

**TOXICOLOGICAL EVALUATION OF SOME INSECTICIDES AND THEIR
COMBINED ACTION WITH AZADIRACTIN AGAINST THE LESSER
MEALWORM *ALPHITOBIOUS DIAPERINUS* (PANZER)**



**THESIS SUBMITTED FOR THE DEGREE
OF
DOCTOR OF PHILOSOPHY
IN THE
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Dedicated
To
My beloved parents

DECLARATION

I hereby declare that the whole of the work submitted as a thesis entitled **Toxicological evaluation of some insecticides and their combined action with Azadirachtin against the lesser mealworm *Alphitobius diaperinus* (Panzer)** for the Degree of **Doctor of Philosophy** in the Institute of Biological Sciences, University of Rajshahi, Bangladesh, is the result of my own investigation. No part of the thesis has been submitted elsewhere for any degree or diploma.

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The Candidate

CERTIFICATE

This is to certify that Sirajum Muneera worked under our supervisions as a Fellow in the Institute of Biological Sciences, university of Rajshahi. We are pleased to forward her thesis entitled **Toxicological evaluation of some insecticides and their combined action with Azadirachtin against the lesser mealworm *Alphitobius diaperinus* (Panzer)**, which is the record of a bonafide research carried out in the Institute of Biological Sciences, University of Rajshahi, Bangladesh.

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ABSTRACT

The toxicity of five (5) commercially formulated insecticides viz. Fenitrothion (organophosphate); Thiodicarb (Carbamet); Deltamethrin (Pyrethroid), Imidacloprid (Neonectinoide) and Nimbicidine (Azadirachtin) were investigated following residual film method (RFM) and treated flour method (TFM). Adults and larvae (1, 10, 20, 30 and 40-days old) of *Alphitobius diaperinus* Panzer were used in the experiment. The mortality data were recorded after 24, 48 and 72 hours of exposure. All tested insecticides were found to be toxic on the beetles.

The insecticides were moderately and less toxic on adults in residual film method having LD₅₀ values as 3082.80 (Imidacloprid), 3033.83 (Fenitrothion), 1229.48 (Deltamethrin), 376.39 (Thiodicarb), and 61.85 (Azadirachtin) µg/cm² after 24 hrs exposure. Azadirachtin and thiodicarb were the most toxic and fenitrothion and imidacloprid were found to be comparatively less toxic to adults in RFM test. The order of toxicity for adults was Azadirachtin > Thiodicarb > Deltamethrin > Fenitrothion > Imidacloprid. The LD₅₀ values for the mature larvae (40 days old) at same test were 361.78 (Deltamethrin), 257.76 (Fenitrothion), 215.48 (Imidacloprid), 78.47 (Thiodicarb) and 25.28 (Azadirachtin) µg/cm² after 24 hours respectively. The toxicity was higher than that of adults and the order of toxicity was Azadirachtin > Thiodicarb > Imidacloprid > Fenitrothion > Deltamethrin. The LD₅₀ values for the newly hatched larvae (1 day old) at same test were 49.74 (Fenitrothion), 19.62 (Imidacloprid), 4.49 (Azadirachtin), 4.43 (Thiodicarb) and 3.05 (Deltamethrin) µg/cm² after 24 hours exposure. Most of the insecticides were found to be highly toxic on 1 day old larvae. The toxicity was higher than mature larvae and the order of toxicity was Deltamethrin > Thiodicarb > Azadirachtin > Imidacloprid > Fenitrothion.

In Residual Film Method (RFM) imidacloprid was less toxic on adults. Deltamethrin was less toxic on mature larvae but highly toxic on newly hatched larvae. Fenitrothion was less toxic on newly hatched larvae than other insecticides. Azadirachtin and thiodicarb were found highly toxic on all stages of beetles.

