease; pesticides affect their requirements for survival. The resulting reduction in biodiversity may ultimately threaten the long term survival of human as a species by reducing food supply and increasing disease. In India 51% of food commodities have been found as contaminated with pesticide residues and out of these, 20% have pesticides residues above the maximum residue level values on a World wide basis (Gupta, 2004; Agnihotri, 1999).

During the last few decades different insecticides have been used against these stored product pests (Quinlan *et al.,* 1980 Green, 1975). However, the extensive use of insecticides has been a major cause of disruption.

The pesticide creates environmental hazards to men, his animals and wild life including pollinator and other non-insects beneficial forms (Smith and Vonden Bosch, 1967). More over often pests have become resistance to the insecticides (Powles and Holtum, 1994; Caseley *et al.,* 1991; Roush and Tabashnik, 1990; Georghiou, 1990; Le Barou and McFarland, 1990 Metcalf, 1989;), Although food grains are still common problems have generated a sustained search for their alternative means of insect control or methods of reducing the amount of insecticides require for the pest management. This will inevitably lead to both increase in cost and also to the possibility of some insecticides accumulating in the environment.

The use of toxic pesticides by Bangladeshi farmers increased by 328 percent during the past 10 years, posing a serious health hazards on human health due to its long-term residual effect, according to a study released by Bangladesh Rice Research Institute (BRRI). The survey, studying the use of toxic pesticides in farmland during 1997 to 2008, showed that in 1997 the use of pesticides in Bangladesh was more than 8,000 tons; it doubled to 16,000 tons in 2000; in 2005-06, it increased to nearly 20,000 tons and in 2008 it rose up to 48,690 tons. The insecticides, being the dominant item, account for 76 percent of the pesticides, and per hectare use of pesticides increase around 598.8 percent and its annual import cost stands nearly at 171.43 million U.S. dollars. The study said the residual effect of these toxic chemicals on vegetables are likely to create different diseases in human bodies including cancer, skin diseases, hypertension and kidney diseases as its long term effect. The use of pesticides in vegetables is likely to grow further in the future unless appropriate alternatives, based on integrated pest management approaches, are developed, warned the study. (Source: I Stock Analyst).

In 1998 more than 500 different pesticide formulations are being used in our environment mostly in agricultural sector (Azevedo, 1998). The non-judicious use of pesticides has led to concerns regarding the potential contamination of

environmental media i.e., air, surface water, ground water, soil and sediment by countless pathways; thus pose risks on the human health and wildlife (Sattler *et al.,* 2007; Worrall and Besien, 2005; Fava *et al.,* 2005; Craven and Hoy, 2005; Kolpin *et al.,* 2004; Sivanesan *et al.,* 2004; Hapeman *et al.,* 2003; Duyzer, 2003; Das *et al.,* 2002; Dubus *et al.,* 2000). It has been estimated by Arias- Este vez *et al.,* (2008) that less than 0.1% of the pesticide applied to crops actually reaches the target pest. The rest enters into the environment gratuitously where it can poison otherwise adversely affect nontarget organisms.

In order to keep the stored grain products free from the infestation of insect pests some control measures are undertaken. The chemical control method is very effective and rapid in creative action. It has been considered the most important and powerful tool in controlling insect pests both in the field and under storage conditions. Snelson (1987) presented a comprehensive review of the chemicals used throughout the world for combating insects for this purposes.

Contact insecticides on grains are degradable by hydrolysis through enzymatic actions (Anderegg and Madisen, 1983; Rowlands, 1970). Some insecticides have repellent effects on insects, which will affect their distribution (Collins *et at.,* 1988). Sub-lethal exposures to insecticides usually have negative effects on an insect's longevity, fecundity and larval survivability. (Taher and Cutcomp 1983; Zettler and Lecato, 1974).

Insecticides resistance, which evolves through the repeated exposure of insects to insecticides, began to emerge as a serious world wide problem about 50 years ago. Today a large number of arthropods have developed resistance to the insecticides in different parts of the world (Poweles and Holtum, 1994; Caseley *et al.,* 1991; Green *et al.,* 1990; Roush and Tabashnik, 1990; Georghiou, 1990; Le Baron and McFarland, 1990). It has therefore, necessary to compliment our reliance on synthetic insecticide with less hazardous, safe and biodegradable substitutes.

One method of combating insecticide resistance is to use a synergist. Pesticide synergism is only one of the several techniques that can be used to control or study pesticide resistance (Kemp and Caseley, 1991; Hammock and Soderland, 1986).

No other control method has been established in Bangladesh so far except pesticides (Hussain, 1996). The use of synthetic pesticides grew enormously over the years and millions of tons of pesticides are being used annually in Bangladesh (Ameen, 1994).Total of 7.35 metric tons of pesticides were imported under 112 trade names that valued Tk. 106 crores during 1992. In 1993, nearly 10,000 metric tons of pesticides were sold to the farmers more than 100 categories of pesticides have been registered and marketed by different corporations in Bangladesh (Hussain, 1996). Insect populations of many species have evolved resistance to insecticides due to their indiscriminate use in pest control strategies. In some cases, insects exposed to one insecticide also developed multi-resistance. However, it takes several years and costs millions of dollars to develop new alternatives.

The effect of pesticides or other synthetic chemicals, or biological system have usually been investigated primarily with single chemical. However, since the 1940's increasingly large amounts of insecticides or herbicides have been developed and marked for insect and weed control. The result of which has increased the probability of these two groups of pesticides acting thus presenting the potential problem of pesticide interaction in biological system (Litchtenstein *et al.,* 1973).

There are several important reasons that underline the potential importance of research and development of possible synergists and their interactive properties.

THESE REASONS ARE

- Increased toxicity of pesticides to pest organism own to synergistic interactivity may mean that the grower is using more pesticides then would be required to control the pest organism (s) to bellow the economic threshold.
- This increase in toxicity as described above many result is sever increase in mortality levels of non-target organism many of which may be beneficial to a grower example for Bees, ladybirds, etc.

- The use of synergists may results in alleviating to some extent the problem of increasingly rapid pesticide resistance evolution, especially in insect pest population in the tropics. The present way of dealing with this problem is just to increase the doses of the insecticide to eliminate the more resistant population. However, the result of this is to apply a greater selection pressure on the population. This ultimately enhances the evolution of resistance in the population and obviously this procedure cannot go on infinitely, science increasing the dose and thus the presence in the environment of a pesticide not only increase the non-target organism's mortality, but levels could be reached where mammals, ultimately man would be seriously affected. Therefore, the introduction of synergists in these systems could be of great benefit both economically and ecologically, especially since tests have shown that synergistic increases in toxicity of insecticides in only towards insects and not mammals (Metcalf, 1992).

Pesticides are chemical substances that are used to kill, repel, or regulate the growth of biological organisms. This diverse group includes insecticides, herbicides, fungicides, nematocides, acaricides, rodenticides, avicides, wood preservatives, and antifoulants. The U.S. Environmental Protection Agency (EPA) recently estimated that > 1.2 billion pounds of pesticides are applied to crops, forests, residential areas, public lands, and aquatic areas in the United States each year (Kiely *et al.,* 2004). The release of these chemicals into the environment creates a potential for unintended adverse health impacts to both humans and non target wildlife. Another concern regarding the wide spread use of pesticide is the development of resistant pest strains to insecticides (Fakoorziba *et al.,* 2009; Alyokhin *et al.,* 2007; Lambkin and Rice, 2006; Prabhaker *et al.,* 2005; Enayeti *et al.,* 2003; Ahmad *et al.,* 2002). Resistant strains have developed through the survival and reproduction of individuals after exposure to a given insecticide and as a result insect pests have now developed tolerance to all major classes of insecticides (Alyokhin *et al.,* 2007; Kristensen *et al.,* 2004; Baki *et al.,* 2002; Pittendrigh and Gaffney, 2001; Wilkins *et al.,* 1995). Resistance within or between whole classes of insecticide is

an ever increasing problem for control of major crop pests; when in order to have a same level of control, the amount of insecticide use needs to be increased. Without development of better and more cost effective means, farm chemicals will remain as the major weapons in our constant battle against the pest population.

Therefore, in order to have some level of control, the amount of the insecticide used needs to be increased. This will inevitably lead both to increase in cost and also to the possibility of residual problems. Therefore the introduction of synergists in these systems could be at great benefit both economically and ecologically. Tests have shown that synergists increase the toxicity of insecticides and their toxicity in only towards insects and not mammals (Metcalf, 1992).

With a view to overcome these problems, a need to find an alternative to this reliance on pesticides has become imperative so that the benefits of insecticides probably outweigh the risks, and to safety to human health, improve the World's food supply and be friendly to the environment.

According to Sawicki and Keilding (1981) at least 350 species of insect pests are now resistant to one or more insecticides throughout the world. The number of species of insects and mites in which cases of resistance were reported through 1975 totaled 364 (Georghiou and Taylor, 1977). Later Georghiou (1986) reported about the involvement of resistance in 414 species of insects and mites.

Since synergists are not in themselves toxic or insecticidal, but are materials used with insecticides to enhance the activity of the insecticides thus reduces the dose level. The use of synergist may result in alleviating to some extent the problem of rapid pesticide use and resistance evolution in insect pests in the tropics (Khot *et al.,* 2008; Wu *et al.,* 2007; Kumar *et al.,* 2002). This hypothesis has lead to start the present study.

Table 1: Some recent works on insecticide synergism against several pest species.

Insecticides	Synergist	Pest species	References
Deltamethrin (Pyrethroid)	PBO	*Culex tritaeniorhynchus* and *Aedes aegypti*, *Anopheles culicifacies*, *A. stephensi* & *A. vagus*.	Fakoorziba *et al.*, 2009.
Acetamiprid and Imidacloprid (Nicotinoid), Pirimicarb(Carbamate)	PBO	*Myzus persicae* (Sulzer) *Aphis gossypii* (Glover)	Thalavaisundaram *et al.*,2008
Cypermethrin (Pyrethroid), Diazinon (OP)	PBO	Cockroach, *Periplaneta americana* L.	Rahman and Akter, 2008
Primicarb (carbamate), Imidacloprid and Acetamiprid (Neonicotinoid)	PBO	Peach potatoes aphid, *Myzus persicae* (Sulzer), Cotton aphid, *Aphis gossypii* (Glover) and Tobacco whitte fly, *Bemisia tabaci.*	Bingham *et al.*, 2008.
Formetanate (Formamidine), Acrinathrin (Pyrethroid), Chlorpyrifos (OP), Methiocarb, Carbofuran and Carbosulfan (Carbamates)	PBO	Western flower thrips, *Frankliniella occidentalis* (Pergande)	Bielza *et al.*, 2007.
Malathion (OP) and Lambda-cyhalothrin (Pyrethroid)	PBO	Lesser grain borer, *Rhizopertha dominica* (Panzar).	Rahman *et al.*, 2007
Methamidophos (OP), Fenvalerate (Pyrethroid), Fipronil(Fiproles), Avermectin (Antibiotics).	PBO TPP and DEM.	18 species of insects (pest of crucifer vegetable crops)	Wu *et al.*, 2007
Furadan 5g (Carbamate)	PBO	Blue Green Algae (BGA)	Islam *et al.*, 2007
Methamidophos and Chlorpyrifos (OP), Fenvalerate (Pyrethroid), Avermectin (Antibiotics), Fipronil(Fiproles), Spinosad (Spinocin), Imidacloprid (Nionicotinoid),	PBO TPP and DEM.	Silver white fly, *Bemisia tabaci* (Gennadius)	Kang *et al.*, 2006
Cyfluthrin, Lambda-cyhalothrin and Tau-fluvalinate (Pyrethroid).	PBO DEF and DEM	Honey bees; *Apis mellifera* L.	Johnson *et al.*, 2006

1.2 Experimental insect

In this investigation the lesser grain Borer *R. dominica* (F.) is used as the test organism to evaluate toxicity of one organophosphate, one pyrethroid, one carbamate and one chlorinated hydrocarbon insecticides and their synergistic effect in combination with a reference synergist Piperonyl butoxide (PBO).

Systematic Position of Experimental insect:

Phylum	:	Arthropoda
Class	:	Insecta
Order	:	Coleoptera
Family	:	Bostrychidae
Genus	:	*Rhyzopertha*
Species	:	*Rhyzopertha dominica* (F.) (Fabricius, 1792)

This beetle is especially important as a major pest of whole cereals even of rough rice (Hill, 1975).

Plate 2: Adult *Rhyzopertha dominica*.

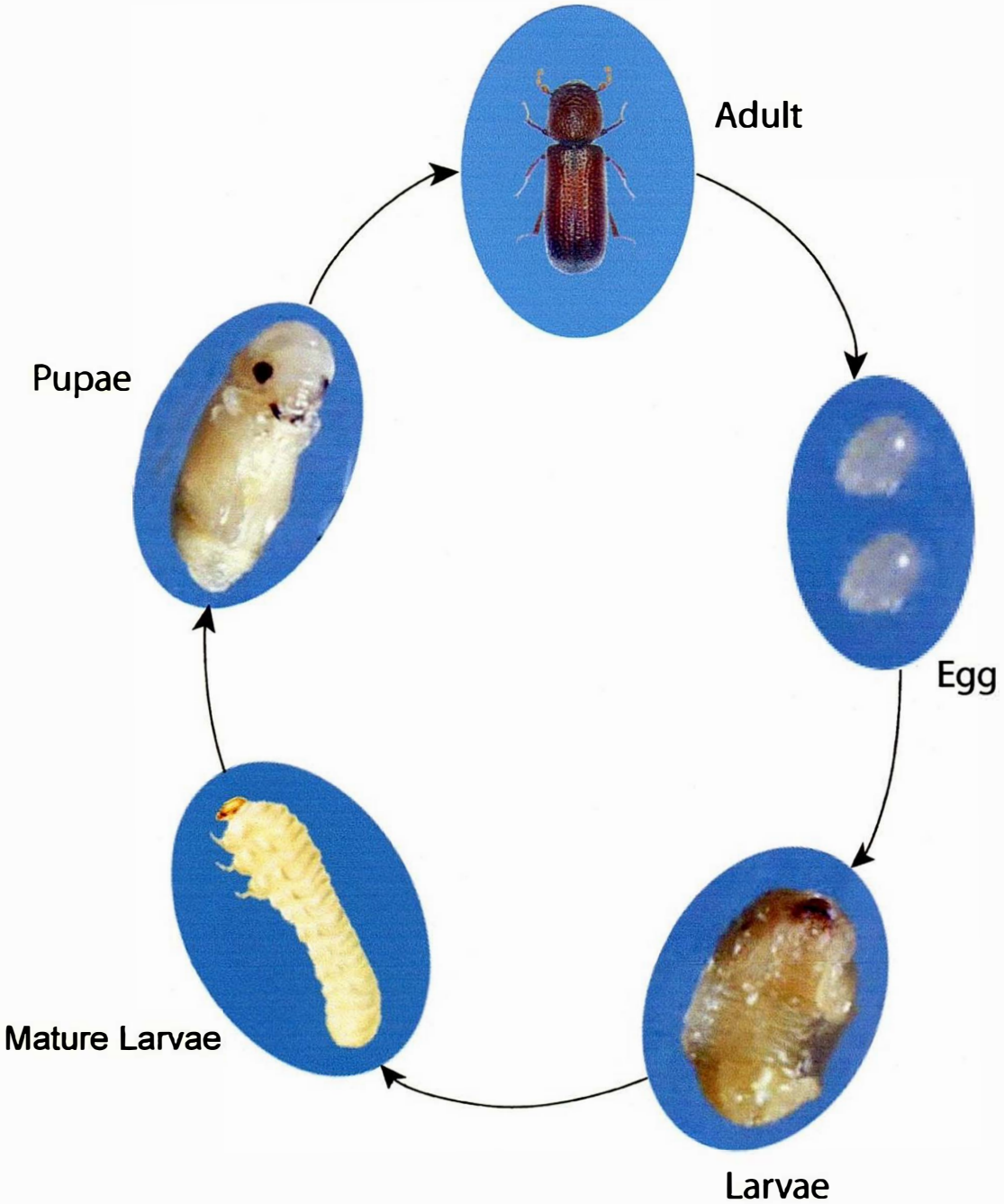


Plate. 3: Life cycle of *Rhyzopertha dominica*

1.3 Biology of Insect

The adults are brown to black, nearly cylindrical, about 1/8 inch long by 1/4 as wide, large head is bent under the thorax and the rear end of the body is blunt. The larvae are grub-like, lie curved, and become about 1/10 inch long, the anterior end much swollen, bearing a small brown head and six short legs (Metcalf & Flint, 1962).

The female lays about 300-500 egg in the grain and under favorable condition a generation may developed in a month. In case of male, there was a pair of two segmented papillae placed almost parallel or convergent to each other whereas, in case of female the paired papillae were three segmented placed diversely at the abdominal tip. (Metcalf & Flint, 1962).

The pre-oviposition period was shortest (7.40 days) with oviposition period being longest, 55.2 days. The post oviposition period was recorded as 1.25 days. Fecundity was 140.33 eggs / female on whole grain. Adult longevity was record to be as 48.07 and 62.84 days for whole and broken grains respectively (Almeda *et al.,* 1994). Total life cycle was recorded to be 33.0 ± 42 days and the larval period was 19.0 ± 2.8 days (Hashem, 1989).

R. dominica laid eggs outside the grains. On emergence from eggs the larvae bore and enter into the whole grain where they complete their total development becoming adults, causing serious damage to stored wheat. Although *R. dominica* infests a large variety of stored products, no detailed information is available so far on the life history of *R. dominica* and extents of damages by it, in Bangladesh. Pensook-Tauthong *et al.,* (1992) reported that both larvae and adult beetles cause serious damage to grains or kernels of burly which have been reduced to mere shells by the feeding of them. The eggs hatch in 5-7 days and the small whitish grubs crawl actively and feed on the flour produced by the boring of the beetles, or bore directly into grain that have been slightly damaged. They complete their growth either within the grain or in the grain dust, then transform to white pupae and change to adult beetle. It takes 23-32 days and 6-8 days for larval and pupal period actively. The life cycle is completed in 40-45 days (Pensook *et al.*, 1984).

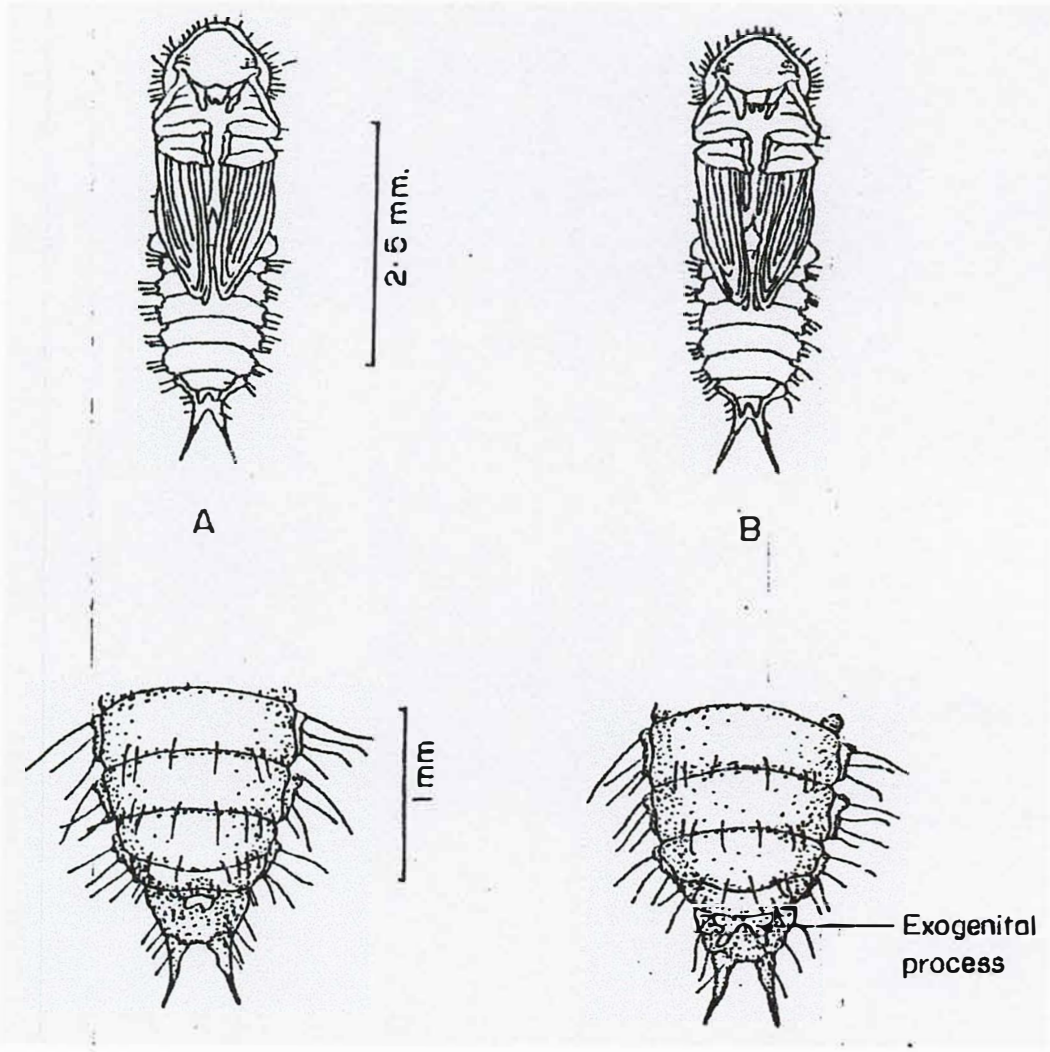
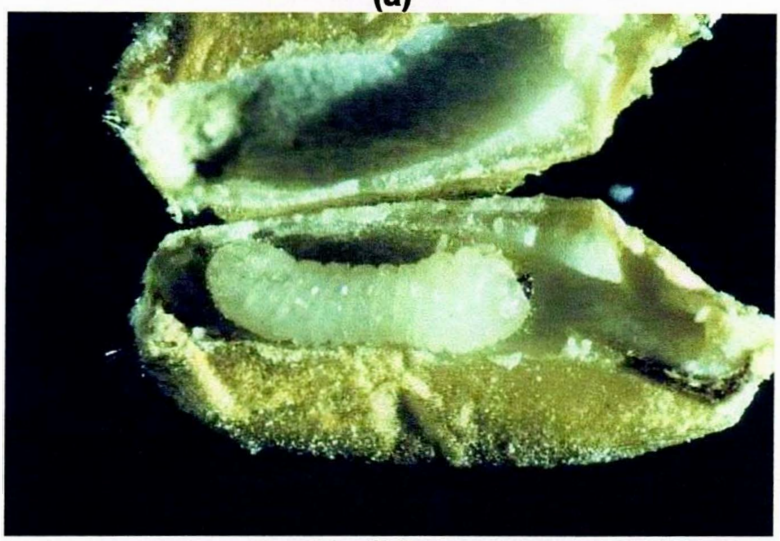


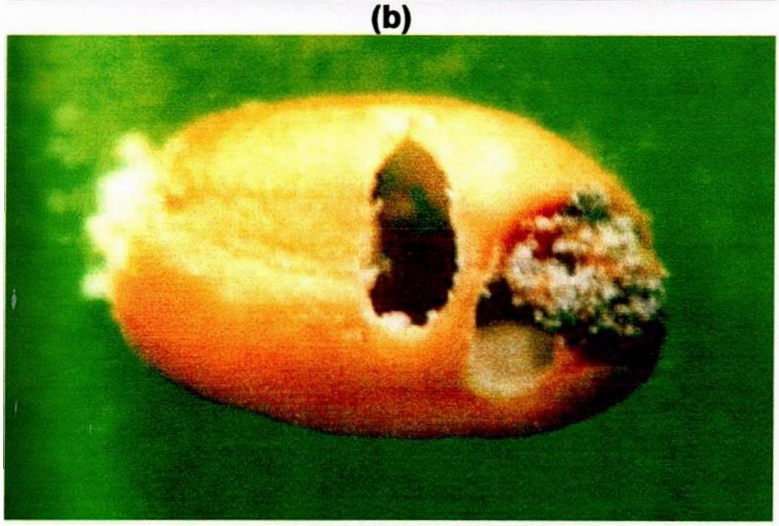
Fig.1: Terminal view of male and female *Rhyzopertha dominica* showing the sexual character.