In treated food method (TFM), the LD₅₀ values after 24 hours for adults were 2044.41 (Deltamethrin), 901.08 (Fenitrothion), 579.83 (Azadirachtin), 359.46 (Imidacloprid) and 194.23 (Thiodicarb) ppm. For the mature larvae these values were 1095.90 (Deltamethrin), 436.41 (Fenitrothion), 340.98 (Azadirachtin), 307.99 (Imidacloprid) and 49.28 (Thiodicarb) ppm. For the newly hatched larvae these values were 168.90 (Deltamethrin), 161.34 (Fenitrothion), 81.42 (Azadirachtin), 22.39 (Imidacloprid) and 14.62 (Thiodicarb) ppm.

In treated food method, thiodicarb was the most toxic, and deltamethrin was the least toxic against all stages of beetles. The order of toxicity on all stages of beetles was, Thiodicarb > Imidacloprid > Azadirachtin > Fenitrothion > Deltamethrin.

In all type of methods for the larvae and adults, the toxicity of all insecticides were increased with time and decreased with age of insects.

Synergistic effect with azadirachtin in combination with all insecticides was evaluated. The synergistic effects were calculated by using co-toxicity coefficient (> 100) values. The interaction between insecticides and synergist was analyzed for co-toxicity co-efficient and through plotting isoboles of the LD₅₀ values. Azadirachtin considerably increased the toxicity of all insecticides in both adults and mature larvae after 72 hours exposure.

The co-toxicity coefficient of all insecticides indicates synergistic action. The co-toxicity coefficient of azadirachtin indicates synergistic action when combined with deltamethrin and imidacloprid on adults in RFM test. The combination of deltamethrin and imidacloprid with azadirachtin was the most effective for controlling adults in residual film method (RFM).

Deltamethrin+ azadirachtin was the best combination for controlling of mature larvae in both residual film method (RFM) and treated flour method (TFM). Imidacloprid+ azadirachtin combination was the best for controlling of adults in both residual film method (RFM) and treated flour method (TFM).

Thiodicarb and imidacloprid was the most toxic at single action. Deltamethrin was less toxic at single action but when combined with azadirachtin then it was the most toxic on *A. diaperinus*.

Chapter – 1

Introduction

The struggle between men and insects began long before the dawn of civilization and it has been continued without cessation to the present time, and undoubtedly will continue, as long as the human race endures. It is because both men and certain insects constantly want the same things at the same time for their existence. For this reason man has been exploring every possible means to control insects (Metcalf and Flint 1962).

The darkling beetle, *Alphitobius diaperinus* Panzer (Coleoptera: Tenebrionidae), is a significant pest in poultry worldwide. This beetle was originally a pest of dried meats and stored grains (Goodwin and Waltman 1996). However, modifications such as higher bird densities and improved ventilation patterns in the poultry industry since the 1950s have resulted in the emergence of this insect as the most commonly encountered beetles in broiler facilities (Lambkin 2005). Now *A. diaperinus* is a major pest in commercial poultry house (Axtell and Arends 1990). This is also cosmopolitan pest (Salin *et al* 2003) and a minor pest of stored products (Hinton and Corbet 1975; Ichinose *et al* 1980). The darkling beetle not only threatens to the health and production of the birds, but it is also thought to be a health risk to humans, producing allergenic sensitivity in individuals who have been in close contact with it for extended periods of time (Schroeckenstein *et al* 1988). In addition to the immense problems associated with *A. diaperinus* inside poultry houses, these beetles have also been known to cause concern in residential areas. Adult beetles, attracted to light migrate into residential areas following litter distribution as fertilizer on pastures or fields near human habitation (Gall 1980, Axtell 1999).

The lesser mealworms have become the most serious pest affecting several types of poultry production systems. They have a high reproductive rate, are difficult to control, vectors of disease, cause considerable damage to insulation in poultry facilities, and may migrate from litter disposal sites to urban housing areas where they are a nuisance. They also may consume considerable amounts of poultry feeds if they are very numerous (Adams 2003).

High populations of *A. diaperinus* in poultry houses sometimes are of concern due to the potential for insects to harbor pathogens that cause poultry diseases (Eidson *et al* 1966; De Las Casa *et al* 1976,). The beetles harbor and potentially spread a wide variety of viral, bacterial and fungal pathogens of poultry and serve as intermediate hosts of parasites parasitizing poultry (Mc Allister *et al* 1995a).

Chemical pesticides are still indispensable in controlling insect pests both in field and storage due to their quick knockdown and killing properties and due to their easy availability to the farmers/growers. The efficacy of insecticides against storage pests varies greatly after treatment (Suchita *et al* 1989, Pinto *et al* 1997). But the indiscriminate and large-scale use of broad spectrum synthetic pesticides has serious demerits including their persistence in the environment (Smith 1970, Wilkin and Fishwick 1981, Jolly *et al* 1989, Bryne *et al* 1994, Laliberte 1995, Bell *et al* 1999, Rajappan *et al* 2000), toxicity to human beings (Anon 1981, Oudejans 1982, Hasanuzzoha 2004), wild life including pollinator and economically beneficial insects (Munakata 1977, Pimentel 1981, 1983), development of insect resistance to the insecticides (Georghiou and Mellon 1983, Champ 1986, Reichmuth 1992) and finally, higher costs (Khan and Mannan 1991). Moreover, both multi-resistance and cross-resistance to pesticides have been reported in a large number of insects (Metcalf 1980, Georghiou and Mellon 1983). It is now a constant concern in the post-harvest ecosystems throughout the world particularly in developing countries including Bangladesh (Champ 1979, Subramanyam and Hagstrum 1995).

In spite of insecticides being the major means of defense against insect pests, the above mentioned problems have generated a sustained search for either alternative means of insect control methods of reducing the amounts of insecticides required for the pest management (Mondal 1984a, Smet *et al* 1990, Burkholder and Faustini 1991).

In this respect, plant materials may play important role because of their low mammalian toxicity to both human and environment (Jacobson 1990). In several countries locally available plant materials are being widely used to protect stored products against insect pests. The plant materials possessing insecticidal properties

are known as botanical pesticide or biopesticide (Rashid *et al* 2006). Botanicals have broad spectrum activity. They are safe, relatively specific in their mode of action, easy to process and use. (Odderskaer *et al* 2003).

Keeping this in mind, the azadirachtin- a botanical pesticide was used in the present experiment in order to investigate whether it causes synergistic effect on the insecticides against *A. diaperinus*. It may be mentioned that limited numbers of research works on the effect of Azadirachtin on beetle species have been conducted (Malik and Naqvi 1984, Mukherjee and Ramachandran 1989, Hu and Chiu 1993, Xie *et al* 1995, Suss *et al* 1997, Khalequzzaman and Nahar 2003, Banu 2004, Das *et al* 2006, Khatun 2010).



Plate 1: Neem tree



Plate 2: Neem fruits

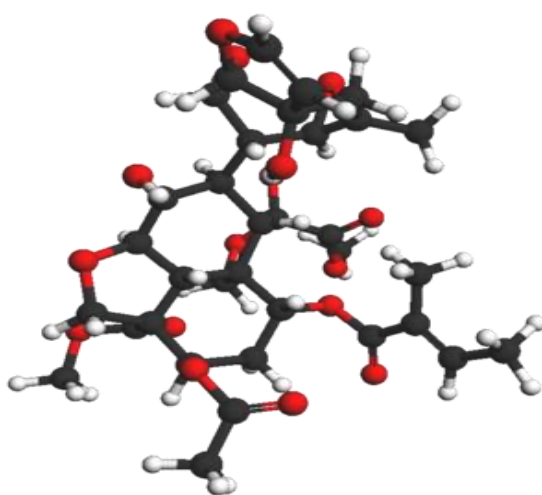


Plate 3: Molecular structure of Azadirachtin



Plate 4: Nimbecidine 0.03%

1.1. Background of the Study

In Bangladesh during the storage of grains and cereals or milled products use of insecticides is limited. Even within this limited application scientific methods or processes are not duly followed. This may be due to lack of proper knowledge, training and skill (Ferdous 2006). Chemical control, in spite of its so many demerits has been the primary method of pest control and is still in use. As mentioned earlier, pesticide use in Bangladesh started from mid-fifties and gained momentum in late 1960 (Alam 1991). Until 2000, 17 companies (national and multinational) were involved in pesticide formulation, repacking, distribution and supply through their own sales and distribution network in Bangladesh (Hasanuzzoha 2004).