A. Male pupa
B. Female pupa
C. Male pupa (abdominal portion enlarged)
D. Female pupa (abdominal portion enlarged)

(a)



(b)



(c)

Plate 4: Eggs laid on the crevices of a wheat grain (a) larvae inside of wheat grain (b) adult remained inside the grain (c).

Begum *et al.* (1974) studied the effect of foods on the life history of *R. dominica* and found that rearing media did not affect the incubation period. The larvae ecdysed 3 times when reared in wheat and maize and 4 to 5 times when reared on flour and rice. The duration of the life cycle was 33.28, 36.19, 41.82 and 46.31 days in wheat, maize, flour and rice respectively. The maximum growth of larvae was on maize followed by wheat, flour and rice. The wheat proved to be the best medium for the development and growth of *R. dominica*. It has 5 to 6 generations per year (Lin, 1958) and the life cycle was completed in about two months (Edwards and Heath, 1964).

1.4 Occurrence and Distribution

The origin of lesser grain borer is tropical (Dell'Orto Trivelli, 1985; Potter, 1935 Schwardt, 1933,) but it is well established in temperate region of the World also. Occurrence of this species has been reported from Egypt (Kascheif 1959). USA (Storey *et al.,* 1983). India (Jacob and Mohan, 1997) Bangladesh (Alam, 1971), Saudi Arabia (Taher and Zuheira, 1987). It is also found in Pakistan (Tariq-Mahmood and Ahmad, 1996; Khalil and Irshad, 1998), Romania (Ghizdava and Deac, 1994), Maxica (Wong-corral, Cortez-Rocha-Mo and Borboa-Florezy,1996). It is now well distributed through out the South and Midwest but not more northern areas. (Metcalf and Flint, 1962).

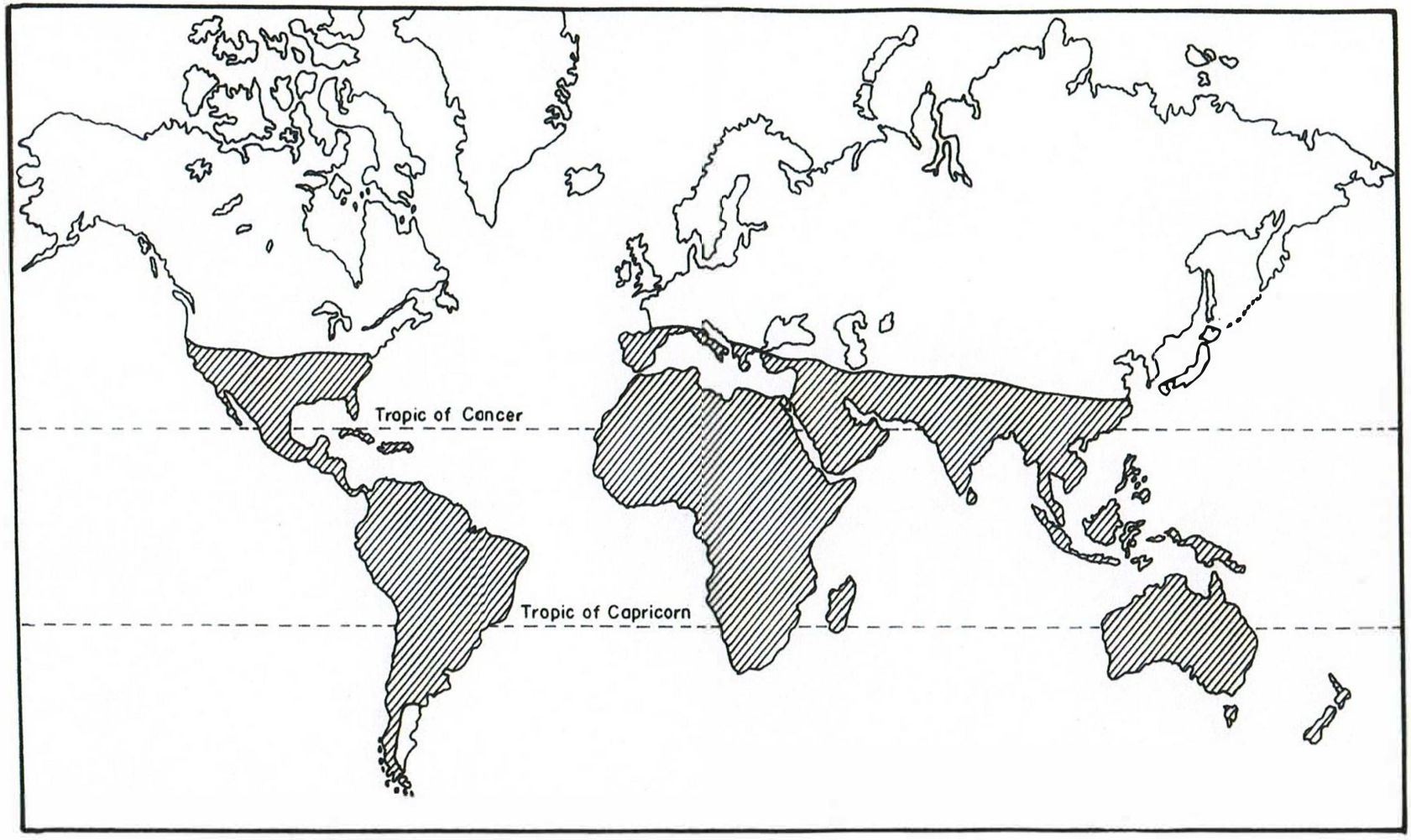


Fig.2: Distribution of *Rhyzopertha dominica* (F)

1.5 Economic Importance

R. dominica is considered to be one of the most destructive pest of stored grain.it attacks wheat and make them unfit for human consumption. Infestation of wheat, and sorghum grains caused by *Trogoderma granarium* and *R. dominica* individually or in mixed population led to the substantial reduction in content of total lipid (Sudesh *et al.,* 1996). Seed germination was significantly reduced with medium and heavy infestation, the bread making qualities of wheat were also affected even at low levels of infestation (Ghizdava and Deac, 1994).

R. dominica caused great loss in seed weight and reduced seed viability of the maize hybrids under storage conditions (Kurdekeni *et al.,* 1993). Percentage at seed damage and loss in weight increased and seed viability decreased markedly with the increase of storage of the infested grains (Kurdikari *et al.,* 1994). Presence of *R. dominica* produces bad odours in stored commonly, which is partially due to production of aggregation pheromones called Dominiculture (Seitz, 1996).

The extent of damage due to this pest in maize seed was reported to be 32 to 53 percent and loss in weight from 0.85 to 1.92 gm (Single and Pande, 1975). The viability of maize seed stored for 11 weeks was reported to markedly reduce to zero percent due to this pest (Demianyk and Sinha, 1987). In maize, variety susceptibility or resistance to storage pests has been related to physicochemical Properties of seed (Sing *et al.,* 1975).

Under storage conditions *R. dominica* causes heavy losses to cereals by feeding and marking circular holes in them and also affect the nutritional and baking quality as well as germination capacity of the grain (Patel and Valand, 1994).

Mookherjee *et al.,* (1968) found six important cereal seeds viz. paddy, wheat, maize, barley, jowar and dajra damaged by storage pests. *S. oryae, R. dominica, T. granarium, T. castaneum, O. surinamensis, S. cerealella, C. cephalonica* and *Laemophloeus minutus*. The authors found that wheat seed got maximum damage. Paddy seeds from the dry region were damaged mostly by *R. dominica*.

The damage varied between 0 to 70%, to 100%, 0 to 100%, 0 to 255, 0 to 22.7% and 0 to 11% in paddy, wheat, maize, barley, jowar and bajra respectively for different ecological zones with an overall average damage of 1.34%, 4.39%, 4.75%, 2.50%, 2.29 and 1.20% for whole India.

Wheat crop suffers heavy losses both in quality and quantity every year during its storage due to pest attacks (Aheer and Ahmad, 1993). Losses caused to wheat and other cereals in stores by the pests vary between 5 to 15 percent (Qayyam and Zafar, 1978). Aheer and Ahmad (1993) tested twelve wheat varieties against *T. granarium* and *R. dominica*, and observed significant differences among varieties for number of bored grain and grain weight loss caused by these pests. Twenty five percent losses in wheat grains during storage due to insect pest were reported by Ahmad (1983).

Presence of *R. dominica* directly affects both quantity and quality of stored grains (Burkholder and Faustine, 1991 Wilbur and Mills, 1985). *R. dominica* F. infests a number of other products besides cereals eg. Seed of white lolus. Pearl millets Pumkin seeds, tamarind seeds, dried fruits and dried wood. (Kapur, 1994).

The damage caused by the Bostrichid, *R. dominica* on 32 wheat varieties was recorded (Saxean and Singh, 1995).

Under storage conditions *R. dominica* causes heavy losses to cereals by feeding and marking circular holes in them and also affect the nutritional and baking quality as well as germination capacity of the grain (Patel and Valand 1994).

The lesser grain borer is the predominant pest species damaging paddy, while *Sitophilus zeamis* is the main pest of stored maize. Estimates of weight losses due to insects in unprotected grain are 34% for maize stored for 8 months and 2.5% for milled rice stored for 3 months (Caliboso *et al.*, 1986). Various species of insects (*Sitophilus* spp., *R. dominica*, *Sitotroga cerealella*) are the main pests of stored paddy, while in the milled rice rodents and birds are of major concern in addition to insect species (*S. oryzae* and *Tribolium castaneum*). Studies revealed losses due to two insects estimate at 3-7% in paddy and 5-14% in milled rice (Rahim-Muda, 1986).

Plate. 5: Wheat is damaged by *R. dominica*.

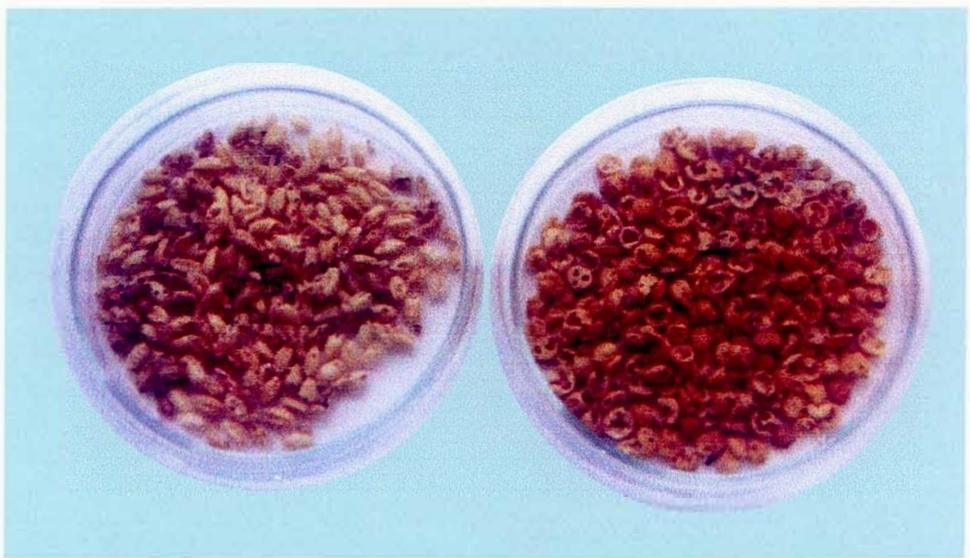


Plate. 6: Cereals infested by *R. dominica*.

Table 2: Insects associated with stored products (Metcalf and Flint, 1962; Alam, 1971; Wilbur and Mills, 1985; Kabir, *et. al.*, 1989; Gorham, 1990).

Species	Common names	Products infested
Lasioderma serricome (F.)	Cigarette beetle/Tobacco beetle	Dried Tobacco, foodstuffs, turmeric
L. testaceum L.	Cheroot beetle	Dried Tobacco, foodstuffs, turmeric
Stegobium paniceum L.	Drug Store beetle	Foodstuffs, stored turmeric, ginger, chili, coriander
Gastrulus indicus L.	Book worm	Printed matter
Rhizopertha dominica (F)	Lesser grain beetle	Rice, wheat, maize, flour
Dinoderus ocellaries (F)	Ghoon beetle	Dried bamboo, furniture, rice, wheat
Prostephanus truncates (Horn)	Larger grain borer	Corn, Soft wheat, dried cassava
Araeocerus fasciculatus (Deg.)	Coffee, bean weevil	Coffee bean seeds
Callosobruchus chinensis L.	Pulse beetle/Oriental cowpea bruchid	Pulses
C. maculates (F)	Spotted cowpea bruchid	Pulses
Bruchus pisorum (L)	Pea weevil	Pulses
Carryon serratus (Oliv.)	Groundnut Borer	Pulses, groundnuts
Acanthoscelides obtectus (Say)	Bean Bruchid	Pulses
Necrobia rufipes (De Geer)	Red-legged ham beetle/copra beetle	Copra, oilseeds, dried fish, rice, wheat, mixed feed
Cryptolestes ferrugineus (Stephens)	Rust-red grain beetle	Grains
C. pussilus (Schonherr)	Flat grain beetle	Grains
Laemophloeus minutus (Oliv.)	Flat grain beetle	Grains
Sitophilus oryzae L.	Rice weevil	Rice, maize, foodstuffs
S. zeamis Mostsch	Maize weevil	Maize, rice, sorghum,mung-bean
Trgoderma granarium Everts	Khapra beetle	Grains, groundnut
Necrobia rufipes (De Geer)	Red-legged ham beetle/copra beetle	Copra, oilseeds, dried fish, rice, wheat, mixed feed
Lophocateres pusillus (Klug)	Siamese grain beetle	Grains, turmeric
Typhaea stercorea L.	Hairy fungus beetle	Maize
Carpophilus dimidiatus F.	Corn sap beetle	Rice, corn, flour
C. hemipterus (L.)	Dried fruit beetle	Dried fruits

Species	Common names	Products infested
Ptinus tectus Boield	Australian spider beetle	Cereal, cereal products and species, often found as scavengers of miscellaneous debris
Oryzaephilus surinamensis (L.)	Saw-toothed beetle	Rice, wheat, peas, flour
O. mercator (Fauvel)	Merchant grain beetle	Wheat, mixed feed
Ahasverus advena (Walt)	Foreign grain beetle	Rice, maize
Paloru subdepressus (Wall)	Depressed four beetle	Grain, flour
P. ratzeburgii (Weissmann)	Small-eyed flour beetle	Cereal products
Alphitobius diaperinus (Panzer)	Lesser mealworm	Rice, wheat
A. laevigatus (F)	Black fungus beetle	Whole grains, wheat bran etc.
Gnathocerus comutus (F)	Broad-horned flour beetle	Grain, flour
G. maxilossus (F)	Slender-horned flour beetle	Grain, flour
Tenebrides mauritanicus L.	Cadelle beetle	Grain, mixed feed
Sitotroga cerealella (Oliv.)	Moth/Angoumois moth	Rice, wheat, maize, flour
Hoffimannophila	Brown house moth	Grain and grain products, cause damage to carpets
Endrosis sarcirtella	White-shouldered house moth	Grain and grain products; damage to carpets
Cadra cautella (Walker)	Almond moth	Grains, dried fruits, almonds
Ephestia (Hub.)	Tobacco moth	Tobacco, dried fruits, cocoa beans
E. (Anagasta) kuehniella (Zell)	Mediterranean flour moth	Flour
Plodia interpuntella (Hubner)	Indian meal moth)	Cereals, pulses dried fruits and fishes
Corcyra cephalonica Staint	Rice moth	Cereals, pulses, dried fruits and fishes
Hypospygia costalis (F.)	Clover Howard	Clover
Pyralis farinalis	Meal snutyl moth	Maize
Doloessa viridis(Zeller)	Green rice moth	Milled rice, maize, sorghum
Tinea pellionella L.	Cloth moth	Woolen-cloth, carpets, skin, feathers
Embidopsocus sp.		Rice, bean
Lipsclelis entomophilus		Rice, maize, mung-bean
L. botrychophillus		Rice, maize, cassava

1.6 Test chemicals

In this investigation four insecticides belonged to the four different groups and one known synergist Piperonyl butoxide (PBO) were used as follow:

Sl.	Commercial Name	Common Name	*Chemical Class*
1.	Dursban 10 EC	Chlorpyrifos	*Organophosphate*
2.	Cythrin 10 EC	Cypermethrin	*Pyrethroid*
3.	Imitaf 20 EC	Imidacloprid	*Chloro/Neonicotinoid*
4.	Sevin 85 SP	Carbaryl	*Organo Carbamet*
5.	*Piperonyl butoxide (PBO)98% technical grade (Chemical Service)*		

Acetone: The solvent has been chosen following the guideline or it is a rather generalist solvent.

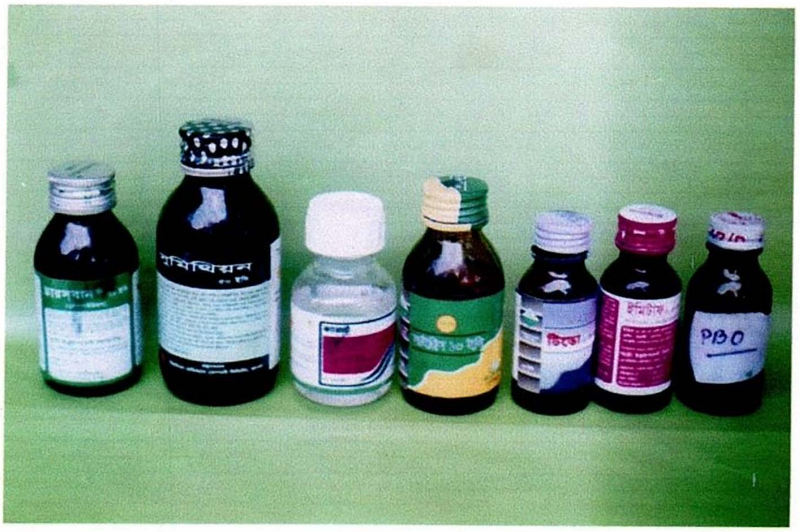


Plate. 7: Tested insecticides and PBO used in the experiment.

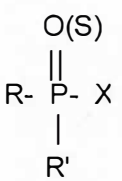
1.6.1. General properties of organophosphorous insecticide

The organophosphate insecticides were developed in Germany during World War II as a substitute for nicotine, an insecticide used against Colorado potato beetle, *Leptinitersa decemlineata*. The discovery of the insecticidal properties of this group was associated with other German studies on related chemicals, the so called 'nerve gases' (sarin, soman and tabun) (Pedigo, 1999). Organophosphorous compounds form an important class of pesticides. More than 1,000 different organophosphorous compounds have been synthesized and evaluated as pesticide of which more than eighty are widely used in agriculture. These compounds are highly toxic to the insects and many of them are non-toxic to vertebrate and do not accumulate in the animal body.

Organophosphates are derived from phosphoric acid and are some of the most toxic insecticides. As opposed to the chlorinated hydrocarbons, they are unstable (in the presence of light) and quickly breakdown into non-toxic compounds. This class of insecticide inhibits the action of several esters splitting enzymes. The organophosphorous insecticides are particularly effective against cholinesterase. This enzyme is widely distributed in the animal kingdom. Adrian *et al.*, (1947) first observed the inhibition properties of organophosphorous esters against cholinesterase. Balls and Jansen (1952) found that the inhibition was attributed to the phosphorylation of the esteratic site, which was initially demonstrated by the action of DFP with chymotrypsin. It is well known that actual thion type insecticides do not inhibit the easterase *in vitro*, but act to increase the efficiency of anti-cholinesterases *in vivo*. Gage (1953) demonstrated that the Cholinesterase inhibition produced from parathion *in vivo* was the oxoanalogue paraoxan. It has been demonstrated that, when paraoxon is presented to chymotrypsin in a dilute solution, the phosphorous of one molecule of the inhibitor becomes locked up in every molecule of the enzyme inhibited and at the same time one free nitrophenate ion appears. The active site of the enzyme evidently splits the molecule of the inhibitor, which is then unable to release the phosphoryl moiety. This causes blockage of the further action, whereas the unchanged enzyme can attach, split and release some thousands of its normal substrate molecules per second.

In animals acetylcholine is believed to be the transmitter in the synapses of the nervous system (Smallman and Mansingh, 1969). Acetylcholine is synthesized in the nerve ending by the action of the cholinacetylase from choline and acetyl co-enzyme-A, which is sterol in synaptic vesicles. These vesicles burst automatically to generate a miniature potential without giving any action to the associated cells. However, by the stimulation of the action current, the burst and release of acetylcholine increases quickly by 100 to 1000 times. This generates synapse or end-plate potential and consequently, the excitation of the post synaptic membrane. The released acetylcholine is then rapidly hydrolised into inactive acetic acid and choline by the action of acetylcholine sterases before the nerve impulse arrives. The original state of the post synaptic vesicle can not reach till the acetylcholine remains in the region of the synaptic cleft. So, acetylcholine inhibition results in the disturbance of the nervous function leading to serve and often lethal damage in the animal body. Generally acetylcholine inhibition interferes with the co-ordination of muscular response in the vital organs with serious symptoms and eventually death.

Organophosphates are characterized as having different alcohols attached to their phosphorous atoms, and the various phosphorous acids produced are termed esters. These esters have different combinations of oxygen, carbon, sulphur and nitrogen, and organophosphates formed from them can be divided into three groups of derivatives: aliphatic, phenyl and heterocyclic. In spite of the enormous structural diversity of organophosphorous insecticides, all the compounds can be represented by the classical hypothetical structure as:

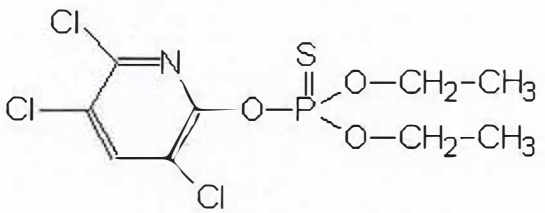


Where R and R′ are short chain alkyl, alkylthio of amide groups and X is labile as ion leaving group.

Chlorpyrifos (Dursban 20EC)

Physical and chemical identity of dursban

Common name	:	Chlorpyrifos.
Commercial name	:	Dursban or Lorsban.
Chemical class	:	Organophosphate.
Chemical name	:	O, O-diethyl O-3, 5, 6-trichloro-2-pyridyl phosphorothioate.
Solubility	:	2 mg/L at 25°C in water, also soluble in acetone and in many organic solvents.
Molecular Weight	:	350.6
Reg No.	:	2912-88-2 (Ap-93)
Produced by	:	Dow Agro Sciences (Indianpolis, Indiana)
Marketing	:	Auto Equipment Ltd. Bangladesh.
Empirical formula	:	$C_9H_{11}Cl_3NO_3PS$
Chemical Structure	:	



Chemical structure of dursban

Chlorpyrifos is a broad – spectrum insecticide commonly known as Dursban or Lorsban, was registered first in 1965 (Odenkirchen and Eisler, 1988) and was first reported in the scientific literature in 1966. It is available in a variety of formulations including granules, wettable powder and emulsifiable concentrate. Chlorpyrifos forms colourless crystals with a mild mercaptan odour; m.p. 42-43.5°C; v.p. 2.5 mPa (25°C). Solubility (25°C): 2 mg/l water; 6.5 gm/kg acetone; 7.9 gm/kg benzene; 6.3 gm/kg chloroform; 450 gm/kg methanol. The rate of hydrolysis in water increases with pH, with temperature, the presence of copper and possibly of other metals that can form chalets. Under laboratory conditions, 50% hydrolysis takes from 1.5 d (water at pH 8 and 25°C) to 100 d (phosphate buffer at pH 7 and 15°C). It is corrosive to copper and brass.