In Bangladesh farmers or grain stockers use pesticide whimsically and irrationally. Most of them do not know the significance of using right pesticides in right time with right dosage using right equipments. These inappropriate and misuse of pesticides have led to the loss of effective pesticides due to the development of resistance (Forrester 1990) and cause human health hazards like nausea and vomiting, stomach pain and diarrhoea, eye and skin irritations, as are detected after pesticide application, and environmental pollution (McInlyre *et al* 1989).

However, in our country the stored product entomologists are trying to get rid of the hazards of conventional neurotoxic insecticides and endeavour is being made to find out potent alternative control measures for insect pests in the food stores, which would be safer for non-target organisms including human beings. In this regard, they are trying to manipulate insect growth and development rather than to kill them within the food grains. The plant materials are being used in this regard as the botanical insecticides. From this effort, the present experiments were planned.

1.2. Objectives of the Study

Pesticides are being used to kill, repel, or regulate the growth of biological organisms. The release of these chemicals into the environment creates a potential for unintended adverse health impacts to both humans and non target wildlife. Mixtures of pesticides are common in the human food supply (National Research Council 1992).

Another concern regarding the wide spread use of pesticide is the development of resistant pest strains to insecticides. Resistance to one or more pesticides has been reported in at least 477 species of insects and mites (Geoghiou and Mellon 1983). 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.

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.

The above problem has generated a sustained search for their alternative means of insect control as methods of reducing the amount of insecticide required for the pest management. The toxicity of azadirachtin is increased with time (Tang *et al* 2001). In toxicology, synergism is defined as the case where the toxicity of two compounds applied together is greater than would be expected from the sum of their individual effects (Olkowski *et al* 1991).

Keeping this in mind the present study was undertaken. The research reported here was initiated to investigate the toxicity of fenitrothion (Sumithion 50 EC), deltamethrin (Decis 2.5 EC), imidacloprid (Confidor 70WP), thiodicarb (Larvin 75WP) and azadirachtin (Nimbicidine .03%) both independently and in combination against *A. diaperinus*. The goal was to minimize the use of insecticides which will ultimately help reducing environmental pollution and human health hazards.

With a view to achieve the above objectives, the following experiments were conducted:

- > To observe the toxicity of deltamethrin, fenitrothion, imidacloprid, thiodicarb and azadirachtin alone on *A. diaperinus* adults and different aged larvae (1, 10, 20, 30 and 40 days old) after 24, 48 and 72 hours in Residual Film Method (RFM) and Treated Food Method (TFM).
- > To observe the toxicity of Deltamethrin, Fenitrothion, Imidacloprid, Thiodicarb combined with Azadirachtin on *A. diaperinus* adults and matured larvae (40 days old) after 72 hours in both Residual Film Method (RFM) and Treated Food Method (TFM).
- > To study the biology of *A. diaperinus*.

Chapter -2

REVIEW OF LITERATURE

2.1 *Alphitobius diaperinus*

The generic name of *Alphitobius* was first published by Stephens in 1829 with the specific epithet *diaperinus*, which was established by Panzer in 1794 (Spilman 1966, Poole and Gentili 1996).

A. diaperinus has been referred to by a multitude of common names. It is commonly called as “Litter beetle”, “Darkling beetle” and “Black bug”- a pest of chicken house, and “Lesser mealworm”- a pest of stored milled products. The beetle is also called as “Cereal mould beetle” (Sterin 1996), shining black wheat beetle, black fungus beetle, black poultry bug, Schmittle beetle, and shiny black moldy grain beetle (Swatonek 1970, Nolan 1982).

They are found in grain bins, mills and poultry houses throughout the world. (Adams 2003). They are thought to have originated in sub-Saharan Africa (Lambkin 2001; Geden and Hogsette 2001)

Darkling beetles have the ability to find nourishment in diverse places. They are voracious predators as well as proficient scavengers (Harding and Bissell 1958). They feed on grains and flour, particularly in damp, musty sites. Poultry houses with deep litter are ideal breeding grounds. Adults have been found feeding on carcasses in poultry house. (Adams 2003). It usually feeds on damp and moldy grain, milled products and spoiled foods. The larvae of *A. diaperinus* ingest chicken feed and other organic matter including dead or moribund chicks (Hickle *et al* 2008). The darkling beetle was previously thought to be exclusively phytophagous or saprophagous until 1958 when it was observed attacking dead and moribund birds (Harding and Bissell 1958). In poultry houses the darkling beetle has been regarded as the best-adapted scavenger (Pfeiffer and Axtell 1980), where, in addition to consuming feed and manure, adults and late-instar larvae prey on other insects, and dead or dying birds (Axtell and Arends 1990). The omnivorous diet of *A. diaperinus* also means that they can compete with the birds for their feed (Roche *et al* 2009). They live in the poultry litter where they eat organic waste (Fabio and Rafael 2011). They feed on almost anything -decaying litter, poultry feed, bird carcasses and even

each after cannibalistic (Adams 2003). They show cannibalism (Lyon 2000, Nahar and Wadud 2000). Larvae are known to feed on bat guano, mold and on sick dead bats, chickens and pigeons (Falomo 1986).

The darkling beetle's mouthparts indicate that the adult beetle is a general feeder, while the larva possesses planar molar surfaces that are adapted for feeding on "cemented" food material. The darkling beetle's propensity for scavenging and continuous feeding could have significant implications concerning their potential as disease vectors (McAllister *et al* 1995a, b).

2.1.1 Habits and behavior:

Habits: Darkling beetles are commonly found in woods or around feed bins. These beetles fly well and are attracted to lights at night but hide during the day. Matured larvae seek sheltered place to pupate. Most of the damage to insulation is done by lesser mealworms seeking a safe place to pupate.

All stages of the darkling beetle are killed by temperatures below 30°F (Adams 2003). They survive on the floor of a broiler production house in the accumulated mix of bedding material, excreta, feathers, spilt feed, carcasses and other debris referred to as litter (Despins and Axtell 1994).

Behavior: Lesser meal worm usually is not distributed evenly throughout a house. They tend to congregate in areas that are most favorable for them. Usually this is where there is adequate moisture or where the litter is looser and deeper. The larvae and adults tend to accumulate under anything laying on or just under the surface of the litter. Floor feeders provide excellent places for them to hide. If nothing is available they will eat around the edges of caked litter. Mealworm larvae and adults avoid very dry or very wet areas but do need some moisture to survive (Adams 2003).

Larvae cluster in dark corners under manure or litter, under feed sacks or under feed in the feed storage areas. Larvae may congregate in areas of higher temperature and moisture, such as near water or wet feed. Late instar larvae may migrate upward and pupate in the insulation of the building dried areas of the manure or litter and in cracks and crevices (Adams 2003). Larvae also bore into wood posts, beams, paneling, drywall, and insulation (Dunford 2000). When the final instar is achieved, the larvae seek out pupation substrates in the earth floor or

insulation for protection against predators and cannibalism from adult and larval conspecifics (Ichinose *et al.* 1980, Despins *et al.* 1987, Geden and Axtell 1987). Geden and Axtell (1987) evaluated the behavior of the larvae in both field and laboratory conditions and found that climbing occurred primarily at night between 2000 and 2400 h, and that it is influenced by both soil availability as a pupation substrate and larval density (Boozer 2011).

The pupae wiggle when disturbed and lay motionless otherwise. They are found in lower, compressed litter, dry manure, or in the soil. They are also found in the insulation and the result is extensive damage to all type of insulation (Adams 2003)

The adults are very active and burrow into litter when disturbed. They are also found crawling on walls, hiding in cracks and crevices or feeding on the underside of bird carcasses. Adult beetles can fly for approximately ½ mile. They lay eggs in the manure and litter, especially under feed and water lines (Adams 2003).