Toxicological effect of chlorpyrifos: Chlorpyrifos have been shown to be detrimental to non-target species including aquatic organisms, waterfowl and terrestrial organisms from surrounding ecosystems (Reinecke and Reinecke, 2007; Yan *et al.,* 2006; Venkateswara, 2006; Mayer, 1987; Tagatz *et al.,* 1982). It has a broad range of insecticidal activity and is effective by contact, ingestion and vapour action, but is not systemic. It has been used for the control of flies, household pests, mosquitoes (larvae and adults) and of various crop pests in soil and on foliage, also used for control of ectoparasites on cattle and sheep. Its volatility is great enough to form insecticide deposits on nearby untreated surfaces. It is non-phytotoxic at insecticidal concentrations. It is degraded in soil, initially to 3, 5, 6-trichloropyridin-2-ol, which is subsequently degraded to organochlorine compounds and carbon dioxide. It persists in soil for 60-120 days (Worthing and Walker, 1987).

It is moderately toxic to mammals and very highly toxic to birds, fish and aquatic invertebrates (US, EPA, 1989). The oral LD_{50} is 95 to 270 mg/kg in rats, 60 mg/kg in mice, 1000 mg/kg in for rabbits and 500 to 504 mg/kg in guinea-pigs. The dermal LD_{50} is greater than 2000 mg/kg in rats and 1000 to 2000 mg/kg in rabbits. The 4 hour inhalation LC_{50} for chlorpyrifos in rats is greater than 0.2 mg/l (Dow El. Co., 1992; Kidd and James, 1991; Gosselin *et al.,* 1984).

Chlorpyrifos has been used commercially in India and Bangladesh for more than a decade particularly to control foliar insects on cotton, paddy fields, pasture and vegetable crops (Rao *et al.,* 2003). Some professional users use chlorpyrifos in food storage areas. However, little is known about the dose responses of lesser grain borer *Rhyzopertha dominica* to this chemical.

1.6.2 . General properties of pyrethroids

Pyrethroids are the fastest developing group of modern insecticides. They are replacing many older insecticides because of their great effectiveness and safety of application. Pyrethriods are not new insecticides; the first, allethrin was developed in 1949 (Pedigo, 1996). Allethrin was synthesized to duplicate the insecticidal activity of a natural product, Cinerin I, a component of the botanical insecticide pyrethrum

Pyrethroids are a class of insecticides of great importance for the protection of man's crops and his health. The large differential toxicity to mammals and insects means that these compounds can be used safely by man to generate very unsafe conditions for insects. Pyrethroids also have very many of the properties required for a deleterious effect on the environment, i.e., ready degradation in soil, virtually zero mobility in soil and rapid metabolism and excretion by animals. No insecticide is perfect but the pyrethroids appear to be more acceptable than many other standard insecticides (Leahey, 1984).

The characteristics of high knockdown, lethal activity, wide spectrum, and good residual activity together with repellent and anti feeding activity of the pyrethroids have enabled them to become widely used for plant protection and public health and vector pest control (Hirano, 1989).

The pyrethroids are active against a wide range of insect pests and have been used on a variety of crops as experimental evidence has shown that they are non- phytotoxic. Their major use in agriculture has been for control of bollworms and leaf worms in cotton, but they have also been successful with lepidopterous pests in fruits and cereals, aphids in cereals and other minor outlets. Further pyrethroids are being introduced for use in soil (Hirano, 1989).

The control of public health pests involves a variety of techniques using pyrethroids, these include, (a) Domestic aerosols/sprays: these generally use powerful knockdown pyrethroids such as tetramethrin, bioallethrin combined compounds providing good kill activity such as remethrin, permethrin and the alpha (α) -cyano pyrethroids (e.g. cypermethrin). (b) Mosquito coils/mats: Bioallethrin gives a 50% knockdown at time of 5.5 minutes. (c) Large scale space sprays: Especially important in urban fly control. Where control of 90% to 100% has been achieved with all pyrethroids tested. (d) Surface treatments: Applied as a residual spray to resting sites has given efficient control. (e) Larvicidal: As pyrethroids are highly effective against mosquitoes, they can be use as surface treatments without toxic effects to fish. (f) Special treatments: pyrethroids have been used for bed nets, clothing impregnation and targets (Carter, 1989).

The symptoms of pyrethroids poisoning are indicative of an attack on the insect nervous system. Insects are not dependent on continuous nervous control of respiration and circulation. Because of this death appears to be due to the irreversible damage to the nervous system occurring when poisoning lasts for more than a few hours.

Many pyrethroids (e.g. Cypermethrin,Lambda-cyhalothrin) are now used in the animal sector as ectoparasiticides and in the household insecticide market, laboratory tests have indicated that pyrethroids are highly toxic to bees. However, in the field this toxicity is diminished by the repellant activity e.g. alpha (α) - cypermethrin. Fluvalinate has been used to control mites in honey bee colonies without adversely affecting the bees (Elliot, 1989).

In 1986 the market share of pyrethroid reached 25% of the total insecticide market for plant protection and can be expected to increase in the future (Hirano, 1989).

Cypermethrin (Cythrin 10EC)

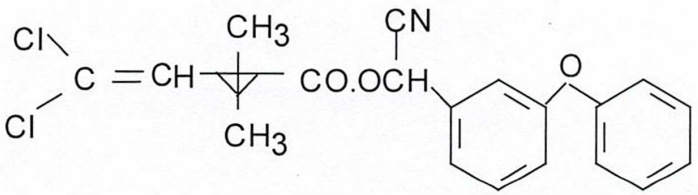
Cypermethrin was launched as a commercial insecticide in 1977. The majority of commercial cypermethrin formulation contains 100g/l of active ingredient. The commercial product is 10% pure (Hill, 1983)

Cypermethrin is a composite pyrethroid; a broad spectrum, non-cumulative insecticide; and, a fast-acting neurotoxin with good contact and stomach action. It is of moderately high toxicity to mammals and readily metabolized with immediate loss of activity. Cypermethrin is not a plant systemic, it is readily degraded on soil or plants but has good residual activity on inert surfaces.

The pure isomers are colourless crystals; the technical material is a viscous yellow-brown semi-solid. The melting points are 60-80°C.

Physical and chemical identity of cythrin

Common name	:	Cypermethrin.
Commercial name	:	Cythrin, Cimbush,Ambush etc.
Chemical class	:	Pyrethroid.
Chemical name	:	(R,S)-alpha-Cyano-3-phenoxybenzyl-2,2-dimethyl (1R,1S)-cis,trans-3-(2,2-dichlorovinyl) cyclopropane-carboxylate
Solubility	:	Soluble in water 9 ug/liter. Soluble in other solvents eg. methanol, acetone, xylene, methylene dichloride
Molecular Weight	:	416.32
Reg No.	:	52315-07-8
Produced by	:	Dow Agro Sciences (Indianpolis, Indiana)
Marketing	:	ACI Bangladesh limited.
Empirical formula	:	$C_{22}H_{19}Cl_2NO_3$
Chemical Structure	:	



Chemical structure of cythrin

Toxicological effect of cypermethrin: Cypermethrin is a synthetic pyrethroid insecticide used to control many pests, including moth pests of cotton, fruit and vegetable crops. It is also used for crack, crevice and spot treatment for control of insect pests in stores, warehouses, industrial buildings, houses, apartment buildings, greenhouses, laboratories and on ships, railcars, buses, trucks and aircraft. It may also be used in non-food areas in schools, nursing homes, hospitals, restaurants, hotels, and in food processing plants and as a barrier treatment insect repellent for horses. Cypermethrin is available in emulsifiable concentrate, ULV, and wettable powder formulations. Technical cypermethrin is a mixture of eight different isomers, each of which may have its own chemical and biological properties.

Cypermethrin is a moderately toxic material by dermal absorption or ingestion. It may cause irritation to the skin and eyes. Symptoms of dermal exposure include numbness, tingling and itching, burning sensation, loss of bladder control, in coordination, seizures and possible death. Pyrethroids may adversely affect the central nervous system. Human volunteers given dermal doses of 130 $ugcm^{-2}$ on the earlobe experienced local tingling and burning sensations. One man died after eating a meal cooked in a 10% cypermethrin concentrate that was mistakenly used for cooking oil. Shortly after the meal, the victim experienced nausea, prolonged vomiting, stomach pains, and diarrhea which progressed to convulsions, unconsciousness and coma. Other family members exhibited milder symptoms and survived after hospital treatment. Rats fed high doses of 37.5 mg/kg of the cis-isomer of cypermethrin for 5 weeks exhibited severe motor in coordination, while 20-30% of rats fed 85 mg/kg died 4 to 17 days after treatment began. Cypermethrin is not a skin or eye irritant, but it may cause allergic skin reactions.

Long-term exposure to cypermethrin may cause liver changes. Pathological changes in the cortex of the thymus, liver, adrenal glands, lungs and skin were observed in rabbits repeatedly fed cypermethrin.

In humans, urinary excretion of cypermethrin metabolites was complete 48 hours after the last of 5 daily doses of 1.5 mg. Studies in rats have shown that cypermethrin is rapidly metabolized by hydroxylation and cleavage, with over 99% being eliminated within hours. The remaining 1% becomes sequestered in body fat. This portion is eliminated slowly, with a half-life of 18 days for the cis-isomer and 3.4 days for the trans-isomer.

1.6.3. General properties of neonicotinoid insecticides

The neonicotinoides introduced to the market in the early 1990s (Nauen and Bretschneider, 2002). These synthetic compounds evolved from the naturally occurring insecticide 'nicotine' the primary alkaloid in tobacco. So the insecticides are related to nicotine in their structure and action at the same

nicotinic acetylcholine receptors (nAChRs) target site (Tomizawa and Casida, 2003). Nicotine was discovered in 1970 by Shell Development Company in California and is still used as a minor insecticide (Soloway *et al.*, 1979).

Many of the remaining gaps in pest control capabilities were filled recently by the neonicotinoids replacing the pyrethroids, chlorinated hydrocarbons, organophosphates (Ops) and methylcarbamates, which have decreased effectiveness because of resistance or increased restrictions due to toxicological considerations (Kagabu, 2003 ; Nauen *et al.,* 2001).

Neurophysiologic studies have confirmed that the nicotine, al neonicotinoids act as agonists at same target site to interfere with normal nerve impulse transmission by binding to post synaptic nicotinergic acetylcholine receptors (nAChR) (Tomizawa and Casida, 2003; Nauen *et al.,* 2001; Zhang *et al.,* 2000; Wollweber and Tietjen, 1999; Chao *et al.,* 1997; Bai *et al.,* 1991). Unlike nicotine however, they show marked selectivity within the insecta as foliar or systematic treatments (Prabhaker *et al.,* 2005).

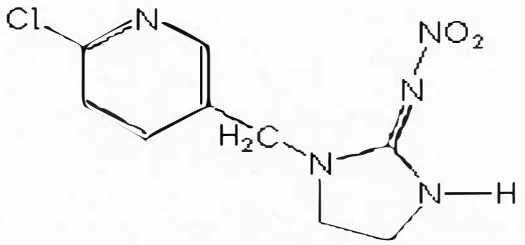
This new group of chemicals has brought diversity to the insecticide arswenal available for both pest and resistance management and has relieved intensive pressure on older conventional chemicals which nicotinoids are now supplanting for insect control on many major crops (Denholm *et al.,* 2002).

Imidacloprid (Imitaf 20 SL)

The first commercially available insecticide from nicotinoid class is imidacloprid. It introduced to market since 1991 and got fully registered by the US Environmental Protection Agency on March 21, 1994 (Elbert *et al.,* 1998). Commercial formulation with imidacloprid as the active ingredient such as Gaucho, Admire, Confidor, Advantage, Merit, Provado, Imicide, Imosol, Imituf, Vision and Premier are available as dustable powder, granular formulations seed dressing (flow able slurry concentrate), soluble concentrate, suspension concentrate and wettable powder (Wang *et al.,* 2005; Meister, 1994).

Physical and chemical identity of imitaf:

Common name	:	Imidacloprid
Commercial name	:	Imitaf, 20SL (flow able slurry concentrate).
Chemical class	:	Chloronicotinyl or Neonicotinoid.
Chemical name	:	1-[(6-chloro-3-pyridinyl) methyl]-N-nitro-2-imidazolidinimine.
Solubility	:	0.51 g/l at 20°C in water; 50.0 - 100.0 g/l in dichloromethane; 1.0-2.0 g/l in isopropanol; 0.5-1.0 g/l in toluene; <0.1 g/l in n-hexane ; 0.061 g/100g in fat
Molecular Weight	:	255.7
Reg No.	:	13826-41-3 (Ap-449)
Produced by	:	Rallis Indian Ltd.
Marketing	:	Auto Equipment Ltd. Bangladesh.
Empirical formula	:	$C_9H_{10}ClN_5O_2$
Chemical Structure	:	



Chemical structure of imitaf

Mode of action: Acetylcholine (Ach) is the endogenous agonist and excitatory neurotransmitter of the cholinergic nervous system. Neurotransmission through the nicotinic cholinergic synapse is mediated in two steps. Firstly, Ach is released form the pre-synaptic membrane by exocytose and interacts with the binding site located at the extra cellular domain of the nAChR/ion channel complex. Secondly, a conformational changes of the receptor molecule leads to opening the ion channel, promoting the influx of extracellular NaC and efflux of intracellular KC to disrupt the equilibrium status of the membrane potential.

In insects, the nAChR is widely and predominantly distributed in the neuropil regions of the central nervous systems (CNS). It is not only responsible for rapid neurotransmission but it is also an important target for insecticide action.

Imidacloprid possesses a unique combination of characteristics viz. a novel mode of action, excellent systematic and contact activity, a wide variety of application methods, low application rates, long residual control, strong binding to soil organic matter and favorable toxicological and environmental profiles. These characteristics make it one of the most widely used insecticides Worldwide (Cox *et al.,* 1998; Kagabu, 1997; Elbert *et al.,* 1996).

The insecticide is a neuroactive chemical causes the blockage in a type of neuronal pathway (nicotinergic) (Ware, 2000) that is more abundant in insects than in warm blooded animals. This blockage leads to the accumulation of acetylcholine, resulting in the insect's paralysis and eventually death (Kidd and James, 1991). Imidacloprid has been registered in 56 countries for foliar and soil application as well as seed treatment (Wang *et al.,* 2005) especially active on aphids, hoppers, thrips, whiteflies, termites, turf insects, soil insects and some species of chewing insects (Kanrar *et al.,* 2006; Kumar *et al.,* 2002; Tomlin, 2000). It is most commonly used in rice, cereal, maize, potatoes, sugar beets, fruits, hops, cotton, tobacco, vegetables and turf (Leal, 2001); in indoor and outdoor cockroach control (Pospischil *et al.,*1999), termite control (Bayer Corporation, 2000) and for the treatment of cats and dogs against fleas (Schenker *et al.,* 2003).

Extensive surveys have shown that resistance to imidacloprid is still restricted to very few species and often very localized in extent, though exceptional cases of resistance and cross resistance have been confirmed in some populations whitefly (*Bemisia tabaci*) and the Colorado potato beetle (*Leptinotarsa decemlineata*) (Alyokhin *et al.,* 2007; Mota-Sanchez *et al.,* 2006; Nauen and Denholm, 2005; Foster *et al.,* 2003).

Toxicological effects of imidacloprid: The imidacloprid active ingredient is considered by the World Health Organisation to be moderately toxic. In laboratory animals, symptoms of acute (short term) oral exposure to imidacloprid included apathy and laboured breathing which lasted for five days. Imidacloprid is moderately toxic to vertebrates.

Imidacloprid has a wide range of uses – soil, seed and foliar. It is used to control sucking insects such as rice-, leaf- and plant hoppers, aphids, thrips and whitefly. It is also effective against soil insects, termites and some species of biting insects, such as rice water weevil and Colorado beetle but has no effect on nematodes or spider mites. It can be used as seed dressing, as soil treatment and as foliar treatment in different crops including rice, cotton, cereals, maize, sugar beet, potatoes, vegetables, citrus fruit, apples and pears, and stone fruit. In European countries such as France, UK, and Holland, imidacloprid is widely used as an insecticide in sugar beet crops. Imidacloprid can be phytotoxic (toxic to plants) if not used according to manufacturers instructions, and it has a tendency to reduce seedling emergence and crop vigour. (U.S. National Library of Medicine, 1995)

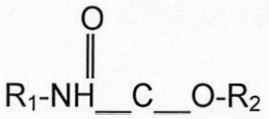
Imidacloprid has been shown to be more toxic to aquatic invertebrates than vertebrates (Overmyer *et al.,* 2005). It is also highly toxic to bees if used as a foliar application (Suchail *et al.,* 2001).

However, imidacloprid has been proved remarkably resilient to resistance and cases that have been reported are still manageable and/or geographically located (Nauen *et al.,* 2008; Denholm *et al.,* 2002; Zhao *et al.,* 2000; Elbert *et al.,* 1996). The existence of strong resistance in some species such as the cotton whitefly (*Bemisia tabacai*) and Colorado potato beetle (*Leptinotarsa decemlineata)* have nonetheless demonstrated the potential of pests to adapt and resist field application of imidacloprid (Alyokhin *et al.,* 2007; Mota-Sanchez *et, al.,* 2006; Prabhaker *et al.,* 2005; Nauen and Denholm, 2005).

The use of synthetic pesticides is currently the most widely adopted method for grain protection but little is known about the dose responses of *Alphitobius diaperinus* to this chemical. Hence the chemical has undertaken in the investigation to explore its performance against the test organism *A. diaperinus.* Imidacloprid is a widely used insecticide with relatively low human toxicity. It has raised concerns because of its possible impact on bee populations, ability to cause eggshell thinning in birds, and reduced egg production and hatching success.

1.6.4. General properties of organo carbamet insecticides

The carbamates or urethanes are a group of salts or esters of nitrogen (N) substituted carbamic acid. They differ structurally from organophosphorus esters sharing a common functional group with the general structutre as-



Where R_1 and R_2 are alkyl or aryl groups.

These compounds have been used as agricultural and household insecticides replacing the more persistent and hazardous organochlorine insecticides. Aldicarb, carbaryl, propoxur and carbofuran are the most commonly used insecticides of carbamate class (Fleischli *et. al.,* 2004; Yang *et al.,* 2000; Hill, 1995; Osteen, 1993). Like organophosphorus, carbamate insecticides also inhibit acetylcholinesterase enzymes and disrupt nerve transmission in vertebrate and in invertebrate species alike. Carbamate compounds bind to the enzyme that is normally responsible for breaking down ACh after it has carried its message across the synapse. When an insect has been poisoned by a cholinesterase inhibitor, the cholinesterase is not available to help break down the ACh and the neurotransmitter continues to cause the neuron to 'fire' or send its electrical charge. This causes over stimulation of the nervous system and the insect dies. Like insects, humans also use ACh as a neurotransmitter and cholinesterase to break it down. Cholinesterase poisoning in humans can be very severe.

Carbamates though react with cholinesterase in a way precisely analogous to the reactions of organophosphates and acetylcholine but the binding is weaker and less stable. In all cases there is an enzyme-complex formation with cholinesterase which is spontaneously hydrolyzed. Thus it is labile, reversible and has short duration with spontaneous hydrolysis occurring within several hours (Clark 2002; Yang *et at.,* 2000).

Carbaryl is a neurotoxic carbamate insecticide. In humans, acute effects of carbaryl exposure include headaches, nausea, incoordination, and difficulty breathing. Carbaryl can cause a variety of behavioral effects, some of which are relatively long-term. It also suppresses several functions of the immune system. Men exposed to carbaryl have more abnormal sperm and lower sperm counts than unexposed men. In female laboratory animals, exposure to carbaryl has caused a variety of reproductive problems, including birth defects in beagle dogs and increased rate of miscarriages in monkeys. Exposure to carbaryl has been associated with a higher incidence of the cancer non-Hodgkin's lymphoma in farmers and brain cancer in children. Nitrosocarbaryl, formed when carbaryl and nitrites react, is a potent carcinogen. Both carbaryl and nitrosocarbaryl cause genetic damage in some test systems, as does carbaryl's primary breakdown product, 1-naphthol.

Sevin 85 SP (Carbaryl)

Sevin is the trade name for a widely used synthetic insecticide containing the active ingredient carbaryl. Carbaryl belongs to the chemical class called carbamates. As insecticides go Sevin is only moderately toxic to mammals and is still widely used in gardens and landscapes. It is, however, highly toxic to honey bees and many other beneficial insects and mites.

Sevin is sold as a powder (dust), granule, and liquid concentrate. Uses include vegetable gardens, landscape plants, lawns, tree fruits and small fruits and the target pest list is broad and includes many common insect and mite pests.

While Sevin insecticide can be used safely we believe there are now safer alternatives that also avoid the harmful effects that broad spectrum chemicals, like carbaryl, can have on beneficial species.

Sevin® is a trademark for the insecticide commonly called carbaryl. One chemical name for the substance is 1-napthyl N-methylcarbamate. The "napthyl" portion of the name refers to its substituted napthalene ring. One

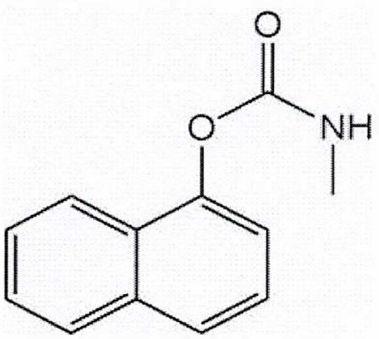
means of manufacturing the pesticide is to react methyl isocyanate with 1-napthol. This is the reaction that so tragically caused thousands of deaths the notorious industrial accident at Bhopal, India, in 1984.[1] It was not the carbaryl, itself, that caused the disaster, but the methyl isocyanate vapors that leaked into the atmosphere outside the plant.

While horrible, the Bhopal disaster does not constitute evidence the pesticide is ineffective and should not be used. To illustrate, the electricity that illuminates our home also has been responsible for many electrocution deaths.

Carbaryl is a wide-spectrum carbamate insecticide which controls over 100 species of insects on citrus, fruit, cotton, forests, lawns, nuts, ornamentals, shade trees, and other crops, as well as on poultry, livestock and pets. It is also used as a molluscicide and an acaricide. Carbaryl works whether it is ingested into the stomach of the pest or absorbed through direct contact. The chemical name for carbaryl is 1- naphthol N-methylcarbamate. Carbaryl is formulated as a solid which varies from colorless to white to gray, depending on the purity of the compound. The crystals are odorless. This chemical is stable to heat, light and acids under storage conditions. It is non-corrosive to metals, packaging materials, or application equipment. It is found in all types of formulations including baits, dusts, wettable powder, granules, oil, molassas, aqueous dispersions and suspensions.

Physical and chemical identity of Sevin

Common name	:	Carbaryl
Commercial name	:	Carbamine, Denapon, Dicarbam, Hexavin, Karbaspray, Ravyon, Septene, Sevin, Tercyl etc.
Chemical class	:	Carbamet
Chemical name	:	Carbamic acid, methyl-, 1-naphthyl ester, Methylcarbamic acid-1-naphthyl ester, 1-Naphthyl methylcarbamate.
Molecular Weight	:	201.24
Reg No.	:	FC5950000
Empirical formula	:	$C_{12}H_{11}NO_2$
Chemical Structure	:	



Chemical structure of Sevin

Mode of Action

Carbaryl is a carbamate insecticide. Like all members of this chemical family, it inhibits the action of an enzyme that is an essential component of insect, fish, bird, and mammal nervous systems. The enzyme, acetyl cholinesterase (AChE), controls the chemical reaction that transforms acetylcholine into choline after acetylcholine has been used to transmit nerve impulses across the junctions between nerves. Without functioning AChE, acetylcholine accumulates and prevents the smooth transmission of nerve impulses. This

causes loss of normal muscle control, and ultimately death. The AChE inhibition is said to be reversible because the carbaryl disassociates from the AChE within several hours. This happens even if death has already occurred. Insecticides in the organophosphate family (malathion and diazinon, for example) also inhibit AChE, but the inhibition is not as readily reversible.

Carbaryl can also affect a number of other enzyme systems in living things. For example, the carboxylesterases (detoxification enzymes), lactic dehydrogenase (enzymes that utilize sugar), and serine esterases (enzymes important to the function of certain immune system components) are all inhibited by carbaryl.

Toxicological effects of Carbaryl: Symptoms of acute carbaryl exposure in humans are malaise, muscle weakness, dizziness, sweating, headache, salivation, nausea, diarrhea, incoordination, and slurred speech. Depression of breathing ability combined with an excess of fluid in the lungs (pulmonary edema) is the usual cause of death when exposure is high.

Carbaryl's acute oral LD_{50} (the dose that causes death in 50 percent of a population of test animals) in rats is 255 milligrams per kilogram (mg/kg) of body weight.5 Extrapolated to the weight of an average 70 kilogram (154 pound) human who is assumed to be as sensitive to carbaryl as are rats, this means that a dose of about 18 grams or two-thirds of an ounce would be fatal.

Lower doses of carbaryl over a longer period of time cause a variety of adverse effects. In humans, ingestion of 0.13 mg/kg/day (less than a thousandth of the LD_{50}) caused abdominal cramps and a decrease in the ability of the kidneys to resorb amino acids. In rats, decreases in weight and body temperature occurred following single injections of doses of less than one-twentieth of the LD_{50}. Similar doses given for two years caused kidney abnormalities in rats as well as dogs. In addition, drinking water contaminated with 10 parts per million (ppm) of carbaryl caused liver pathologies and reduced blood clotting activity in rats and single sublethal doses in rabbits reduced their heart rate and caused changes in their electrocardiograms.

Humans are exposed to carbaryl through consuming contaminated food and water, using carbaryl in homes, gardens, and offices, through drift, and through occupational exposure. Carbaryl is the tenth most commonly detected pesticide in U.S. food residues surveys. It has been found in groundwater, surface water, and fog. Almost 60 million applications of carbaryl-containing insecticides are made annually in homes and gardens. Workers in carbaryl manufacturing facilities, agricultural workers, and pet handlers are all occupationally exposed to carbaryl and have suffered adverse effects, including sperm abnormalities, AChE inhibition, diarrhea, and coughing. Carbaryl is well-absorbed by skin, particularly skin of young animals. Protective clothing can be difficult to effectively launder and transmits more carbaryl under hot, sweaty conditions.

A wide variety of nontarget animals, plants, and microorganisms are affected by carbaryl exposure. The number of sublethal effects that occur at low exposures is particularly striking. Beneficial arthropods, fish, birds, a variety of crop plants, and nitrogen-fixing microorganisms are all affected by carbaryl. Only one ecosystem study has been done with carbaryl, but it indicated that the effects on individual species result in persistent effects on ecosystems.

1.6.5. General properties of piperonyl butoxide (PBO)

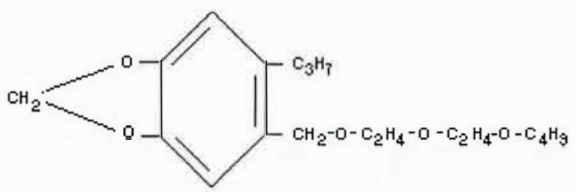
piperonyl-butoxide is the first effective and commercially viable synergist to be developed in 1947 using naturally occurring safrole as the key raw ingredient (Wachs,1947). The chemical has been used in conjunction with household insecticides such as pyrethrins, pyrethroids, organophosphates, rotenone and carbamets to increase the insecticidal activity (Fakoorziba *et al.,* 2009; Rahman *et al.,* 2007; Young *et al.,* 2006; El-Merhibi *et al.,* 2004; Huang and Subramanyam, 2003; Kumar *et al.,* 2002; Zhao *et al.,* 2000; Tozzi, 1998; Knowles, 1991).

Piperonyl-butoxide is a yellowish pale brown oily liquid particularly insoluble in water. Its boiling points is 180°C at 1 mm Hg and miscible with most organic solvent. The technical grade is 90-92% pure and grade comprises ≥ 85% m/m

piperonyl - butoxide and ≤ 15% m/m related compounds. It is stable to light and resistant to hydrolysis. Its molecular weight is 1 mm Hg (Metcalf and Flint, 1962). It is non corrosive, changes colour with faint characteristic odor when put in an iron container. It is stable to light under normal atmospheric conditions and very stable hydrolytic influences (Anon, 1993).

Physical and chemical identity of Piperonyl- buoxide:

Common name	:	Piperonyl- buoxide
Chemical name	:	3, 4-methylenedioxy-6-propylbenxyl n-butyl diethylene glycol ether.
Other name	:	1,3-Benzodioxole, 5-[[2-(2-butoxyethoxy)ethoxy] methyl]-6-propyl; Toluene, α-[2-(2-butoxy etho xy) ethoxy]-4,5-(methyl lenedioxy)-2-propyl- butacide; Butocide; Butyl carbitol 6-propylpiperonyl ether; ENT 14,250; Pyrenone 606; 6-Propylpiperonyl butyl diethylene glycol ether.
Solubility	:	Soluble in water.
Molecular Weight	:	356.4538
Reg No.	:	51-03-6
Empirical formula	:	$C_{19}H_{30}O_5$
Chemical Structure	:	



Chemical structure of piperonyl butoxide

Piperonyl-butoxide (PBO) is short lived in the environment and has a low to moderate potential to contaminate groundwater if it used properly (Arnold, 1998). It is plus product in protecting the environment from the unnecessary build up of the insecticide residue (Anon, 1993). PBO is moderately toxic to fish and to low in toxicity to birds (Osmitz and Hobson, 1998).

PBO is considered minimally toxic and is not thought to cause significant symptoms in most people following short term oral or skin exposures. In two different studies, human volunteers were given oral and dermal doses of PBO. After monitoring the volunteers for 3 days, researchers found no evidence of toxicity or changes in normal metabolic functions. However, sensitive individuals may experience skin irritation and moderate gastro-intestinal symptoms if they are exposed to PBO. It is low to very low in toxicity to mammals when eaten or inhaled or absorbed by skin (Moretto, 1995; Breathnach, 1988).

Piperonyl-butoxide has varied group of crop tolerance. The synergistic effect of PBO in particular on pyrethroids is well known and the subject has been reviewed by Sawicki (1962, Hewlett (1960) and Metcalf (1955). The effect of piperonyl-butoxide as synergist on malathion and its analogues (Ware and Roan, 1958; Rai *et al.,* 1956; March *et al.,* 1952) carbamates (Moorefield, 1958) and some other organophosphorous insecticides have also been studied.

With the advent of the house flies showing a pronounced resistance to DDT, a search for synergist for this type of compound was made by March *et al.,* (1952). Rai *et al.,* (1956) initiated an investigation on the possible action of piperonyl-butoxide and other organic phosphorus compounds such and diazinon, Bayer L, 13/59 and malathion on two strains of house flies. He observed a significant synergistic effect with diazinon and Bayer L, 13/59 but a pronounced antagonism with malathion.

When piperonyl-butoxide was newly introduced its factor of synergism with pyrethrin was known only when used against the housefly. Dove (1947) first performed experiments with houseflies with maximum 1 part pyrethrins in 10 part of piperonyl-butoxide. The ratio was used in later works with insects and proved to be the most useful both in efficacy and cost. There are four sets of circumstances which affects the amount of synergism displayed by a mixture namely; (1) the manner in which the insecticide reaches the insect (2) the way in which the insect reaches the insect varies with the technique of the tests.

These can be grouped roughly into three categories viz. (a) tropical application, contact sprays and aerosols (b) exposure to deposits of insecticide and finally (c) exposure to insecticidal dusts (3) the ratio in which the two substances are present and (4) the nature of the constituents particularly when powder products are formulated (Dove, 1947).

Resmethrin and bioresmethrin synergizede with piperonyl-butoxide have been tested in Australia as protectants of wheat (Ardley, 1976; Ardley and Desmarchelier, 1978). Residues from application rates of both pyrethroids killed all introduced lesser grain borer. Sawicki (1962) and Hewlett, (1961) recorded some factors of synergism for house flies treated tropically with solution of pyret hrins and piperonyl-butoxide in refined kerosene. Burnett (1961) recorded low factors of synergism with young adult flies (*Glossina morsitans* West) tropically applied with kerosene solution of pyrethrins and piperonyl-butoxide Synergism of carbamates by piperonyl-butoxide against larvae of Culex pipiens fatigans Wide. was also observed by Georghiou and Matcalf (1967). Increased toxicity of sevin to house fly *Musca domestica* L. was also observed when combined with piperonyl-butoxide (Moorefield, 1958).

Synergism was exhibited in mixtures of piperonyl butoxide and allethrins (Jones, *et al.,* 1952), piperonyl butoxide and pyrethrins (Wachs, 1947) against houseflies. It is reported that commonly known synergists of pyrethrum, including piperonyl butoxide piperonylcyclone, sesamin, MkG_{264} and n-propyl isom also synergise the synthetic pyrethroids, allethrin and cyclethrins.

Metcalf (1967) reported that synergistc action of piperonyl butoxide when it was combined with a number of alkoxyphenyl N-methylcarbamates and deltamethrin against *Rhyzopertha dominica* and some other stored product pests. In combination with fenitothrion, phenopthrin, permithrin piperonyl butoxide gave virtually complete protection of *T. castaneum, T. confusum* and *Sitophilus zeamais* during 1 month storage. Cyfluthrin plus piperonyl butoxide was effective as synergist against some stored product insects (Arthur, 1994; Pospischil and Smith, 1994; Collins, 1990; Bengston *et al.,* 1987).

Besides, synergistic activity of piperonyl butoxide in combination with some commercially available pesticides has been studied against several pest species by a number of investigators as shown in table 1.

1.7. Penetration and distribution of insecticides

Penetration through the integument is of great importance because of its possible influence on the selective action of insecticides as between insect and mammals (Wilkinson, 1976). An insecticide applied to insect cuticle comes first into contact with the outer layer of epicuticular wax which overlies more polar layers consisting largely of chitin and tanned protein. The mechanism of penetration of insecticides through insect cuticle is controversial. However, according to Moriarty (1969), on entry via the cuticle the insecticide passes into the haemolymph, which rapidly transports it through the insect body. As it spreads it may be sorbed on to proteins dissolved and stored in lipid rich tissues, activated or detoxified. Insecticides may also move laterally in the integument and reach the target site via the tracheal system (Welling *et al.,* 1971)

1.8. Insect resistance to insecticides

Resistance is the ability of an insect to survive a dose of insecticide that would be lethal to members of a normal population (Muggleton, 1984). Those that show reduced susceptibility are more likely to survive an exposure to the insecticide and are hence more fit than the others in the same population. Such insects pass on their reduced susceptibility to their offspring and within a few generations, true resistance may develop.

Melander (1984) was first to report the appearance of resistance in insects to insecticides. Insect resistance began to receive scientific attention following the introduction of DDT after World War II, when resistant strains of the house fly, *Musca domestica* L., appeared almost simultaneously in Sweden and Denmark in 1946, mosquitoes (*Culex pipiens* L. in Italy, *Aedes sollicitans* Walker in Florida) the bed bug (*Climex leuctularius* L.) in Hawaii in 1947, and human body louse (*Pediculus humanus humanus* L.) in Korea and Japan in 1951 (Brown and Pal, 1971).

Most early examples of insecticide resistance were found in insect vectors of human diseases because of the widespread use of DDT, lindens and dieldrin in vector control program. However, by 1970 insecticide resistance was demonstrated in 118 pests of crop, forest and stored products as compared with 166 pests of humans or animals (Brown and Pal, 1971) and by 1980, resistance was established in 260 agricultural pests compared to 168 pest of humans and animals (Georghiou, 1986).

Insecticides are indiscriminately used to suppress insect populations in stored grains (Storey *et al.,* 1984) increased reliance on insecticides or stored grain has lead to development of resistance in adults of several species of beetles and moths (Subramanyam *et al.,* 1989; Beeman *et al.,* 1982; Zettler, 1982; Haliscak and Beeman, 1983; Arthur *et al.,* 1988; Summer *et al.,* 1988; Binns, 1986; Bondnaryk *et al.,* 1984) Resistance to insects to contact insecticides is now a widespread occurrence and is increasing globally (Zettler and Cuperus, 1990; Prickett *et al.,* 1990; Collins, 1990; Taylor, 1989; Navarro *et al.,* 1986)

According to Georghiou and Taylor (1977), the number of species of insects and acarines in which resistant strains have been reported has increased from 1 in 1908 to 364 in 1975. Resistance to one or more pesticides has been reported in at least 477 species of insects and mites (Geoghiou and Mellon, 1983). Budiansky (1986) reported that the number of insects which exhibited resistance to pesticides was 7 in 1935 and had swelled to 462 in 1986 all over the world. The total number of species in which resistant strains appeared by the end of 1989 has reached 504, of which 481 are injurious and 23 beneficial of the 481 injurious species, 283(58.8%) are of agriculturally importance and 198(41.2%) of medical or veterinary importance (Georghiou, 1990). Major factors contributing to resistance to insecticides may be behavioural, physiological or biochemical which are summerized in Table 1 (Hodgson and Motoyama, 1984).

Table 3: Causes of resistance development to insecticides in insect.

Category	Examples of sites/ Activity
A. BEHAVIOURAL	Resting behaviour olfactory behaviour
B. PHYSIOLOGICAL	
1. Reduced penetration	Cuticle
2. Increased storage	Adipose tissues
3. Reduced nervous sensitivity	Impulse transmission & Knockdown
C. BIOCHEMICAL	
1. Reduction in Sensitivity	Cholinesterase
2. Receptor by pass	HCN in scale insect
3. Increase in non-oxidative enzymes	Glutothione transferase & esterase
4. Increase in oxidative enzymes	Cytochrome p-450 mono-oxygenase.

Georghiou (1983) and Pluthers and Therlkeld (1981) have referenced to the importance of physiological factors. Melander (1914), however, emphasized that insecticide resistance are often associated with an enhanced enzymatic detoxifying mechanism.

1.9. An introduction to synergism

The term 'synergism' has been derived from the Greek word "synergos" means working together. It describes the special case of joint action where one of the ingredients is nontoxic at the dose used, but has the effect of increasing potency of the other component of the mixture so that their combined effect is greater than the amount of their individual effects.

Busvine (1971) defined a 'Synergist' as a compound that is not toxic to the target organism on its own but increases toxicity when applied with another toxic compound, while Palpp (1979) defined 'Synergist' as a compound that increases toxicity when mixed with a toxic compound whether it is toxic or not.

Olkowski *et al.*, (1991) defined synergism or synergistic effect as the action of two different effects acting together to create a greater effect than the sum of the actions produced by each acting independently.

Ferdous (1995) stated that when certain compounds are jointly administered to living organisms, the potency of combination is greater than might be expected form the potencies of the component if administered separately. The phenomena concerned arte included in the term of synergism and potentiation.

The first systematic discussion of the joint action of insecticides was made by Bliss (1939), who distinguished different types of joint toxic action. Independently joint action refers to poisons applied at the same time but acting quite independently on different physiological mechanisms. Similar joint actions, refers to poisonous action at the same physiological site, in such a way that one may be substituted for the other at a certain proportion. Bliss (1939) was largely responsible for introducing the statistical calculation of independent action and synergism. The mixture of several components produces synergism when the lethal effect of the mixture is greater than the sum of the lethal effects of each component considered separately.

Plackett and Hewlett (1952) discussed the joint action more thoroughly and introduced a new classification as well as new methods of calculation. Horsefall (1934) suggests the use of mixture of the chemicals on various proportions; a system used by Le Pelley and Sullivan (1936) and expended by Diamond and Horsefall (1955). Dove (1947) demonstrated that the efficacy of pyrethrum could be increased by mixing it with piperonyl butoxide (PBO). Hewlett (1960) reviewed the joint action of insecticides. Sun and Johnson (1960a) studied the synergistic and antagonistic actions of insecticide-synergist combination and there mode of action.

Piperonyl butoxide (PBO) was the first commercially viable synergist to be developed (Wachs, 1947). Since then many compounds were developed but

few remained. Nowadays best known synergists are sesamex, MGK_{264}, SKF_{525}, S, S, S-tributylphosphorotrithioate (DEF), diethylmaleate (DEM), triphenyl phosphate (TPP) etc (Sacher *et al.,* 1968). The methylenedioxyphenyl (MDP) compounds such as sesamex, sesamine, sesamoline, sulfoxide and propyl isome are also the most important insecticide synergists (Pap *et al.,* 2001).

Synergists have practical importance in three respects (MetCalf, 1967).

- ❖ In the more economical or efficient control of insects by a mixture.
- ❖ In increasing the spectrum of activity of an insecticide
- ❖ In restoring the activity of an insecticide against resistant strains of insect.

Benz (1971) proposed a quantitatively based definition for the classification of synergism as follow;

1. **Independent synergism** (Independent action with zero correlation): Here two compounds act independently and do not interfere each other.
2. **Sub-additive synergism:** This is the system of two components which together produce an effect greater than the independent effect but less than the algebraic sum of the two single effects. A weak potentiating effect is necessary to produce such a result.
3. **Suplimental synergism:** Here two effective components together produce an effect greater that their algebric sum.
4. **Potentiating synergism:** This type of synergism has a component C which cause the effect Mc and synergist (s) which alone cause no effect, i. e. Ms = 0, but which in combination produce an effect which is significantly greater than Mc. This is found when sub lethal doses of a chemical insecticide are mixed with a pathogenic microorganism.
5. **Collective synergism:** In this system each of the two components alone causes no measurable effect but together produce a significant effect.

6. **Temporal synergism:** This is said to occur if two components applied jointly kill insects quicker than either component separately. This system may be incorporated in economic synergism where two components together reduce damage more than each component alone.

Antagonism: Antagonism is the opposite of synergism. It is the situation where the combined effect of two or more compounds is less toxic than the individual effects. For example: 4+6<10. Antagonistic effects are the basis of many antidotes for poisonings or for medical treatments. For example: ethyl alcohol (ethanol) can antagonize the toxic effects of methyl alcohol (methanol) by displacing it from the enzyme that oxidizes the methanol.

In comparison, a synergistic effect is the situation where the combined effect of two chemicals is much greater than sum of the effects of each agent given alone. For example: 2+2>4 (may be 10 times or more).

Modes of action: Mechanism of synergism in insecticides has been much investigated (Hewlett, 1968). Many details remain to be worked out. However, according to Veldstra (1956) synergistic action is caused by the blocking of "site of loss" (a mechanism by which an active substance can be lost before it reaches to the effective receptor). If two insecticides (similar in chemical composition and action) differ in their toxicity at the site of action, this difference will not necessarily be reflected in their related toxicities unless they are both equally affected by the "site of loss". The substance with lower toxicity than the other at the site of action may show a greater toxicity in tests if less is lost before it reaches the sites of action. Blocking "site of loss" by synergists will allow the insecticides to reach the site of action and show their "true" relative toxicities.

According to Sun and Johnson (1960b) the synergistic effect of sesamex and related compounds was due to the inhibition of biological oxidation. The synergism of certain group of compounds may be inhibition of other enzymatic detoxification. In the absence of synergist the insect is able to metabolize

some or most of the insecticide to non-toxic compounds or to compounds less insecticidal than the insecticide. The synergist reduces this metabolism and thus a greater portion of insecticide dose exacts a toxic effect in the presence of synergist than in its absence. The sites of detoxification and of toxic action appear in general to be separate and thus an understanding of the synergism does not depend on knowledge of the mode of toxic action of the insecticide. Moreover, the same enzyme complex within the insect appears to be responsible for oxidative detoxification of insecticides of widely different chemical structures so that a given synergist may synergize insecticides differing widely in their mode of action.

Metcalf (1967) reviewed much of the literature on the mode of action of synergist. The synergism by bezodioxoles of carbamates, pyrethroids and certain organophosphorous compounds is accounted for by the benzodioxoles depressing metabolism of the insecticides than can be regarded as oxidative in board sense. Oxidative metabolism converts certain other organophosphorous insecticides into compounds of higher insecticidal potency than the original insecticides.

Synergists have been used since 1947 to enhance the efficacy of insecticides in insect control (Wachs, 1947). Synergists inhibit the enzymes responsible for toxicant degradation (Hewlett, 1968). Sacher *et al.,* (1968) reported that the synergistic compounds such as piperonyl-butoxide, sesamex, MGK_{264}, SKF_{525}, TTP, DEM, DEF etc readily inhibit the activated enzymes (Metcalf, 1967).

It is now generally recognized that a synergist produces its synergistic effect by biochemically inhabiting the detoxification enzymes (metabolic enzymes) within the insect body, thus increasing the effectiveness of insecticides (Willoughby *et al.,* 2007; Young *et al.,* 2005).

The pest species posses some detoxification enzymes such as esterases, carboxylesterases, phosphorotriester hydrolases, carboxylamidases, epoxide hydrolases and cytochrome P_{450} mono-oxygenases enable insects to 'transform insecticides to less biological active compounds that can then be eliminated by excretion (Ranson *et al.,* 2002; Jensen, 2000; Oppenoorth, 1985).

Though it has been reported that synergist's block the specific enzyme systems (Roush and Daly, 1990) they are not entirely specific to a single detoxification enzyme class. For example: PBO is a well established inhibitor of cytochrome P_{450} mono-oxygenases but is also a moderately effective inhibitor of esterases in some insects (Gunning *et al.,* 1998). S, S, S-tributylphosphorotrithioate (DEF) is a strong esterase inhibitor but it is also a substrate for microsomal mono-oxygenases and has a limited capacity to inhibit those (Sanchez-Arroyo *et al.,* 2001).

Diethylmaleate (DEM) inhibits GSH S-transferases indirectly through the depletion of glutathione and this depletion may have other cellular effects that alter toxicological responses such as enhancing oxidative stress (Cnubben *et al.,* 2001). It may also act as a microsomal mono-oxygenase inhibitor (Arends, 1987). Further, piperonyl butoxide and MGK_{264} are commonly used with carbamates, organophosphates and pyrethroids for the control of different pests (Sahay *et al.,* 1996; Singh and Agarwal, 1994). Piperonyl-butoxide and MGK_{264} usually exert their synergistic action with synthetic pesticides by inhibiting the mixed function oxidase activity which detoxifies xenobiotics or they may increase the penetration of the toxin which results in a high titer of toxin at the active site.

Gunning *et al.,* (1998) have reported that in the cotton aphid (*Aphis gossypii,* Hemiptera; Aphididae), piperonyl butoxide acted as an inhibitor of esterase, since it is a well known inhibitor of mocrosomal mono-oxygenase which are involved in the metabolism and detoxification of virtually all insecticides (Hemingway and Ranson, 2000; Feyereisen, 1999).

In this investigation synergism will be considered as the cooperative action of two compounds of a mixture so that the total effect is greater than the sum of the effect of the compounds used independently.

The effects on synergism of altering the amount of synergist has previously been noted increasing the dose of synergist enhanced the synergistic ratio when synergist and insecticide were co-applied (Pap *et al.,* 2001).