2.1.2 Origin & distribution:

A. diaperinus is believed to have originated in sub-Saharan Africa in association with bird nests and bat caves (McFarlane 1971, Vaughan *et al* 1984, Lambkin 2001). It has been imported into temperate regions via commerce, in stored food products (Crook *et al* 1980).

They are cosmopolitan in distribution. *A. diaperinus* was first known as a secondary pest usually found in flour-mill basements infesting damp or musty flour or grain, preferring cereal products that are slightly out of condition (USDA 1953). It is believed to have first infested Indiana brooder houses from crushed corn cobs that were used as insulation for the walls (Gould and Moses 1951) as well as in Maryland from corn cob litter (Harding and Bissell 1958). Although *A. diaperinus* is well known as a pest of seeds, grain, feed, and cereal, this beetle has a long list of hosts worldwide, including an assortment of other plant and animal matter (Crook *et al* 1980).

2.1.3 Life cycle & Biology

The life cycle of *A. diaperius* is temperature dependent. The life cycle varies from one to three months depending on environmental condition. The temperature in the poultry house and the accumulation of feed and organic matter promote ideal conditions for beetle infestation (Fabio and Rafael 2011).

For egg- There is a marked reduction in egg hatch below 70°F.

For larvae- development time from egg to adult increases with decreasing temperature, eg.

At 100°F – 42 days

At 80°F – 58 days

At 60°F – 97 days

Most Larvae develop through 5 – 9 instars, the number is increased with lower temperature (Dunford 2000). Temperature is more important than moisture in rates of development and survival. Moisture level of about 12% is optimum (Lyon 2000).

Optimum Life stages:

The life cycle depends on the temperature and moisture (Adams, 2003). The mean incubation, larval and pupal period are 6.6, 68.8 and 8.4 days respectively (Das *et al.* 1986). Development from egg to adult is completed at stored condition (27°C) within 70-79 days and at poultry house (21-35°C) 40-80 days (Lyon 2000, Das *et al.* 1986).

2.1.3.1 Egg: The egg of the darkling beetle, when first laid, is creamy white in color and darkens with age. It is oval in shape and ranges in length from around 1.0 to 1.4 mm, with average width approximately 0.44 mm (Plate-6) (Preiss 1969). As development progresses the egg shape alters from oval to slightly concave. When laid, the eggs are anchored into cracks and crevices by the female darkling beetle, via a clear sticky substance (Wilson and Miner 1969). The eggs are often laid in clusters. Temperature is an important factor in egg development. Egg hatch can occur anywhere from around 3-13 days after oviposition. The highest rate of egg hatch occurs at 30°C (Rueda and Axtell 1996). Relative humidity also plays a role in egg hatch. The highest percent of egg hatch occurred at a relative humidity of 68-71% (Barke and Devis 1969; Preiss and Davidson 1968).

2.1.3.2 Larva: The larvae (plate- 7) ostensibly resemble true mealworms (*Tenebrio* spp.). They have three pairs of legs and segmented bodies that taper posteriorly (Dunford and Kaufman 2006). A newly hatched larva is about 1.5 mm in length and white in color (Wilson and Miner 1969, Francisco and Prado 2001). As it grows and the cuticle hardens, the larva darkens to a brownish color (Francisco and Prado 2001).

The larvae grow to about 10 mm in length before pupating. The duration of the larval period is dependent on temperature, and can be from 22.4 to 133 days at temperatures ranging from 35 to 20°C. No larval development is observed at temperatures as low as 17°C (Rueda and Axtell 1996). The number of larval instars is also highly variable, ranging from 6 to 11 instars. Wilson and Miner (1969) observed 11 larval instars at a temperature of 15.5°C; however, at 26.6°C only a single larva reached the 9th instar (Dunford and Kufman 2006).

2.1.3.3 Pupa: The pupae (Plat- 8) are initially white in color, but change to tan within a day (Barké and Davis 1969). Female pupal length was found to be significantly different from that of the male, with female lengths averaging 5.9 mm while males averaged 5.5 mm (Preiss 1969). Sexual dimorphism occurs in the pupal stage (Barké and Davis 1969). The difference between sexes can be observed on the ventral posterior section of the abdomen, where the female has a pair of non-sclerotized fleshy projections that are not observed in the male (Barké and Davis 1969, Preiss 1969). These projections are second valvifers (Barké and Davis 1969). The pupal stage can be found in the soil floors of broiler houses, as well as in the insulation, particularly when population numbers are high (Safrit and Axtell 1984, Geden and Axtell 1987). The pupal stage lasts about 4 to 17 days, depending on temperature. Mean pupal development times under lab conditions were 17.0, 8.0, 5.5, 4.0 and 4.1 days at temperatures of 20, 25, 30, 35, and 38°C respectively (Rueda and Axtell 1996). Legs appear to be tucking alongside (Adams 2003).

2.1.3.4 Adult: Lesser mealworm adults (Plate-9) are broadly oval, moderately convex, lack or brownish-black and usually shin in appearance (Kaufman *et al.* 2005). A newly enclosed adult darkling beetle is soft bodied and reddish brown in color. In laboratory conditions it has been shown to take an average of 7d for the cuticle of the darkling beetle to harden and darken to its characteristic color (Wilson and Miner 1969, Preiss and Davidson 1971). However, Hopkins *et al* (1992) found that in their laboratory environment an average of only 5 d was required for completion of this tanning process (Wilson and Miner 1969). Color can be variable depending on age or 'strain'. Length is approximately 5.8 to 6.3mm. Antennae are densely clothed with short yellowish hairs, with the terminal segment lighter in color. The head is deeply emarginated in front, with a distinct clypeal groove, and the surface is coarsely punctured. Eyes are also emarginated. The pronotum is twice

as broad as long, slightly narrowed from base to apex with sides feebly curved and narrowly margined. The elytra have moderately impressed striae with finely punctured, feebly convex intervals. Elytral punctures are sparsely and nearly as large as those of the striae. The ventral surface of the insect body is dark radish-brown, with the prosternal process horizontal between coxae and having a prominent apex (Kaufman *et al.* 2005). The sex of the darkling beetle adult can be determined based on the shape of the metathoracic tibial spines. The male has one straight and one curved metathoracic tibial spine, while both spines are straight on a female. A male can also be recognized by the deeply emarginated posterior edge of the 8th sternite, which is straight on a female (Barké and Davis 1969). The mean life-span of *A. diaperinus* has been reported as greater than 400 d (Preiss and Davidson 1971), after mating, within six to ten days a female beetle has the potential to lay more than 2000 eggs (Adams 2003). Most of the adult's life span is 14-16 months and each female laid 1059 to 1874 fertile eggs in her life time. Some adults survive longer than 16 months and some females laid very few eggs (Falomo 1986).