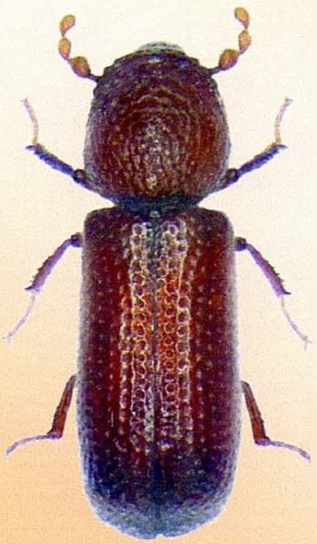
In this thesis synergism will be considered as the co-operative action of two compounds of a mixture so that the total effect is greater than the sum of the effect of the compounds used independently.

Leahey (1984) discovered that of a knock down resistance (kdn) factor is present and knorckdown time should be greatly lengthen in resistant adults as compares to susceptible adults. This he discovered after synergising DDT, permethrin, cypermethrin, deltamethrin, and fervalerate with DMC (1,1-di-(4-chlorophenyl) ethanol], piperony1 butoxide and DDE (S,S,S-tributyl phosphorotrithioate) which all failed to increase thetoxicity values apperciably when applied to a resistant strin of diamond backmoth (Platella xylostella).

1.10 Objectives

1) To assess the toxicity of some widely used insecticides such as cglorpyrifos (dursban 20 EC), cypermethrin (cythrin 10 EC), imidachloprid (imitaf 20 SL) and carbaryl (sevin 85 SP) against the lesser mealworm *R. dominica*.

2) To investigate the synergist or antagonistic effect of piperonyl-butoxide when combined with the selected insecticides against the lesser mealworm *R. dominica*.



Chapter Two

Materials and Methods

CHAPTER-2

MATERIALS AND METHODS

2.1. MATERIALS

To maintain the culture of beetles, the following laboratory materials were used:

i. Plastic Jars.
ii. Beakers.
iii. Petri dishes.
iv. Sieves.
v. Sable hair brush.
vi. Pieces of cloth.
vii. Spoon.
viii. Sieves.
ix. Rubber bands
x. Potato slices, etc.
xi. Yeast.

All the Beakers and Petri dishes were sterilized by keeping in an oven for about six hour at 120^0C (Khan, 1981).

2.2 Collection of test insects

The adults of *Rhizopertha dominica* L. were collected form flour mills of different areas to be mentioned in Benodpur Bazar, Shaheb Bazar, Uposhahar under Rajshahi City Corporation (RCC)

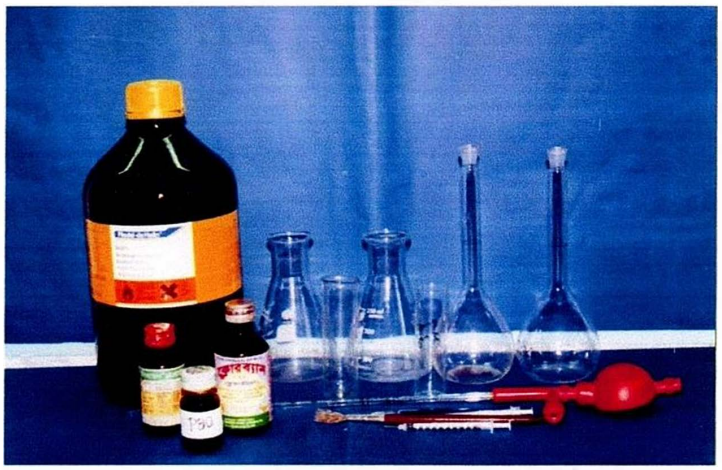


Plate 8: Glassware's used in the experiment.

Plate 9: Culture of the lesser grain borer,*R. dominica* in an incubator.

Wheat flour and corn flour were blended by an electric blender and sterilized in an oven at 120°C for 30 minutes. The mixture was then allowed to cool down and the yeast was mixed well and sieved.

2.3 Culture of *Rhyzopertha dominica* F.

The grains were cleaned by sieving and then sterilized in an oven at 100^0 C for 8 hours. A total of 1200 adults of *R. dominica* were collected and checked under stereo-binocular microscope to avoid other species, if any. The collected adults were divided into 3 groups, each having 400 adults of different ages and were kept in separate beaker (250 ml) containing 400g of wheat grains for ovipositor. After 72 hours adults were sieved out and eggs were collected and released in sterilized wheat in plastic container(36 mm diam. and 24 mm height).. The plastic containers were maintained in the laboratory at room temperature and relative humidity to ensure constant and ample supply of insects of known ages.

Culturing of insects under laboratory condition is an important and useful way to observed wide variety of problems associated with insects infesting stored products. The knowledge concerning biology and habitats of both insects and its enemies helps to some extent for solving storage problem (Mondal and Parween, 1997). The population of *R. dominica* are not flourished in a culture that has become mouldy. Occurrences of enemies of this species under laboratory conditions are likely to be more serious than that of field conditions (Goodrich, 1921). For research purposes, care must be taken to schedule culture time and avoid overpopulation of cultures, disease, parasites and other stresses. Particular attention should be given to sanitation in the culture room and avoidance of cross contamination, specially from grain that has not been disinfected. For reproducibility of results, insects must be unstressed, of similar genetic background, and free of disease. Conditions in the culture room should be standardized and consistent for temperature and relative humidity (Wright *et al.*, 1989).Keeping these points in mind the culture of R. dominica was prepared as follows:

About 1000 of beetles were released in 250g of food in a beaker. The adult beetles were collected form the original stock by sieving through as U.S. standard No. 16 or 20 sieve. The healthy and active beetles were taken in the

beaker. The mouth of the beakers was covered by thin cloth and rubber band. The beetles were placed in an incubator at 30°C ± 0.5°C without controlling the light and humidity. Slices of potato were kept within jars for humidity control, which were replaced when necessary.

These cultures were examined regularly and only healthy cultures were kept. Infested culture if any observed was readily discarded. Dead individuals also were removed from the cultures.

2.4 Preparation of the food medium

In order to prepare the food media 500g of Fresh grains of 'white' variety wheat were collected from the seed stores of Bangladesh Agriculture Development Corporation (BADC), Sopura, Rajshahi. The grains were cleaned by sieving and then sterilized in an oven at 100°C for 8 hrs. The sterilized wheat were kept in a plastic container (36 mm diameter, 24 mm height). For 15 days to equilibrate with the moisture content of the experimental laboratory with the minimum of 13%. All the glassware and sieves were sterilized in an oven at 600°C for 4hr before use.

2.5 Establishment of culture

The original culture of *R. dominica* were raised from the insects collected from the wheat flour mills, Shaheb Bazar, Rajshahi, in 2008. A total of 1200 adults of *R. dominica* were collected and checked under sterio-binocular microscope to avoid other species, if any. The collected adults were divided into 3 groups, each having 400 adults of different ages and were kept in separate beaker (250 ml) containing 400g of wheat grains for oviposition. After 72 h. adults were sieved out and eggs were collected, and released in sterilized wheat in plastic container. The plastic containers were maintained in the laboratory at room temperature and relative humidity to ensure constant and ample supply of insects of known ages.

2.6 Preservation of culture

Frass, spoiled substances, fecal materials, excretory products etc. gradually accumulate in the culture media and make it dirty, unhealthy and damped. To avoid such unhealthy condition, the original cultures were sieved after every 2-3 months and freshly sterilized wheat grains were added to the culture.

2.7 Maintenance of the culture

To maintain the culture and to avoid from predators and parasites following precautions were followed:

a) Food media were sterilized at 100^0C before use.

b) Care was taken to make sure that all the media used in the experiments being sterilized gained the temperature ranged between 80^0C-100^0C (Mondal and Parween 1997).

c) The moisture content of the grains was maintained at 13%.

d) The containers used for the heat sterilization of food medium were well sealed before heating and allowed to cool down at room temperature to prevent evaporation of moisture.

e) Mouth of the culture boyam was covered with a filter paper. A piece of cloth was then tied on the top with the help of gutter.

f) All the glasswares and equipment were regularly cleaned and sterilized before and after use.

g) Cultures were rotated regularly by preparing new cultures and old media were discarded.

h) All the experimental beakers, petridishes and vials were placed on a tray containing mineral oil to avoid mite infestations.

When sufficient numbers of adults were produced, they were separated from the food medium for experiment.

Dose mortality experiments were done by surface film technique method. Before setting experiments pieces of paper were placed inside the culture beaker/jar to allow the beetle to crawl on to it. The adults crawled beetle was then collected in a small beaker with the help of a camel hairbrush.

2.8 Insecticidal application

Same age of adult *R. dominica* were used in the experiment. Different doses were used for both the insecticides, each with five replications. In each replication 50 adults *R. dominica* were used. Insecticides were diluted in acetone and pilot experiments were done according to the indications made by the produces for the users, to obtain doses in which mortality rate was in between 10 to 84%.

The actual doses were calculated from the amount of insecticide present in 1ml of the solution and then the amount of active ingredient was also worked out. The calculated amount of the active ingredient of the insecticide was expressed as

A control experiment was maintained in which treatment was made only with the solvent. The mortality of the *R. dominica* was recorded after 24 hours of treatment.

Plate 10: Preparetion of dose

2.9 Bioassay of the insecticides

.Selected doses were prepared prior to the experiment. Beetles were collected in the shortest possible time before setting up the experiments. Finally washed and properly autoclaved Petri dishes were marked with a permanent marker for different doses. According to the results obtained from the pilot experiment doses were prepared of which 1ml of each of the doses was poured down on the Petri dish (9cm; r = 4.5cm) with a one ml syringe (Hamilton Bonaduz). A control experiment was maintained in which treatment was made only with the solvent. The Petri dish then allowed drying by evaporation of the solvent 50 insects was released within each Petri dish. Then the Petri dish kept within an incubator at 30°C ± 0.5°C for 24 hours. Mortality of the beetles was recorded after 24 hours of treatment.

2.10 Bioassay of insecticide and synergist mixtures

Each insecticide and a synergist were mixed in acetone at 1:1, 1:5 and 1:10 applied as mentioned in section the lowest dose of the insecticide was taken proportionate to that of the synergist to make the combined dose.

2.11 Cleaning of glassware

Petri dish, tube, conical flask were washed with a detergent solution as routine cleaning of glassware. Distilled water was used for final wash and ringed carefully, pencil marks, gum were removed effectively from the glass were by xyline. Sometimes the dirty or spotted glassware were cleaned and washed by recommended clearing solution was prepared in laboratory as follows:

Potassium dichromate	100 gm
Hydrochloric acid	500 ml
Water	1000 ml

Glassware's were sterilized in the incubator. Sterilization was accomplished by placing in the incubator for 15 to 20 minute at 80°C.

2.12 Analysis of data

The invested data analyzed by following method:

2.12.1 Probit analysis

The percent mortality was subjected to statistical analysis according to Finny (1971) and Busvine (1971). The dose mortality relationship was expressed as a median lethal dose (LD_{50}).

During probit mortality calculation percent mortality of the adult beetles were corrected by using Abbott's (1925).

$$P = \frac{P_0 - P_c}{100 - P_c} \times 100$$

Where, P_t = Corrected mortality %

P_o = Observed mortality % and

P_c = Control mortality %

Probit analysis was done according to Busvine (1971) using a software developed in the Department of Agricultural and Environmental Science, University of Newcastle Upon Tyne, United Kingdom, which adapted the traditional calculations to automatic computation. No provisional graph or tables are required. Heterogeneity is tested by a chi-squared test, if the probability is greater than 5% an automatic correction of heterogeneity is introduced. The program also calculates confidence limits for LD_{50}.This data is entered into a linear regression program which fits a regression line on to a probit log dose concentration graph. % mortality and dose concentration can be determined from this graph using the probit transformation table (Busvine, 1980).

The median leathal dose (LD_{50}) was calculated by using a Probit analysis program. The LD_{50} values of the insecticides are inversely related to the toxicity of the insecticide i.e. higher the LD_{50} value lower the toxicity of the insecticide.

2.12.2. Determination of co-toxicity co-efficient

Using the formula described by Sun and Johnson (1960a)

$$\text{Co toxicity coefficient} = \frac{LD_{50} \text{ of toxicant alone}}{LD_{50} \text{ of toxicant in the mixture}} \times 100$$

When the co-toxicity coefficient of a mixture is 100, the effect of this mixture indicates probability of similar action. If the mixture gives a coefficient significantly greater than 100, it indicates a synergistic action. On the other hand, when a mixture gives a co-toxicity coefficient less than 100, the effect of the mixture indicates an antagonistic action.

2.12.3. Construction of isobolograms

The regression lines and isoboles were drawn using the Fig-P (Biosoft) package. Isobolograms for the mixtures of insecticides were constructed according to the methods described by Hewlett (1960). This was done as follows: using the LD_{50} values for each ratio, the concentration of each individual compound in the mixture was plotted. Isobole lines below the additive line indicate synergism. Isoboles were drawn by free and curve fitting.

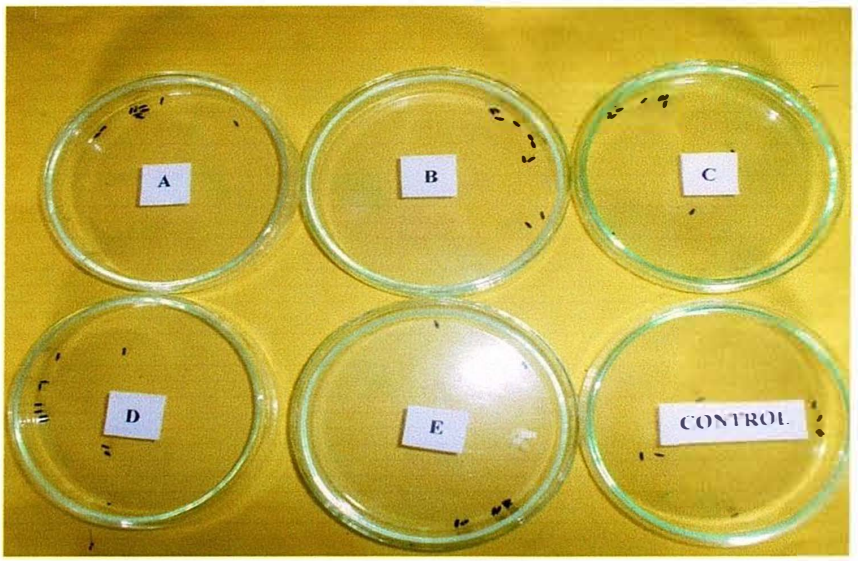
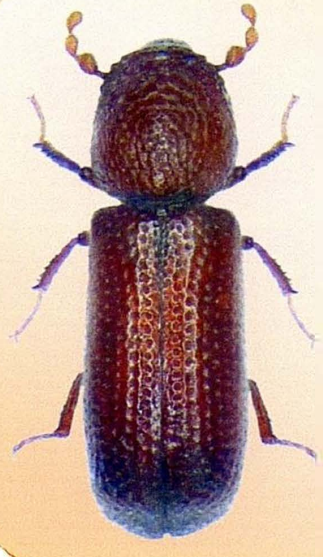


Plate 11: Dead *R. dominica* adults in the Petri dish treated with insecticide after 24 hours of application.

Plate 12: Dead *R dominica* adults in the Petri dish treated by insecticide with PBO after 24 h.



Chapter Three

Results and Observations

CHAPTER-3

RESULTS AND OBSERVATION

3.1. LETHAL EFFECT OF INSECTICIDES

The lesser grain borer, *Rhyzopertha dominica* adults were exposed to different concentrations of Chlorpyrifos (Dursban), Cypermethrin (Cythrin), Imidachloprid (Imitaf) and Carbaryl (Sevin). The toxicity of the insecticides has been recorded properly and the results have been estimated in this chapter. The effect of several concentrations of each insecticide and a synergist piperonyl-butoxide (PBO) at different ratios has also been recorded accordingly.

3.1.1. LETHAL EFFECT OF ORGANOPHOSPHATE INSECTICIDES

Chlorpyrifos (Dursban)

The effect of five different doses of chlorpyrifos on adults after 24h of exposure has been estimated in appendix table I. The final doses exposed to the adults were 0.469, 0.2345, 0.1172, 0.0586 and 0.0293 µg cm^{-2} which gave 84%, 68%, 60%, 52%and 26 % mortality respectively. The LD_{50} value for the adult has been recorded as 0.07450 µgcm^{-2}.The LD_{50} value along with 95% confidence limits, regression equation, chi-squared value have been estimated in the (table 4) No significant heterogeneity observed. A regression line has been drawn by plotting on a log probit paper where probit mortality (Empirical probit) on Y-axis and log dose on X- axis was plotted as shown in (Fig. 3.)

3.1.2. LETHAL EFFECT OF PYRETHROID INSECTICIDES

Cypermethrin (Cythrin)

A serial of five different doses of cypermethrin containing active ingredient as 3.93, 1.96, 0.982, 0.491 and 0.245 μgcm^{-2}, which gave 96 %, 80%, 64 %, 30 % and 20 % mortality of the adult beetles. The residual LD_{50} value of cyermethrin has been calculated as 0.7182 μgcm^{-2} after 24h of exposure.(Appendix table II)

The LD_{50} values along with 95% confidence limits, regression equations and chi-squared value have been estimated in the table 4. Regression lines have been drawn as shown in (Fig. 4). The result of goodness-of- fit also indicates the model fits well.

3.1.3. LETHAL EFFECT OF IMIDACLOPRID INSECTICIDES

Imidacloprid (Imitaf)

Mortality data obtained from the exposure of imidacloprid (Imitaf) to the adult have been presented in appendix table III. Effective doses selected for the final treatment of adult were 9.83, 4.92, 2.46, 1.23 and 0.615 μgcm^{-2}.which gave 88%, 75%, 58%, 38% and 17% mortality for the adult respectively. The LD_{50} value of imidacloprid for the adults has been calculated as 1.9997 $\mu g\ cm^{-2}$ after 24h of exposure.

The LD_{50} values along with 95% confidence limits, regression equation and chi-squared value of have been estimated in the table 4. Regression lines have been drawn in (Fig. 5).

3.1.4. LETHAL EFFECT OF CARBARYL INSECTICIDES

Carbaryl (Sevin)

Mortality data obtained from the exposure of carbaryl (Sevin) to the adult have been presented in appendix table IV. Effective doses selected for the final treatment of adult were 16.71, 8.350, 4.177, 2.088 and 1.044 μgcm^{-2}.which gave 96%, 72%, 48%, 36% and 20% mortality for the adult respectively. The LD_{50} value of imidacloprid for the adults has been calculated as 3.651361µg cm^{-2} after 24h of exposure (table-4).

The LD_{50} values along with 95% confidence limits, regression equation and chi-squared value of have been estimated in the table 4. Regression lines have been drawn in (Fig. 6).

Table 4. $LD_{50,}$ 95% confidence limits, regression equation and χ^2 value of 4 insecticides against *R. dominica* adult after 24h of exposure.

Insecticide	LD_{50} (µg cm^{-2})	95% confidence limits		Regression equations	χ^2 value (df=3)
		Upper	Lower		
Chlorpyrifos	0.07450	0.1044	0.0531	Y = 3.943227 + 1.211645 X	2.7112
Cypermethr.	0.7182	0.87444	0.5898	Y = 3.16517 + 2.142868 X	1.91024
Imidachlorp.	1.9997	2.52485	1.5838	Y = 2.77864 + 1.707447 X	0.272495
Carbaryl	3.6513	4.51994	2.9496	Y = 3.938334 + 1.88755 X	3.676903

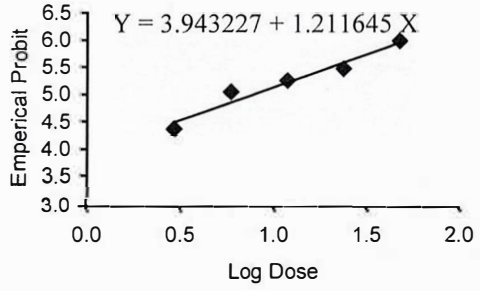


Fig. 3: Regression lines of probit mortality on log dose of chlorpyrifos used against *R. dominica* adult after 24h of exposure

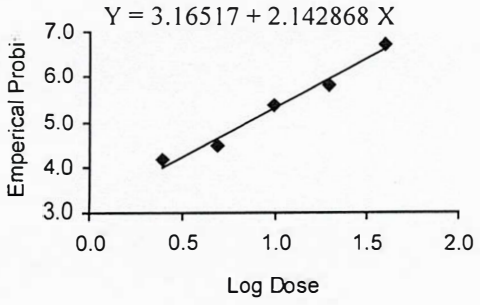


Fig. 4: Regression lines of probit mortality on log dose of cypermethrin used agains *R. dominica* adult after 24h of exposure

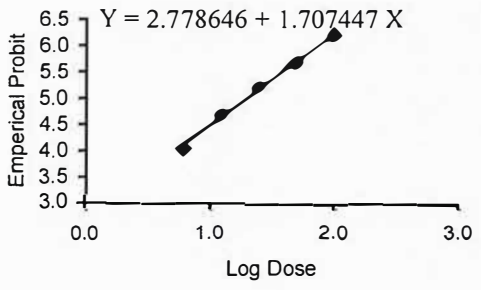


Fig. 5: Regression lines of probit mortality on log dose of Imidacloprid used against *R. dominica* adult after 24h of exposure

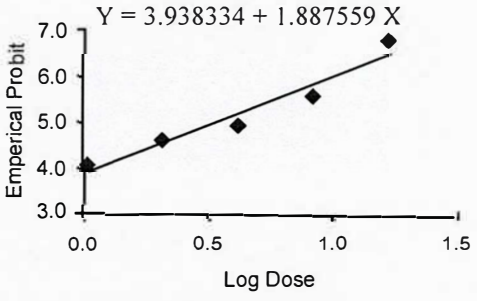


Fig. 6: Regression lines of probit mortality on log dose of Sevin used against *R. dominica* adult after 24h of exposure

3. 2. JOINT ACTION OF INSECTICIDE AND PIPERONYL BUTOXIDE

3.2.1. LETHAL EFFECT OF ORGANOPHOSPHATE AND PBO MIXTURE

Keeping the lowest dose of the insecticide constant, combined doses of each insecticide and synergist (PBO) were prepared at the ratios of 1:1, 1:5 and 1:10. Five serial dilutions of each ratio were made and the mortality percentage was recorded after 24h of exposure.

3.2.1. (A) Chlorpyrifos and Piperonyl butoxide

Effects of five different combined doses of chlorpyrifos and piperonyl butoxide on *R. dominica* adult at the ratios of 1:1, 1:5 and 1:10 have been estimated in appendix table V-VII. The dose concentrations were 0.149, 0.0745, 0.0372, 0.0186, 0.0095 μgcm^{-2} at ratio 1:1; 0.447, 0.225, 0.112, 0.056, & 0.028 μgcm^{-2} at ratio 1:5; 0.8195, 0.4097, 0.2078, 0.1024 and 0.0512 μgcm^{-2} along with at the ratio 1:10. The mortality percentage were recorded as 16-40% at 1:1, 18-44 % at 1:5 and 20-48% at 1:10 after 24h of exposure. (Appendix tables V-VII)

The LD_{50} values of the mixture (Chlorpyrifos + PBO) of different ratios have been calculate as 0.3218, 0.6186 and 0.8237 μgcm^{-2} after 24h of application respectively. LD_{50} values along with 95% confidence limits, regression equations and chi-squared values have been estimated in the table 5. The lowest LD_{50} value has been obtained from the ratio 1:1. Regression lines of different ratios on log probit mortality and the log dose concentrations have been plotted as in the Fig. 7.

Synergistic action of Chlorpyrifos + PBO

The LD_{50} value of chlorpyrifos alone has been calculated as 0.0745 μgcm^{-2} for the adults respectively (Appendix table V-VII) The LD_{50} values of the mixture of different ratios has been calculated for the adult as 0.3218 μgcm^{-2} at 1:1, 0.6186 μgcm^{-2} at 1:5 and 0.8237μgcm^{-2} at 1:10 (Table 5).

To compare the LD_{50} values of the mixtures the LD_{50} values of the insecticide and synergist have been separated as ratios to calculate co-toxicity coefficient according to the formula described by Sun and Johnson (1960b). The co-toxicity coefficients were determined as 46.30, 72.25 and 99.59, for adult (Table 6)

The present investigation (Co-efficient values) shows that all ratios of chlorpyrifos and piperonyl butoxide offered antagonistic action to the adult. It has been observed that the toxicity of the chlorpyrifos has been decreased as the ratio (amount) of PBO is increased. The free hand curve fitting of isobologram (Fig. 8) run above the additive line indicating that the properties of the mixtures were antagonistic at all of the ratios of chlorpyrifos: PBO. The individual LD_{50} value of chlorpyrifos for adult was 0.0745 μgcm^{-2}. But in the mixture, the share of chlorpyrifos was 0.1609, 0.1031and 0.0748$\mu g\ cm^{-2}$ at ratios of 1:1, 1:5and 1:10 when in order to have a same level of control the amount of insecticide use needs to be increased as much as 115.97%, 38.38 % and 0.40% respectively.

Reduction of active ingredients in the doses was calculated using the formula as

$$a - s = r \quad \text{.....(1)}$$

$$\% \text{ or reduced a. i.} = {}^{r}/_{a} \times 100 \quad \text{.....(2)}$$

Where a = LD_{50} value of the active ingredient alone

s = Share of the active ingredient in the LD_{50} value of the mixture.

r = reduced amount of the a. i. to kill 50% of the test insects.

Table 5. LD_{50}, 95% confidence limits, regression equation and χ^2 values of dose mortality experiments of different ratios of Chlorpyrifos (Darsban) with PBO against adult *R. dominica* with 24h of treatment.

Ratios	LD_{50} value ($\mu g\ cm^{-2}$)	95% confidence limits		Regression equations	χ^2 value (df=3)
		Upper	Lower		
1:1	0.3218	1.2324	0.0840	Y = 3.28226 + 0.684996 X	0.4965
1:5	0.6186	1.8113	0.2113	Y = 3.766681 + 0.68845 X	1.6174
1:10	0.8237	2.0871	0.3251	Y = 3.69968 + 0.6787348 X	0.7745

Table 6. Co-toxicity coefficient of piperonyl butoxide (PBO) with chlorpyrifos applied in different ratios on adult *R. dominica* after 24h of application.

Insecticide LD_{50} ($\mu g\ cm^{-2}$)	Ratio Insecticide: PBO	Combined LD_{50} ($\mu g\ cm^{-2}$)	Insecticide LD_{50} ($\mu g\ cm^{-2}$)	PBO LD_{50} ($\mu g\ cm^{-2}$)	Cotoxicity coefficient
0.0745	1:1	0.3218	0.1609	0.1609	46.30
	1:5	0.6186	0.1031	0.5155	72.25
	1:10	0.8237	0.0748	0.7488	99.59

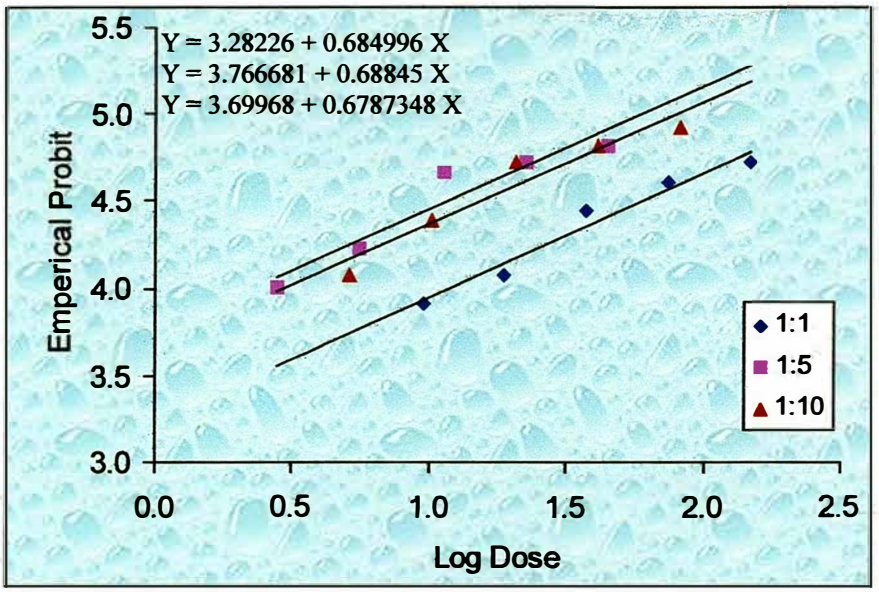


Fig. 7: Regression lines of probit mortality on log dose of the mixture of chlorpyrifos and piperonyl- butoxide at the ratio of 1:1, 1:5 and 1:10 against *R. dominica* adult after 24h of exposure

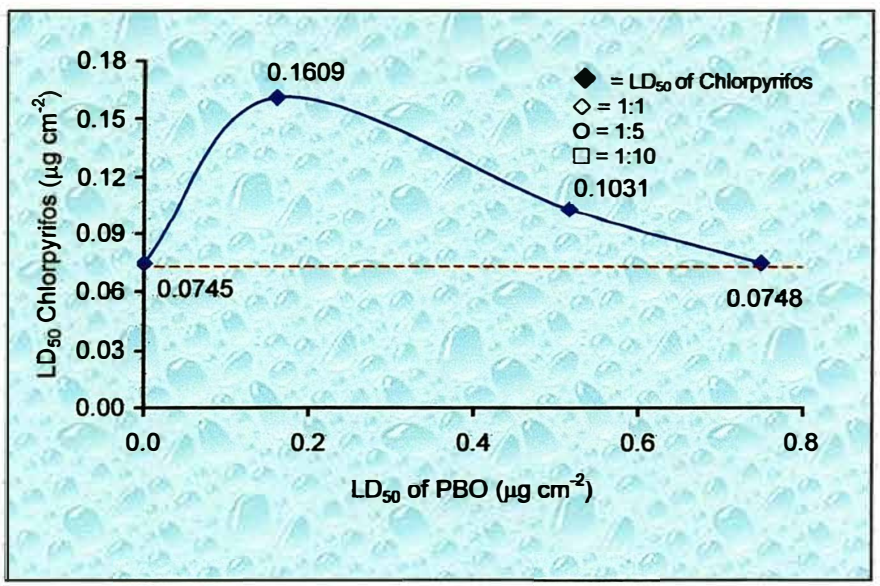


Fig. 8: Isobolograms of LD_{50} of Chlorpyrifos and piperonyl butoxide applied on *R. dominica* adults.

3.2.2. LETHAL EFFECT OF PYRETHROID AND PBO MIXTURE

3.2.2. (A) Cypermethrin: Piperonyl butoxide (PBO)

The effect of combined doses of cypermethrin and piperonyl butoxide on *R. dominica* adult at different ratios has been estimated in appendix table VI. The dose concentrations were 1.4364, 0.7182, 0.3591, 0.1795, 0.0897 at μgcm^{-2} at 1:1; 4.3092, 2.1546, 1.0773, 0.5386, 0.2693 μgcm^{-2} at 1:5 and 7.9002, 3.95, 1.975, 0.987, 0.4935μgcm^{-2} at the ratio 1:10.The mortality percentage was recorded as 18-72%, 20-86%, 30-96% at the ratios respectively. The LD_{50} values of the mixture have been calculated as 0.4560, 0.8446 and 1.0488 μgcm^{-2} for adult respectively. Highest mortality observed at the ratio 1: 10.

LD_{50} values along with 95% confidence limits, regression equations and chi – squared values have been estimated in the table 16. Regression lines of different ratios on log probit mortality and the log dose concentrations have been plotted in the Fig. 9.

Synergistic action of Cypermethrin + PBO

The LD_{50} value of the cypermethrin alone has been calculated as 0.7182 μgcm^{-2} for the adult respectively (Appendix tables VIII-X.)

The LD_{50} value of the mixture have been calculated as 0.4560, 0.8446 and 1.0488 μgcm^{-2} for adult respectively

(Appendix tables VIII-X)The co-toxicity coefficients were determined as 315.00, 510.44and 753.62for adult respectively. (Table 8)

The co-toxicity coefficients of mixtures of each ratio are greater than 100. Following the principle of Sun and Johnson (1960b) the present investigation shows that cypermethrin and PBO produced synergistic action with the adults at all ratios. It has been observed that the toxicity of the insecticide was increased when the ratios of PBO were progressive.

The isoboles (Fig. 10) have run below the additive line indicating synergistic action of the mixture with adult. The LD_{50} value of cypermethin with adult was 0.7182 μgcm^{-2} but in the mixtures, the share of cypermethrin was 0.2280, 0.1407 and 0.0953 μgcm^{-2} at the ratios 1:1, 1:5 and 1:10 when PBO causes reduction the dose level of 68.25%, 80.40% and 86.73% respectively.

Table 7. LD_{50}, 95% confidence limits, regression equation and χ^2 values of dose mortality experiments of different ratios of Cypermethrin (Cythrin) with PBO against adult *R. dominica* with 24h of treatment.

Ratios	LD_{50} value (µg cm^{-2})	95% confidence limits		Regression equations	χ^2 value (df=3)
		Upper	Lower		
1:1	0.4560	0.61917	0.33583	Y = 2.903613 + 1.263668 X	0.9553
1:5	0.8446	1.07134	0.66596	Y = 3.437418 + 1.686196 X	1.4003
1:10	1.0488	1.32374	0.83104	Y = 3.014231 + 1.945469 X	0.2108

Table 8: Co-toxicity coefficient of piperonyl-butoxide (PBO) with cypermethrin (cythrin) applied in different ratios on adult *R. dominica* after 24h of application.

Insecticide LD_{50} (µg cm^{-2})	Ratio Insecticide: PBO	Combined LD_{50} (µg cm^{-2})	Insecticide LD_{50} (µg cm^{-2})	PBO LD_{50} (µg cm^{-2})	Cotoxicity coefficient
0.7182	1:1	0.4560	0.2280	0.2280	315.00
	1:5	0.8446	0.1407	0.7035	510.44
	1:10	1.0488	0.0953	0.9530	753.62

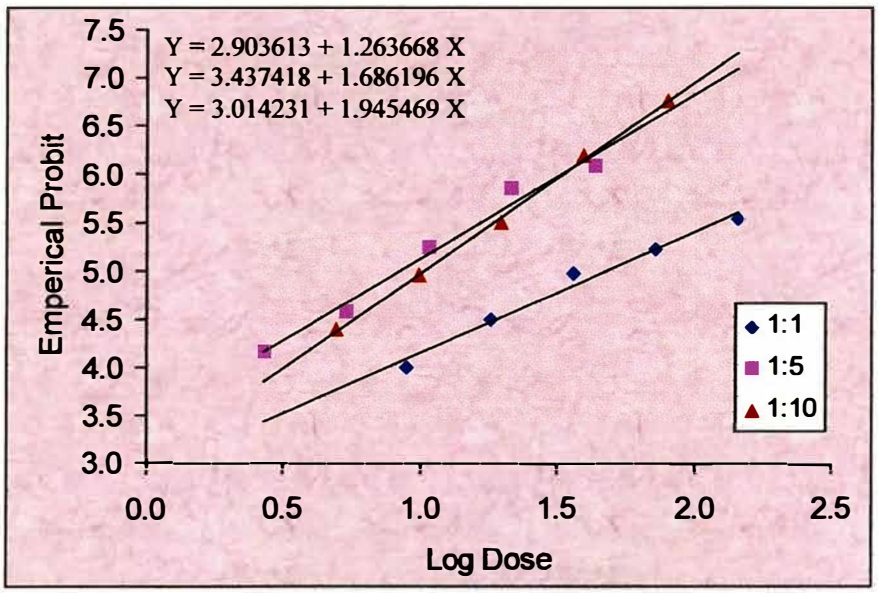


Fig. 9: Regression lines of probit mortality on log dose of the mixture of cypermethrin and piperonyl- butoxide at the ratio of 1:1, 1:5 and 1:10 against *R. dominica* adults after 24h of exposure

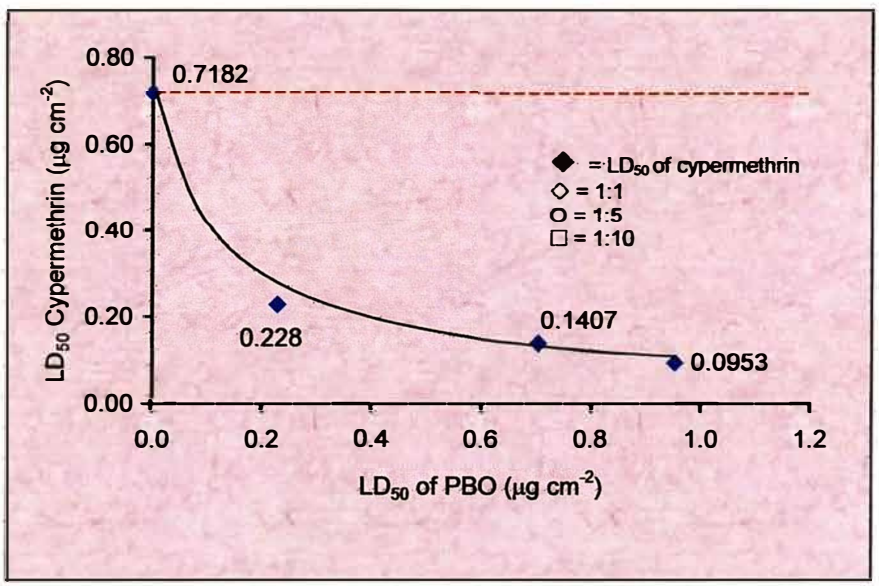


Fig. 10: Isobolograms of LD_{50} of Cypermethrin and piperonyl butoxide applied on *R. dominica* adults.

3.2.3. LETHAL EFFECT OF IMIDACLOPRID AND PBO MIXTURE

3.2.3 (A) Imidacloprid: Piperonyl butoxide (PBO)

The combined dose concentrations of imidacloprid were 3.67, 1.837, 0.918, 0.459 and 0.229 μgcm^{-2} at 1:1; 11.022, 5.511, 2.755, 1.377 and 0.688μgcm^{-2} at 1:5, and 20.207, 10.103, 5.05, 2.525 and 1.26 μgcm^{-2} at the ratio 1:10 for adults. Against these concentrations the mortality percentage were recorded as 16-80%, 22-88% and 26-94% respectively (Appendix tables XI- XIII) Highest mortality observed at the ratio of 1:10 The LD_{50} value of the mixture have been calculated as 0.9979, 1.5629 and 2.5989μgcm^{-2} respectively .

LD_{50} values along with 95% confidence limits, regression equations and chi–squared values have been estimated in the table 24. Regression lines of different ratios on log probit mortality and the log dose concentrations have been plotted in the Fig. 11. All values were insignificant indicating good fit of the regression lines.

Synergistic action of imidacloprid + PBO

The LD_{50} value of imadacloprid alone has been calculated as 1.9997 μgcm^{-2} for adult (Appendix table iv). The shares of imidacloprid in the LD_{50} values of mixtures have been separated as ratios. The co-toxicity coefficient was determined as 400.82, 767.63 and 846.25 for adults (Table 10). The coefficient values are greater than 100 that indicate that imidacloprid + piperonyl butoxide offered synergistic action to the adults at all the ratios. The mixture was highly synergistic to adults at the ratio 1:10 (Coefficient value 846.25).

The free hand curve fitting of isobologram (Fig. 12) have run below the additive line indicating synergistic action of the mixture with adult at all the ratios of imidacloprid: PBO. The individual LD_{50} value of imidacloprid for adult was1.9997 μgcm^{-2} but in the LD_{50} value of the mixture, the share of imidacloprid for adult was 0.4989, 0.2605 and 0.2363 μgcm^{-2} at the ratios 1:1, 1:5 and 1:10 when PBO causes reduction of dose level 50.09%, 21.84% and 29.96% respectively.

Table 9. LD_{50}, 95% confidence limits, regression equation and χ^2 values of dose mortality experiments of different ratios of Imidacloprid (Imitaf) with PBO against adult *R. dominica* after 24h of treatment.

Ratios	LD_{50} value ($\mu g\ cm^{-2}$)	95% confidence limits		Regression equations	χ^2 value (df=3)
		Upper	Lower		
1:1	0.99796	1.29766	0.76747	Y = 3.535997 + 1.465302 X	1.0541
1:5	1.56292	2.06352	1.18377	Y = 3.144272 + 1.554291 X	4.9845
1:10	2.59894	3.33327	2.02639	Y = 4.244848 + 1.820536 X	1.6340

Table 10. Co-toxicity coefficient of piperonyl-butoxide (PBO) with Imidacloprid (imitaf) applied in different ratios on adult *R. dominica* after 24h of application.

Insecticide LD_{50} ($\mu g\ cm^{-2}$)	Ratio Insecticide: PBO	Combined LD_{50} ($\mu g\ cm^{-2}$)	Insecticide LD_{50} ($\mu g\ cm^{-2}$)	PBO LD_{50} ($\mu g\ cm^{-2}$)	Cotoxicity coefficient
1.9997	1:1	0.9979	0.4989	0.4989	400.82
	1:5	1.5629	0.2605	1.3025	767.63
	1:10	2.5989	0.2363	2.3630	846.25

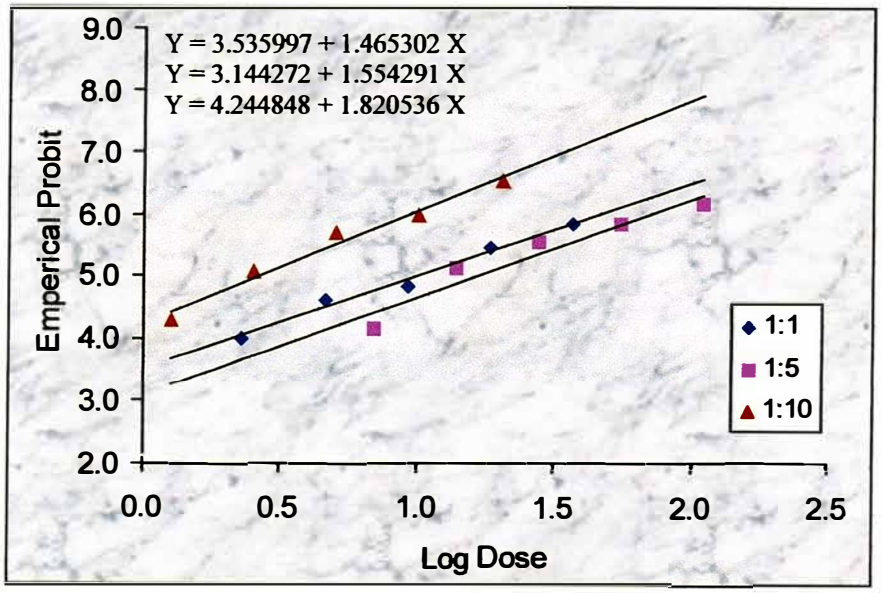


Fig. 11: Regression lines of probit mortality on log dose of the mixture of imidacloprid and piperonyl- butoxide at the ratio of 1:1, 1:5 and 1:10 against *R. dominica* adults after 24h of exposure

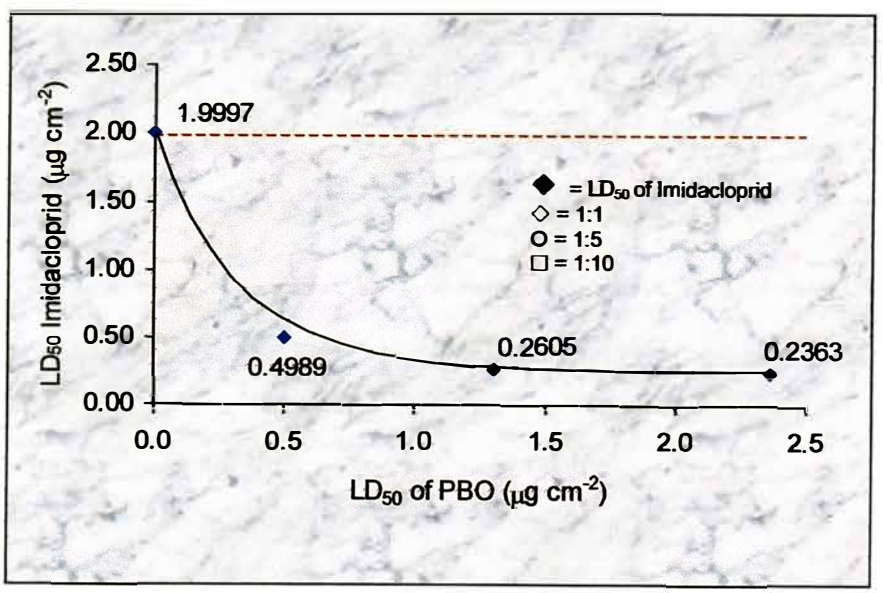


Fig.12: Isobolograms of LD_{50} of Imidacloprid and piperonyl butoxide on R. dominica adults.

3.2.4. LETHAL EFFECT OF CARBARYL AND PBO MIXTURE

3.2.4. (A) SEVIN: Piperonyl butoxide (PBO)

The combined dose concentrations of carbaryl were 7.302, 3.651, 1.825 , 0.912 and 0.456µgcm^{-2} at 1:1; 21.906, 10.953, 5.4765, 2.738and 1.369 µgcm^{-2} at 1:5, and 40.161, 20.08, 10.04, 5.02 and 2.51µgcm^{-2} at the ratio 1:10 for adults. Against these concentrations the mortality percentage were recorded as 24-80%, 26-88% and 30-96% respectively (Appendix tables XIV-XVI) Highest mortality observed at the ratio of 1:10. The LD_{50} value of the mixture have been calculated as 1.4884, 3.4161 and 5.2441µgcm^{-2} respectively.

LD_{50} values along with 95% confidence limits, regression equations and chi–squared values have been estimated in the table 11. Regression lines of different ratios on log probit mortality and the log dose concentrations have been plotted in the Fig. 13. All values were insignificant indicating good fit of the regression lines.

Synergistic action of Sevin + PBO

The LD_{50} value of imadacloprid alone has been calculated as 3.6513µgcm^{-2} for adult (Appendix tables III). The shares of carbaryl in the LD_{50} values of mixtures have been separated as ratios (Table 12). The co-toxicity coefficient was determined as 490.63, 641.36 and 765.95 for adults (Table 12). The coefficient values are greater than 100 that indicate that sevin + piperonyl butoxide offered synergistic action to the adults at all the ratios. The mixture was highly synergistic to adults at the ratio 1:10 (Coefficient value 765.95).

The free hand curve fitting of isobologram (Fig. 14) have run below the additive line indicating synergistic action of the mixture with adult at all the ratios of carbaryl: PBO. The individual LD_{50} value of carbaryl for adult 3.651 µgcm^{-2} but in the LD_{50} value of the mixture, the share of carbaryl for adult was 1.488, 3.416 and 5.244µgcm^{-2} at the ratios 1:1, 1:5 and 1:10 when PBO causes reduction of dose level 59.24%, 6.87% and 2.96% respectively.

Table 11. LD_{50}, 95% confidence limits, regression equation and χ^2 values of dose mortality experiments of different ratios of Carbaryl (Sevin) with PBO against adult *R. dominica* after 24h of treatment.

Ratios	LD_{50} value (µg cm^{-2})	95% confidence limits		Regression equations	χ^2 value (df=3)
		Upper	Lower		
1:1	1.48847	1.992286	1.11206	Y = 3.451457 + 1.320449 X	0.6717568
1:5	3.416114	4.496558	2.595282	Y = 4.185543 + 1.526538 X	0.7794876
1:10	5.244146	6.77193	4.061039	Y = 3.732674 + 1.760970 X	0.8106918

Table 12. Co-toxicity coefficient of piperonyl-butoxide (PBO) with Carbaryl (sevin) applied in different ratios on adult *R. dominica* after 24h of application.

Insecticide LD_{50} (µg cm^{-2})	Ratio Insecticide: PBO	Combined LD_{50} (µg cm^{-2})	Insecticide LD_{50} (µg cm^{-2})	PBO LD_{50} (µg cm^{-2})	Cotoxicity coefficient
3.6513	1:1	1.4884	0.7442	0.7442	490.63
	1:5	3.4161	0.5693	2.8467	641.36
	1:10	5.2441	0.4767	4.7673	765.95

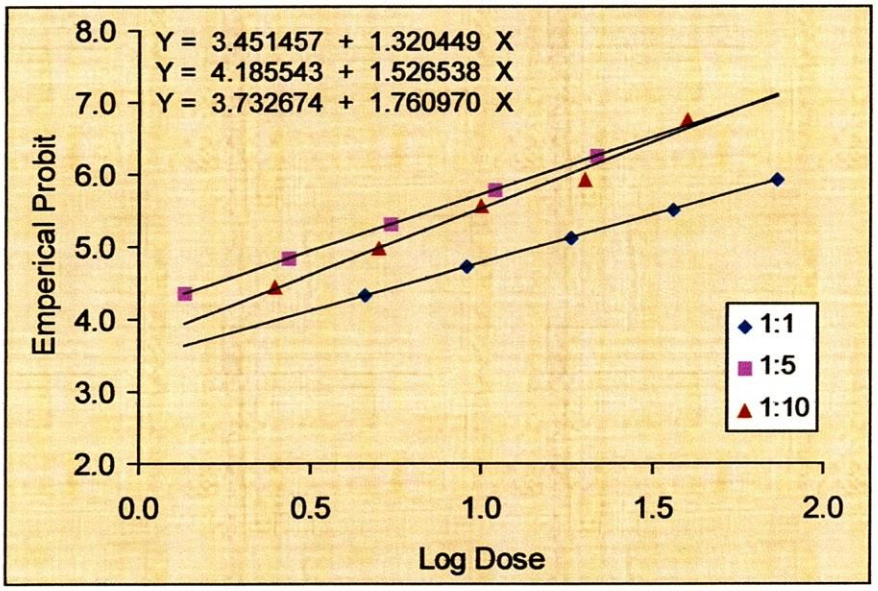


Fig. 13: Regression lines of probit mortality on log dose of the mixture of carbaryl (Sevin) and piperonyl- butoxide at the ratio of 1:1, 1:5 and 1:10 against *R. dominica* adults after 24h of exposure

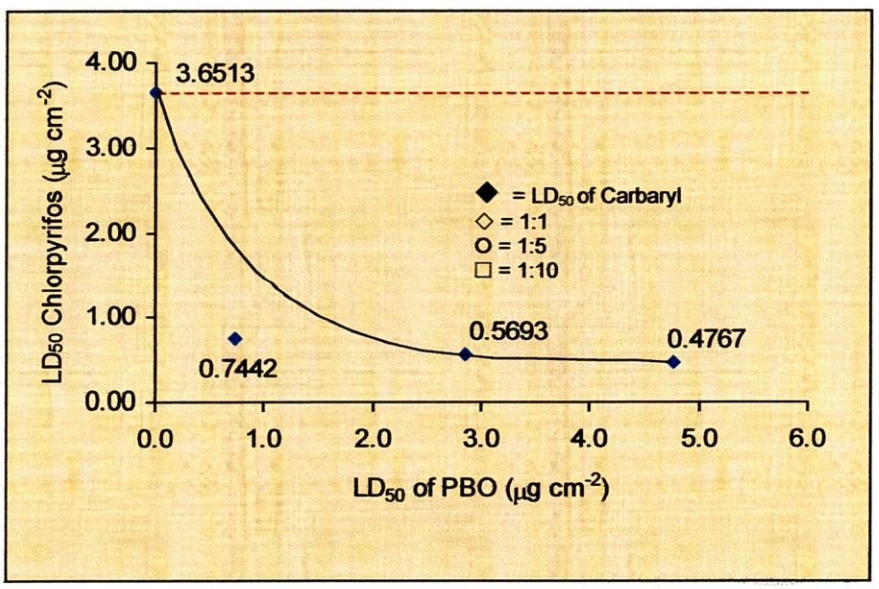


Fig.14: Isobolograms of LD_{50} of Carbaryl and piperonyl butoxide on R. *dominica adults.*

Plate 13: Various type of deformed adults, on medium treated with insecticides.

Chapter Four

Discussion

CHAPTER-4

DISCUSSION

4.1 GENERAL DISCUSSION

The residual film method was adopted in this investigation to assess the toxicity of four insecticides belonging to four different chemical class: (Chloropyrifos 50 EC) Organophosphate; Imidacloprid (Imitaf 20EC) Organochlorine; Cypermethrin (Cythrin 2.5 EC) Pyrethroid; Carbaryl (Sevin 85 SP) Carbamate, against the lesser grain borer, *Rhyzopertha dominica*. The synergistic or antagonistic action of piperonyl butoxide in combination with the selected insecticides was assessed in this investigation.

However, residual bioassay tests provide data that represents exposure of the beetles to a dry residue of the insecticide on a substrate that closely stimulates field applications.

It was hypothesized by Johnson (1975) that organophosphorus compound induced delayed neurotoxic ester (NTE) in nervous system, but not to of AChE. Lotti and Johnson (1978) suggested that measurements of the degree of inhibition of both NTE and AChE in hen brain provide a guide to the evaluation of delayed neurotoxicity of organophosphorus esters.

The increasingly widespread occurrence of resistance to insecticides is a serious threat to the control and management of many important insect pests (Brown and Brogdon, 1987). There has been mounting interest in the use of synergists to reduce some of these resistance incidences by combined application (El-Guindy *et al.,* 1983). The use of piperonyl butoxide, triphenyl phosphate and other compounds to suppress resistance to pyrethroids and organophosphates has been documented (Dyte and Rowlands, 1967). Experimentally, a number of other materials, such as caffeine (Nathanson, 1984), and a wide range of compounds containing heterocyclic moieties have shown synergism with a range of insecticides against insects (Wilkins and Khalequzzaman, 1993).

The first synergist was introduced in 1950 to increase the effectiveness of a plant-derived insecticide, pyrethrin, since then many materials have been introduced (Ware, 1975). Bodnaryk *et al.,* (1984) found a characteristics feature of the synergism of permethrin by chlorodimeform was the considerably steeper slopes of the dose response curves of mixtures compared with that of permethrin alone. Evidently, chlorodimeform not only enhances the toxicity of permethrin but also decreases the heterogeneity of response of *Tribolium castaneum* to permethrin.

Sun and Johnson (1960b) proposed a co-toxicity coefficient to quantity the synergist. If one of the components does not elicit mortality, the co-toxicity coefficient to quantity adapts a simplified expression identical to the synergism factor, which is the ratio of the concentration causing 50% mortality (LD_{50}) of a molecule alone to the LD_{50} of this molecule with the synergist. Earlier Macht (1929) based on the pharmacological effects measured on living organisms. Colin and Belzunces (1992) using sub-lethal doses of agrochemicals demonstrate a synergistic effect between an insecticide and a fungicide.

The synergistic action of some pyrethroid synergists were studied by Rai *et al.,* (1956) and Ware and Roan (1958) on malathion. Effect of piperonyl-butoxide in combination with several insecticides was studied by Bengston *et. al.* (1987); Ishaaya *et al.,* (1983). The effects of some synergists on the potency and metabolism of bromophos and fenitrothion on *T. castaneum* were studied by Hadaway *et al.,* (1962); Dyte and Rowlands, (1967); Hewlett (1968); Sacher *el. al.* (1968) reported that the activating enzymes are readily inhibited by synergistic compounds such as piperonyl butoxide, sesamex, MGK 264, SKF 525 etc. Synergistic and antagonistic actions of piperonyl-butoxide in combination with malathion, cypermethin and fenitrothion were studied on *T. confusum.* Results indicated that piperonyl butoxide produced synergism with malathion. It also produced similar effect with cypermethrin but they were less pronounced.

Piperonyl butoxide has very good crop tolerance. It is formulated as mixtures, aerosols, concentrates, oil based solutions, emulsion etc. Hewlett (1960) and Sawicki (1962) reviewed that the synergistic effect of piperonyl butoxide in particular, on pyrethroids is well known. The effect of piperonyl butoxide as synergist on malathion and its analogous and some other organophosporus insecticides has also been studied (Rai *et al.,* 1956; Ware and Roan, 1958).

Georghiou and Metcalf (1961) revealed that the synergistic effect of piperonylbutoxide for the carbamate, when applied to the house fly strain, together with its action in decreasing the rate of detoxification of the carbamate, suggest the piperonyl butoxide acts by blocking the carbamate detoxification mechanism. This synergist has been assigned a similar role in pyrethrin synergist.

Synergists are able to increase the activity of some insecticides by inhibiting the enzymes within the insect, which are responsible for their detoxification. Synergists, therefore, have often been used as an *in-vivo* approach for the monitoring of resistance development as well as for the identification of resistance mechanisms involving insecticide detoxication (Brindley and Salim, 1984; Osman and Brindley, 1981). The results indicated that there was an increase in the rate of mortality of the lesser grain borer with the increase of insecticide with the synergist dose. The median lethal dose (LD_{50}) was calculated by using a probit analysis program. The LD_{50} values of the insecticides are inversely related to the toxicity of the insecticides i.e. higher the LD_{50} value lower the toxicity of the insecticide.

4.1.1 BIOASSAY WITH INSECTICIDES ALONE

Chloropyrifos : The application of organophosphate insecticides to broiler houses to control *A diaperinus* increased during mid 1960s and early 1970. Malathion, and dichlorvos were generally recommended for application as sprays. Recently chlorpyrifos had received registration by EPA for use in poultry facilities (Steelman, 2008).

OP compounds undergo various metabolic reactions in living organisms. Major biotransformation reactions are common to compounds possessing

similar structures and are mediated mainly by mixed function oxidizes glutathione, s-transferases, and arylesterases. Recent studies revealed that these enzymes show clear stereoselectivity in the metabolism of optically active OP compounds (Ohkawa, 1982).

The history of the organophosphates so far has been one of the exploration by which the first hazardous member of the series have been replaced by insecticides of lower mammalian toxicity. This development has revealed the importance of a fuller knowledge of the fate of the chemical within the organism whereby advantage may be taken of difference between its metabolism by insect and by mammal. After successful development of organophosphate as insecticides other compound carbamet develops which acts as anticholinesterases too, among which physiological properties of this alkaloid arises, because it is a phenylester of methylcarbamic acid.

In the present investigation commercial formulation of Chlorpyrifos (Dursban 20EC) was tested against the 7 day old adult *R. dominica*. The result was in the general agreement with Rahman and Akter (2004) who observed that LD_{50} value of chloropyrifos has been calculated as 0.896µg cm^{-2}. The LD_{50} of the present investigation was recorded as 0.1241 µg after 24 hours. The difference of the result is probably due to the difference in the emulsifiable concentrate formulation as product formulation affect efficacy of insecticide (Kaufman *et. al.*, 2008). They used chlorban 20 EC but in this investigation dursban 20 EC was used.

Rahman *et al.*, (2007) also tested malathion (57 EC) against the lesser grain borer *Rhizopertha* and obtained LD_{50} value as 1.267 µg cm^{-2} suggesting that malathion was less toxic than chlorpyrifos. Similar study has been introduced by Kaufman *et al.,* (2005) who used tetrachlorvinfos as an organophosphate insecticide against susceptible laboratory reared strain of *A. diaperinus*. The 48h LD_{50} values for tetrachlorvinfos were recorded as 0.080 µgcm^{-2} for adult and 0.070 µgcm^{-2} for larvae. The investigation was conducted at 26.5^0C. If the temperature would elevate, the same mortality might be obtained at 24h of exposure, as found in the present investigation.

The present finding is also support the investigation of Khalequzzaman and Nahar (2001), who tested chlorpyrifos against another stored grain product pest *Tribolium castaneum*. They obtained the LD_{50} value of chlorpyrifos as 0.0138 μgcm^{-2} for adult. The larvae were not included in this bioassay.

The results of this investigation are in general agreement with those derived from laboratory studies of the Steelman (2008) who conducted toxicity trails using chlorpyrifos to compare the susceptibility of resistant and laboratory susceptible population of *A. diaperinus* to chlorpyrifos by residual film method at $21^0C \pm 2$. He obtained 24h LD_{50} values for the susceptible laboratory population as 0.097µg/gm for adult and 0.07 µg /gm for larvae. The variation in the result could be attributed to factor such as he used technical grade of chlorpyrifos but in this investigation commercial formulation was used.

Cypermethrin: Toxicity trials were conducted using several effective pyrethroid insecticides against several pest species (Lambkin and Rice, 2006 Amweg *et al.*, 2005) Cypermethrin is widely used and served as an important compound from a historical perspective.It is regiatered for structural pest management in public health applicaions to control cockroaches, mosquitoes, ticks and flies including house fly in poultry farms (Kid and James 1991)

In the present investigation susceptibility of *R. dominica* to cypermethrin was assessed. The LD_{50} values were calculated as 0.7182 μgcm^{-2}. (Table-4) The result is the general agreement with those derived from laboratory studies of Steelman (2008) who investigated the toxicity of pyrethroid insecticides, cyfluthrin, permethrin and cypermethrin on both adult and larvae of the beetle population collected broiler chicken production farms in Arkansas that having different insecticides application history. LD_{50} values of the susceptible strains had been calculated as 0.035 μgcm^{-2} (Cyfluthrin), 0.064 μgcm^{-2} (Cypermethrin) and 0.073 μgcm^{-2} (permethrin) for the adult. Present investigation also follows (Hasan, 2012; Ali, 2011) Baki *et al.*, (2002) tested Lambda cyhalothrin against house fly, *Musca domestica* and obtained LD_{50} as 0.029 μgcm^{-2}, which is similar to the present investigation. But when the doses were exposed to *Tribolium castaneum* (Kalequzzaman and Nahar, 2001) the formulation gave

the LD_{50} value as 0.24 μgcm^{-2}. It suggests that toxicity of product formulation varies species to species.

Several investigators regard the synergist PBO and simazine as specific inhibitors of microsomal oxidases (Lichtenstein *et al.* 1973 and Ali *et al.* 1977) It was found that PBO synergies different insecticides through inhibition of hydrolytic esterases (Devonshire, 1977; Dittarich *et al.*, 1979). Several researchers studied the effect of piperonyl butoxide on toxicity to insects. It is now generally recognised that PBO produces its synergistic effect by inhibiting the detoxification enzymes within the insect body (Casida, 1970; Benke and Wilkinson, 1971; Jao and Casida, 1974; Davenport and Wright, 1985). It has been suggested that carbamates and natural pyrethroids are all detoxified primarily by microsomal oxidases (Jao and Casida, 1974).

Plapp (1979) reported four formamidines (Chlordimeform, Amitraz, U-42662 and U-4650) as synergistic for the synthetic pyrethroids, permethrin, fenvalerate and decamethrin against larva of the bull worm and the tobacco budworm. Chlordimeform and U-42662 were the most and fenvalerate by 2-15 times actives. Dittarich and Studer (1981) working the *Spodoptera littoralis* found that chlordimeform was synergistic with monocrotophos and resmethrin in some cases. Mixtures of Amitraz with endosulfan (Weighton and Kerry 1979) methomyl (Kerry and Weighton 1979a) or pyrethoids (Badmin and Knigt 1979) were synergistic. Amitraz permethrin mixture was synergistic to larvae of *Spodoptera littoralis* (Kerry and Weighton, 1979b).

Imidacloprid: Imidacloprid is a new insecticide introduced to market at the beginning of 1990 (Bai *et al.,* 1991) and classified by the US. Environmental Protection Agency (EPA) in both toxicity class II and class III. Its modes of action are different from that of pyrethroids and organophosphates (Abbink 1991; Bai *et al.*, 1991). A large number of diverse studies have been conducted on the effects of imidacloprid on insects due to the fact that it was one of the first neonicotinoid insecticides developed. In many cases the dose - response is anomalous with extremely low dose causes either a promotion or inhibition of the endpoint of interest and higher dose causes the opposite

response (Guez *et al.*, 2001; Lambkin *et al.*, 2001; Matsuda *et al.*, 2001 Armenguad *et al.*, 2000) Imidacloprid (Imitaf) has not been used yet in the beetle management in the world. In the present investigation the toxicity of this insecticide was examined against the lesser grain borer. LD_{50} value has been calculated as 1.9997 $\mu g/cm^2$ for adult.(Table 4).The present finding with imidacloprid agrees with the result of Paul *et al.* (2006). Wu *et al.* (2007) studied the toxicity of imidacloprid in susceptible F_{21} progeny of *Diaeretiella rapae* (Hymenoptera: Aphidiidae) strains using the dry film method and LD_{50} values were calculated as 0.17 mg/L based on mortality at 24h. Wang *et al.* (2005) tested imidacloprid against Asian born *beetle Anoplophora glabripennis* through the contact feeding bioassay and reported the LD_{50} value of imidacloprid as 5.1 ppm after 24h of exposure. Hasan (2012) tested imidacloprid (tedo) against *R. dominica* and obtained LD_{50} value as 0 .8214$\mu g\backslash cm^{-2}$ suggesting that imitaf was comparatively toxic than imidacloprid (imitaf) (1.9997 μgcm^{-2}) as found in the present investigation

It is apparent that the imidacloprid produces high topical toxicity to *R. dominica* in the laboratory and that neonicotinoid like imidacloprid has significantly higher activity on this beetle. This also agrees with the results of the previous workers. (Van Herk *et al.*, 2008; James, 2003; Suchail *et al.*, 2001)

Carbaryl: Among the insecticides belonging to the carbamate class, Cararyl was introduced to market in 1967 by the FMC Corporation and Bayer AG (Tomlin, 2003) for the control of soil-dwelling and foliar-feeding insects and nematodes. It had not yet been registered for structural pest management or to apply poultry houses to control housefly or lesser mealworm. Very few researches have been done on the toxicological effect of chemicals belonging to carbamate class on beetles. Steelman, (2008) studied the efficacy of carbaryl on *A. diaperinus,* the 24h topical LD_{50} values were calculated as 0.444 $\mu g\ cm^{-2}$ for adult. In the present investigation the author obtained 24h LD_{50} values for the susceptible laboratory population of *R. dominica* as 3.651 $\mu g\ cm^{-2}$ for adult lesser grain borer, *R dominica* (Table-4) The difference of

the result is probably due to the difference in the emulsifiable concentrate formulation as product formulation affect efficacy of insecticide (Kaufman *et al.*, 2008). Hasan (2012) tested carbofuran (iridan) against *R. dominica* and calculated LD_{50} value of 4.6399 μg cm^{-2} suggesting carbofuran (iridan) was less toxic than carbaryl (Sevin) as found in the present investigation (3.651μg $cm^{-2)}$

4.1.2 BIOASSAY WITH INSECTICIDES AND SYNERGIST

Synergists are chemical that enhance the potency of other active ingredients. So, synergists have become favourite to many modern scientists. Use of synergists with insecticide could be promising in controlling the insects as many are major pest population (Bengston *et al.*, 1987). The toxicity of diazinon + synergist (PBO) against cockroaches *Periplaneta americana* L. was found to be more than that of Rahman and Akter (2008). The toxicity of malathion + synergist (PBO) against the lesser grain borer, *R. domonica* was found to be more than the insecticide does alone (Rahman *et. al.* 2007). The co-toxicity coefficients are >100, indicating the synergistic properties of the mixture. In the present studies

the mixture of chloropyrifos + PBO showed the antagonistic results (Fig. 8) But the isobole suggest that the synergistic properties of the mixture increased with the increase amount of synergist(PBO). The cotoxicity coefficients are <100 (Table 6) suggesting that the toxicity of this insecticide is not limited by P_{450} mediated detoxification. This implies that P_{450s} involved in metabolism of this insecticide is different. Sun and Johnson (1960a) suggested that the synergistic effect of sesamex and related compound was due to the inhibition of biological oxidation. The sma mechanism of action may also occur in the combinations tested in this investigation.

PBO is the most important pyrethroid synergist and a classical inhibitor of the microsomal mixed function oxidase (MFO) system, involved in the detoxification of every pyrethroid in mammals (Tanaka 1993). Cypermethrin is known to have a very high insecticidal activity either alone or in combination with synergist against various species of beetles as well as disease carrying insects (Baki *et al.*, 2002; Rahman *et al.*, 2007). At high doses PBO

increased the effectiveness of pyrethrin to houseflies by 300-fold (Testa and Jenner 1981). Kumar *et. al.* (1991) showed that PBO was an effective synergist with natural pyrethrins and synthetic pyrethroids due to its ability to inhibit the pivotal detoxifying enzymes-the monooxygenase and non specific esterase mediated detoxification were retarded in *A. diaperinus*.

From the present investigation it has been found that Cypermethrin was still the most toxic adulticide. Besides, the mixture of PBO + cypermethrin showed dramatic synergism at different ratios. The degree of synergism was increasing in relation to the increament (insecticide: synergist) ratios (Fig. 8). It was suggested by Sun and Johnson (1960a) that the synergistic effect of sesamax and related compound was due to the inhibition of biological oxidation. The same mechanism of action may also occur to the combinations tested in this investigation.

Kumar *et al.*, (1991) have shown that piperonyl butoxide is an effective synergist with natural pyrethrin and synthatic pyrethroids due to its ability to inhibit the pivotal detoxifying enzymes- the monooxgenases. The results of the present study should be considered that P_{450} monooxigenase and non-specific esterase- mediated detoxification were retarded in *R. dominica*.

The synergist effect of PBO on active ingredients of imidacloprid varied depending on the different species of insects. For example, no synergism of PBO on the resistance to imidacloprid was found in the tobacco feeding strain of *Myzus persicae* Sulzer (Nauen *et al.*, 1996). Lower synergistic effect was found on the susceptibility to the imidacloprid in population of *Bamisia tobacy* where as high level of synergism was observed in the field population of the same species (Kang *et al.*, 2006).

In this investigation it was observed that imidacloprid when used with PBO offered significant synergism resulting low LD_{50} values for imidacloprid. From the isoboles (Fig. 12.) and co-toxicity coefficient value (Table 10) it may be noticed that the PBO in imidacloprid solution behaved as a synergist from 1:1 to 1:10 ratios. The result of the present investigation is to some extent similar to the result of a previous studies on *Culex quinquefascitus* Say (Liu *et al.*, 2004).

The result is also similar to that of Paul *et. al.* (2006) who reported the toxicity of six novel insecticides including with PBO on adult and larval *Aedes aegypti* (L.). Imidacloprid was strongly synergized by PBO in *Aedes aegypti* (L.) adults.

In the present investigation it is evident that PBO interacted synergistically with imidacloprid in *R. dominica*. The results also suggest the possibility of PBO as an effective synergist with imidacloprid against adult lesser grain borer.

The synergistic effect of PBO on active ingradients of imadacloprid varied depending on the different species of insects. For example, no synergism of PBO on the resistance to imadacloprid was found in the tobacco feeding strain of *Myzas persicae* Sulzer (Nauen *et al.*, 1996). Lower synergistic effect was found on the susceptibility to the imidacloprid in insectarium population of *Bamisia tabacy* where as high level of synergism was observed in the field population in the same species(Kang *et al.*, 2006)

Carbaryl is cholinesterase inhibitors. The previous study of Steelman (2008) shows that carbaryl was highly toxic to *A. diaperinus* adult with (LC_{50} = 0.141). From the synergistic perspective it has been found that PBO had some effect on the toxicity carbaryl in *R. dominica* adult with increasing values of co-toxicity coefficient (Table 12).Isoboles suggest that the synergism was increased with the inctement of synergist(PBO) in the mixture (Fig. 14)

According to Hewlet (1960) and Metcalf (1967) synergist inhibits enzymes responsible for toxicants degradation. Sun and Johnson (1962b) reported that in the absence of synergist, the insect is able to metabolize some or most of the insecticides to non toxic compounds or to compounds that are less insecticidal. But synergists reduce this metabolism and thus a greater portion of the insecticides exert its (pesticides) toxic effect towards the target pests.

Therefore, employing synergists with insecticides could be promising in controlling the insects that could be of great benefit both economically and ecologically. Bengston, *et al.*, (1987) clearly indicated that the newer and more active pyrethroids such as deltamethrin respond in an economical

manner and in an environmental-safety manner to synergism by PBO. Kumar, *et al.*, (1991) have shown that PBO was an effective synergist with natural pyrethrins and synthetic pyrethroids due to its ability to inhibit the pivotal detoxifying enzymes- the monooxigenases.

In this study chloropyrifos, cypermethrin, imidacloprid and carbaryl in combination with PBO evidently brought down the tolerance level in both the life stages of the beetle tested (except the mixture of chloropyrifos + PBO failed to enhance the toxicity of insecticides.). So, from the present investigation it is clear that PBO could be employed with chloropyrifos, cypermethrin, imidacloprid and carbaryl in endemic areas, where continuous application is needed. The results of this investigation also suggest the possibility of PBO as an effective synergist with cypermethrin, imidacloprid and carbaryl against *R. dominica* adults.

SUMMARY

The foremost focal point in this study was to establish a baseline of contact toxicity of four insecticides from four different chemical classes' viz. chlorpyrifos (Darsban) organophosphate; cypermethrin (Cythrin) pyrethroid ; imidacloprid (Imitaf) neonicotinoid and carbaryl (Sevin) organocarbamet by residual film technique. Another focal point of this study was to evaluate the effect of piperonyl-butaoxide on the toxicity of these insecticides against the lesser grain borer *Rhyzopertha dominica.*

The dose mortality relationship was expressed as median lethal dose (LD_{50}). The LD_{50} values are inversely related to the toxicity of the insecticides i.e, higher the LD_{50} value to lower to toxicity or lower the LD_{50} value higher the toxicity of the insecticide. The LD_{50} values estimated here are 0.07450, 0.7182, 1.9997and 3.6513 μgcm^{-2} for chlorpyrifos, cypermethrin, imidacloprid and carbaryl respectively when the doses were exposed to adult beetles. Heterogeneity was tested by chi-square test. All values were insignificant indication good fit for the regression equation. Pearson x^2 values are lower than 7.82 ($df=3$, $P<0.05$) indicating a significant fit between the observed and expected regression lines.

Variations in the toxicity among the insecticides were observed even the toxicity of the different insecticides was different in adults. At 0.469 μgcm^{-2} dose of chlorpyrifos caused 84 % mortality of *R. dominica* adults where as At 0.0293 μgcm^{-2} concentration of chloropyrifos caused 26 % mortality of adults.

Among the insecticides tested in this investigation, Chlorpyrifos was most toxic and Carbaryl was least toxic to adults. Cypermethrin and Imidacloprid. were the intermediate between the Chlorpyrifos and Carbaryl. Depending on the LD_{50} values, the insecticides can be arranged as Chlorpyrifos (LD_{50} = 0.0745$\mu gcm^{-2)}$ > cypermethrin (LD_{50} = 0.7182 μgcm^{-2}) > Imidacloprid (LD_{50} = 1.9997 μgcm^{-2}) > and Carbaryl (LD_{50} = 3.6513 μgcm^{-2}) with adults.

Finally the study was conducted to determine the synergistic or antagonistic effect of piperonyl-butoxide in combination with the insecticides. According to the formula described by Sun and Johnson (1960a), the co-toxicity coefficient values of the combined doses of chlorpyrifos + PBO at different ratios were > 100, indicating that the properties of the mixtures were antagonistic towards the adults.

The individual LD_{50} value of chlorpyrifos with adult was 0.0745 μgcm^{-2}. But in the mixture, the share of chlorpyrifos was 0.3218, 0.6186 and 0.8237 μgcm^{-2} at the ratios of 1:1, 1:5 and 1:10 respectively, indicating PBO causes reduction of dose level. Isoboles (Fig.11) also suggest the mixture of chlorpyrifos+PBO was antagonistic to adults at respective ratios and that causes increased of dose level of 115.97%, 38.38 % and 0.40% respectively.

Cypermethrin and piperonyl butoxide offered synergistic action with the adults at all the ratios.(Table 8 , Fig.10). The cotoxicity coefficient values were > than 100 (Table 10) and the isoboles run below the additive line (Fig. 10) indicating that the properties of the mixtures were synergistic at all the ratios. LD_{50} value of Cypermethrin alone was 0.7142 μgcm^{-2} but in the mixture the share was 0.2280, 0.1407 and 0.0953 μgcm^{-2} when PBO causes reduction of dose level of 68.25%, 80.40% and 86.73% respectively.

Corresponding results were obtained from the mixture of Imidacloprid (Imitaf) and piperonyl butoxide, which gave the synergistic action with adult.(table10 , Fig.12)The cotoxicity coeficient values were > than100 (Table 10) and the isoboles run below the additive line (Fig.12) indicating that the properties of the mixture were synergistic with adult at all the ratios. LD_{50} value of imitaf alone was 1.9997 μgcm^{-2} but in the mixture the share was 0.4989,0.2605 and 0.2363 μgcm^{-2}, when PBO causes reduction of dose level of 50.09%,21.84% and 29.965 respectively

Carbaryl and piperonyl butoxide offered synergistic action with the adults.(Fig.14) It has been observed that the toxicity of the insecticide was increased as the ratios of piperonyl butoxide increased. LD_{50} value of Carbaryl

alone was 3.6513µgcm^{-2} but in the mixture the share was 1.488, 3.416 and 5.244 µgcm^{-2} when PBO causes reduction of dose level of 59.24%, 6.87% and2.965 at 1:1, 1:5 and 1:10 ratios respectively.

However, piperonyl butoxide is a pesticide synergist and acts as a potent inhibitor to cytochrome P_{450} and non-specific esterase (Moores *et al.,* 2009). The present data showed a dramatic synergism when PBO was combined with all the insecticides tested here. (except Chloropyrifos).This result indicate that Cypermethrin, Imitaf and Carbaryl hold promise for control *R. dominica.*

The presence of pesticide in the environment not only increases the non target organism mortality but also their levels can be reached where mammals ultimately can be seriously affected (Pauli *et al.,* 1999; Grue *et al.,* 1982). The present way of dealing with this problem is just to increase the toxicity of doses towards the target organisms using the less amount of insecticide than to be required alone. Thus the present study was aimed at standardizing a protocol for finding out insecticidal or synergistic effect of PBO against *R. dominica.*

In summary, the present results indicate that PBO is an effective synergist to cypermethrin, imidacloprid and carbaryl which enhanced their toxicity against *R. dominica.* And chlorpyrifos showing antagonistic result in mixture. Mixing PBO with these insecticides minimizes the amount of applied active ingredients and leads to economic and environmental benefits. The results of the present study should be considered in the current control programs to combat *R. dominica* in and around mills, processing plants, storehouses and granaries as well as in the poultry production facilities.

Chapter Five

References

CHAPTER FIVE

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Chapter Six

Appendices

APPENDICES

Appendix table I: Effect of Chlorpyrifos (Dursban 50EC) on R. dominica adults after 24 hours of exposure.

Dose µg cm^{-2}	Log dose	Num.	Kill	% kill	Corr %	Emp probit	Expt probit	Work probit	Weight	Final probit
0.469	1.6711	50	42	84	84	5.99	5.9599	6.02	23.55	5.968
0.2345	1.3701	50	34	68	68	5.47	5.5920	5.44	29.05	5.603
0.1172	1.0691	50	30	60	60	5.25	5.2240	5.28	31.35	5.238
0.0586	0.7679	50	26	52	52	5.05	4.8559	5.07	31.35	4.873
0.0293	0.4668	50	13	26	26	4.36	4.4880	4.36	27.9	4.508
Contr.		50								

Y = 3.943227 + 1.211645 X Log LD_{50} IS 0.8721803 LD_{50} is 0.07450	No significant heterogeneity Chi-squared is 2.711205 with 3 degrees of freedom 95% Conf limits are 0.0531321 to 0.1044729

Appendix table II: Effect of Cypermethrin (Cythrin) on *R. dominica* adults after 24 hours of exposure.

Dose µg cm^{-2}	Log dose	Num.	Kill	% kill	Corr %	Emp probit	Expt probit	Work probit	Weight	Final probit
3.93	1.5943	50	48	96	96	6.75	6.6305	6.72	11.9	6.581
1.96	1.2922	50	40	80	80	5.85	5.9734	5.87	23.55	5.934
0.982	0.9921	50	32	64	64	5.36	5.3206	5.34	30.8	5.291
0.491	0.6910	50	15	30	30	4.48	4.6659	4.47	30.05	4.646
0.245	0.3891	50	10	20	20	4.16	4.0093	4.16	21.95	3.999
Contr.		50								

Y = 3.16517 + 2.142868 X Log LD_{50} is 0.8562499 LD_{50} is 0.7182075	No significant heterogeneity Chi-squared is 1.91024 with 3 degrees of freedom 95% Conf limits are 0.5898882 to 0.87444

Appendix table III: Effect of Imidacloprid (Imitaf) on *R. dominica* adults after 24 hours of exposure.

Dose µg cm^{-2}	Log dose	Num.	Kill	% kill	Corr %	Emp probit	Expt probit	Work probit	Weight	Final probit
9.83	1.9925	50	44	88	88	6.18	6.2056	6.12	18.5	6.180
4.92	1.6919	50	38	76	75	5.67	5.6823	5.67	27.9	5.667
2.46	1.3909	50	30	60	58	5.2	5.1581	5.19	31.7	5.153
1.23	1.0899	50	20	40	38	4.69	4.6339	4.68	30.05	4.639
0.615	0.7888	50	10	20	17	4.05	4.1098	4.05	23.55	4.125
Contr.		50								

Y = 2.778646 + 1.707447 X Log LD_{50} is 1.30098 LD_{50} is 1.99977	No significant heterogeneity Chi-squared is 0.272495 with 3 degrees of freedom 95% Conf limits are 1.583882 to 2.524859

Appendix table IV: Effect of Carbaryl (Sevin) on *R. dominica* adults after 24 hours of exposure.

Dose µg cm^{-2}	Log dose	Num.	Kill	% kill	Corr %	Emp probit	Expt probit	Work probit	Weight	Final probit
16.71	1.2229	50	48	96	96	6.75	6.2682	6.53	18.5	6.246
8.3500	0.9216	50	36	72	71	5.55	5.6905	5.55	27.9	5.678
4.177	0.6208	50	24	48	47	4.92	5.1138	4.91	31.7	5.110
2.088	0.3197	50	18	36	35	4.61	4.5365	4.6	29.05	4.541
1.044	0.0187	50	10	20	18	4.08	3.9594	4.10	20.25	3.973
Contr.		50								

Y = 3.938334 + 1.887559 X Log LD_{50} is 0.5624548 LD_{50} is 3.651361	No significant heterogeneity Chi-squared is 3.676903 with 3 degrees of freedom 95% Conf limits are 2.949692 to 4.519942

Appendix table V: Effect of Chlorpyrifos (Dursban) in mixture of Piperonyl butoxide 1:1 on *R. dominica* adults after 24 hours of exposure.

Dose µg cm^{-2}	Log dose	Num.	Kill	% kill	Corr %	Emp probit	Expt probit	Work probit	Weight	Final probit
0.149	2.1731	50	20	40	39	4.72	4.7832	4.71	30.8	4.771
0.0745	1.8721	50	18	36	35	4.61	4.5691	4.6	29.05	4.565
0.0372	1.5705	50	15	30	29	4.45	4.3545	4.45	26.6	4.358
0.0186	1.2695	50	10	20	18	4.08	4.1403	4.09	23.55	4.152
0.0095	0.9777	50	8	16	14	3.92	3.9328	3.92	20.25	3.952
Contr.		50	1							

Y = 3.282267 + 0.6849963 X LOG LD_{50} is 2.507653 LD_{50} is 0.3218498	No significant heterogeneity Chi-squared is 0.4965029 with 3 degrees of freedom 95% confidence limits are 0.0840482 to 1.232476

Appendix table VI: Effect of Chlorpyrifos (Dursban) in mixture of Piperonyl butoxide 1:5 on *R. dominica* adults after 24 hours of exposure.

Dose µg cm^{-2}	Log dose	Num.	Kill	% kill	Corr %	Emp probit	Expt probit	Work probit	Weight	Final probit
0.447	1.6503	50	22	44	43	4.82	4.9112	4.81	31.7	4.902
0.225	1.3521	50	20	40	39	4.72	4.7022	4.71	30.8	4.697
0.112	1.0492	50	19	38	37	4.67	4.4898	4.69	27.9	4.489
0.056	0.7482	50	12	24	22	4.23	4.2788	4.21	25.1	4.281
0.028	0.4471	50	9	18	16	4.01	4.0677	3.99	21.95	4.074
Contr.		50	1							

Y = 3.766681 + 0.688451 X LOG LD_{50} is 1.79144 LD_{50} is 0.6186434	No significant heterogeneity Chi-squared is 1.617472 with 3 degrees of freedom 95% confidence limits are 0.2112887 to 1.81136

Appendix table VII: Effect of Chlorpyrifos (Dursban) in mixture of Piperonyl butoxide 1:10 on *R. dominica* adults after 24 hours of exposure.

Dose µg cm^{-2}	Log dose	Num.	Kill	% kill	Corr %	Emp probit	Expt probit	Work probit	Weight	Final probit
0.8195	1.9135	50	24	48	47	4.92	5.0076	4.92	31.85	4.998
0.4097	1.6125	50	22	44	43	4.82	4.7963	4.81	30.8	4.794
0.2078	1.3176	50	20	40	39	4.72	4.5895	4.71	29.05	4.594
0.1024	1.0103	50	14	28	27	4.39	4.3738	4.39	26.6	4.385
0.0512	0.7092	50	10	20	18	4.08	4.1625	4.09	23.55	4.181
Contr.		50	1							
Y = 3.699681 + 0.6787348 X LOG LD_{50} is 1.915798 LD_{50} is 0.8237553				No significant heterogeneity Chi-squared is 0.7744894 with 3 degrees of freedom 95% confidence limits are 0.3251226 to 2.087129						

Appendix table VIII: Effect of Cypermethrin (Cythrin) in mixture of Piperonyl butoxide 1:1 on *R. dominica* adults after 24 hours of exposure.

Dose µg cm^{-2}	Log dose	Num.	Kill	% kill	Corr %	Emp probit	Expt probit	Work probit	Weight	Final probit
1.4364	2.1572	50	36	72	71	5.55	5.6139	5.55	27.9	5.629
0.7182	1.8562	50	30	60	59	5.23	5.2330	5.25	31.35	5.249
0.3591	1.5552	50	25	50	49	4.97	4.8521	4.99	31.35	4.868
0.1795	1.2540	50	16	32	31	4.5	4.4710	4.51	27.9	4.488
0.0897	0.9528	50	9	18	16	4.01	4.0898	3.99	21.95	4.107
Contr.		50	1							
Y = 2.903613 + 1.263668 X LOG LD_{50} is 1.65897 LD_{50} is 0.4560053				No significant heterogeneity Chi-squared is 0.9553413 with 3 degrees of freedom 95% confidence limits are 0.3358331 to 0.6191792						

Appendix table IX: Effect of Cypermethrin (Cythrin) in mixture of Piperonyl butoxide 1:5 on *R. dominica* adults after 24 hours of exposure.

Dose µg cm^{-2}	Log dose	Num.	Kill	% kill	Corr %	Emp probit	Expt probit	Work probit	Weight	Final probit
4.3092	1.6343	50	43	86	86	6.08	6.2059	6.02	18.5	6.193
2.1546	1.3333	50	40	80	80	5.85	5.6960	5.82	27.9	5.685
1.0773	1.0323	50	30	60	60	5.25	5.1860	5.24	31.7	5.178
0.5386	0.7312	50	17	34	34	4.59	4.6759	4.57	30.05	4.670
0.2693	0.4302	50	10	20	20	4.16	4.1659	4.17	23.55	4.162
Contr.		50	0							

Y = 3.437418 + 1.686196 X LOG LD_{50} is 0.9266906 LD_{50} is 0.8446768	No significant heterogeneity Chi-squared is 1.400372 with 3 degrees of freedom 95% confidence limits are 0.6659643 to 1.071347

Appendix table X: Effect of Cypermethrin (Cythrin) in mixture of Piperonyl butoxide 1:10 on *R. dominica* adults after 24 hours of exposure.

Dose µg cm^{-2}	Log dose	Num.	Kill	% kill	Corr %	Emp probit	Expt probit	Work probit	Weight	Final probit
7.9002	1.8976	50	48	96	96	6.75	6.7439	6.71	10.4	6.706
3.95	1.5965	50	44	88	88	6.18	6.1490	6.17	20.25	6.120
1.975	1.2955	50	35	70	69	5.5	5.5541	5.47	29.05	5.534
0.987	0.9943	50	25	50	48	4.95	4.9588	4.94	31.7	4.948
0.4935	0.6932	50	15	30	27	4.39	4.3640	4.39	26.6	4.363
Contr.		50	2							

Y = 3.014231 + 1.945469 X LOG LD_{50} is 1.020715 LD_{50} is 1.048854	No significant heterogeneity Chi-squared is 0.210804 with 3 degrees of freedom 95% confidence limits are 0.8310498 to 1.323741

Appendix table XI: Effect of Imidacloprid (Imitaf) in mixture of Piperonyl butoxide 1:1 on *R. dominica* adults after 24 hours of exposure.

Dose µg cm^{-2}	Log dose	Num.	Kill	% kill	Corr %	Emp probit	Expt probit	Work probit	Weight	Final probit
3.67	1.5646	50	40	80	80	5.85	5.8654	5.8	25.15	5.828
1.837	1.2641	50	34	68	68	5.47	5.4154	5.45	30.05	5.388
0.918	0.9628	50	22	44	44	4.85	4.9642	4.84	31.7	4.946
0.459	0.6618	50	18	36	36	4.64	4.5135	4.62	29.05	4.505
0.229	0.3598	50	8	16	16	4.01	4.0613	3.99	21.95	4.063
Contr.		50	0							

Y = 3.535997 + 1.465302 X LOG LD_{50} is 0.9991133 LD_{50} is 0.9979604	No significant heterogeneity Chi-squared is 1.054104 with 3 degrees of freedom 95% confidence limits are 0.7674738 to 1.297666

Appendix table XII: Effect of Imidacloprid (Imitaf) in mixture of Piperonyl butoxide 1:5 on *R. dominica* adults after 24 hours of exposure.

Dose µg cm^{-2}	Log dose	Num.	Kill	% kill	Corr %	Emp probit	Expt probit	Work probit	Weight	Final probit
11.022	2.0422	50	44	88	88	6.18	6.3259	6.13	16.8	6.318
5.511	1.7412	50	40	80	80	5.85	5.8500	5.8	25.15	5.850
2.755	1.4401	50	36	72	71	5.55	5.3741	5.52	30.8	5.382
1.377	1.1389	50	28	56	55	5.13	4.8980	5.15	31.35	4.914
0.688	0.8375	50	11	22	20	4.16	4.4217	4.18	27.9	4.446
Contr.		50	1							

Y = 3.144272 + 1.554291 X LOG LD_{50} is 1.193939 LD_{50} is 1.562929	No significant heterogeneity Chi-squared is 4.984581with 3 degrees of freedom 95% confidence limits are 1.183776 to 2.063521

Appendix table XIII: Effect of Imidacloprid (Imitaf) in mixture of Piperonyl butoxide 1:10 on *R. dominica* adults after 24 hours of exposure.

Dose µg cm^{-2}	Log dose	Num.	Kill	% kill	Corr %	Emp probit	Expt probit	Work probit	Weight	Final probit
20.207	1.3054	50	47	94	94	6.55	6.6097	6.54	11.9	6.621
10.103	1.0044	50	42	84	84	5.99	6.0670	5.96	21.95	6.073
5.05	0.7032	50	38	76	76	5.71	5.5242	5.66	29.05	5.525
2.525	0.4022	50	27	54	53	5.08	4.9815	5.06	31.7	4.977
1.26	0.1003	50	13	26	24	4.29	4.4373	4.3	27.9	4.427
Contr.		50	1							
Y = 4.244848 + 1.820536 X LOG LD_{50} is 0.414797 LD_{50} is 2.598945				No significant heterogeneity Chi-squared is 1.634022 with 3 degrees of freedom 95% confidence limits are 2.026392 to 3.333271						

Appendix table XIV: Effect of Carbaryl (Sevin) in mixture of Piperonyl butoxide 1:1 on *R. dominica* adults after 24 hours of exposure.

Dose µg cm^{-2}	Log dose	Num.	Kill	% kill	Corr %	Emp probit	Expt probit	Work probit	Weight	Final probit
7.302	1.8634	50	40	80	80	5.85	5.9099	5.87	23.55	5.912
3.651	1.5624	50	35	70	69	5.5	5.5130	5.47	29.05	5.514
1.825	1.2612	50	30	60	59	5.23	5.1160	5.21	31.7	5.116
0.912	0.9599	50	21	42	41	4.77	4.7188	4.76	30.8	4.719
0.456	0.6589	50	12	24	22	4.23	4.3220	4.23	26.6	4.321
Contr.		50	1							
Y = 3.451457 + 1.320449 X LOG LD_{50} is 1.17274 LD_{50} is 1.48847				No significant heterogeneity Chi-squared is 0.6717568 with 3 degrees of freedom 95% confidence limits are 1.11206 to 1.992286						

Appendix table XV: Effect of Carbaryl (Sevin) in mixture of Piperonyl butoxide 1:5 on *R. dominica* adults after 24 hours of exposure.

Dose µg cm^{-2}	Log dose	Num.	Kill	% kill	Corr %	Emp probit	Expt probit	Work probit	Weight	Final probit
21.906	1.3405	50	44	88	88	6.18	6.2559	6.12	18.5	6.231
10.953	1.0395	50	40	80	80	5.85	5.7850	5.83	26.6	5.772
5.4765	0.7384	50	32	64	63	5.33	5.3140	5.31	30.8	5.312
2.738	0.4374	50	24	48	47	4.92	4.8429	4.94	31.35	4.853
1.369	0.1364	50	13	26	24	4.29	4.3720	4.29	26.6	4.393
Contr.		50	1							

Y = 4.185543 + 1.526538 X LOG LD_{50} is 0.5335324 LD_{50} is 3.416114	No significant heterogeneity Chi-squared is 0.7794876 with 3 degrees of freedom 95% confidence limits are 2.595282 to 4.496558

Appendix table XVI: Effect of Carbaryl (Sevin) in mixture of Piperonyl butoxide 1:10 on *R. dominica* adults after 24 hours of exposure.

Dose µg cm^{-2}	Log dose	Num.	Kill	% kill	Corr %	Emp probit	Expt probit	Work probit	Weight	Final probit
40.161	1.6037	50	48	96	96	6.75	6.6380	6.72	11.9	6.556
20.08	1.3027	50	41	82	82	5.92	6.0829	5.88	21.95	6.026
10.04	1.0017	50	36	72	71	5.55	5.5279	5.52	29.05	5.496
5.02	0.7006	50	25	50	49	4.97	4.9729	4.96	31.7	4.966
2.51	0.3996	50	15	30	29	4.45	4.4180	4.45	27.9	4.436
Contr.		50	1							

Y = 3.732674 + 1.76097 X LOG LD_{50} is 0.7196748 LD_{50} is 5.244146	No significant heterogeneity Chi-squared is 0.8106918 with 3 degrees of freedom 95% confidence limits are 4.061039 to 6.77193