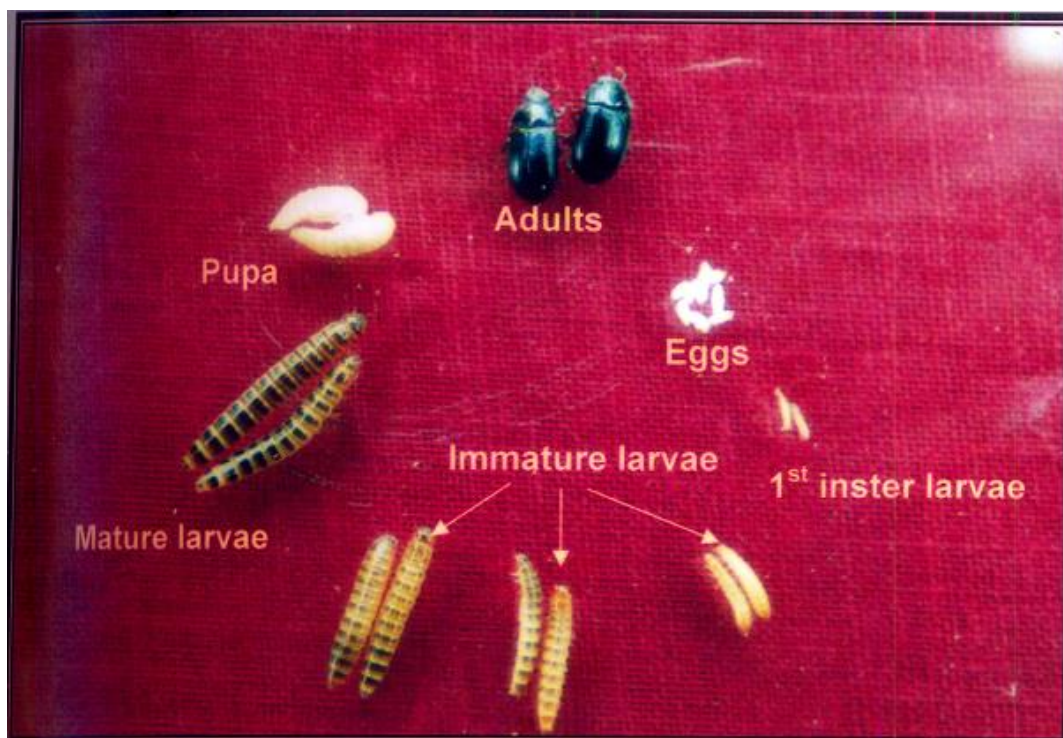


Plate 5: Lifecycle of *A. diaperinus*.

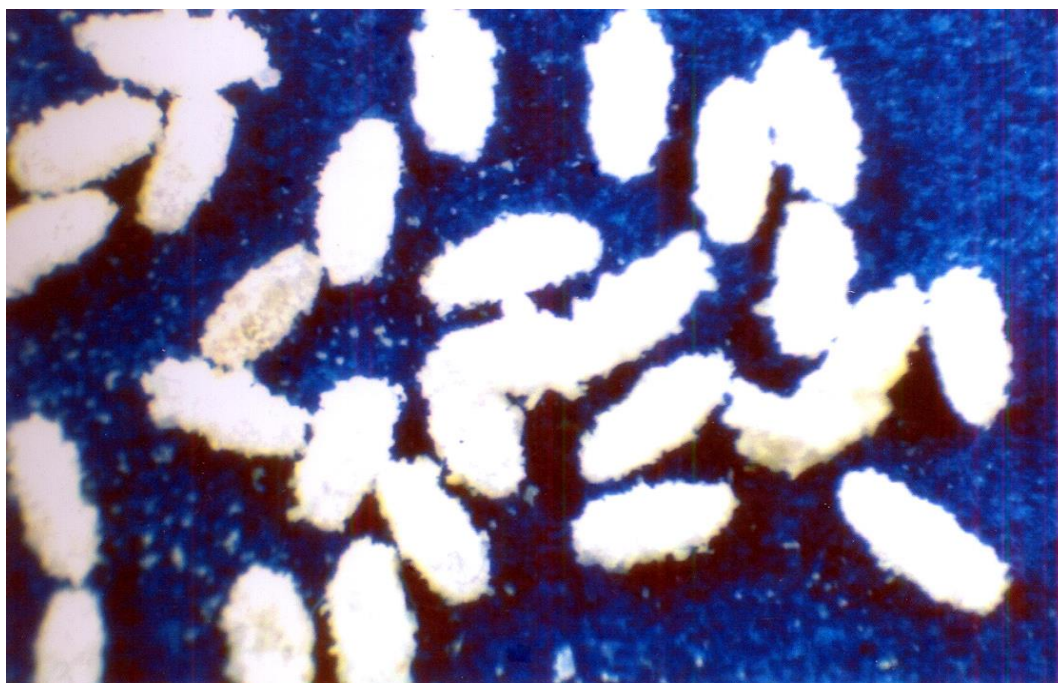


Plate 6: Eggs of *A. diaperinus*



Plate 7: Mature larvae of *A. diaperinus*.



Plate 8: Pupae of *A. diaperinus*

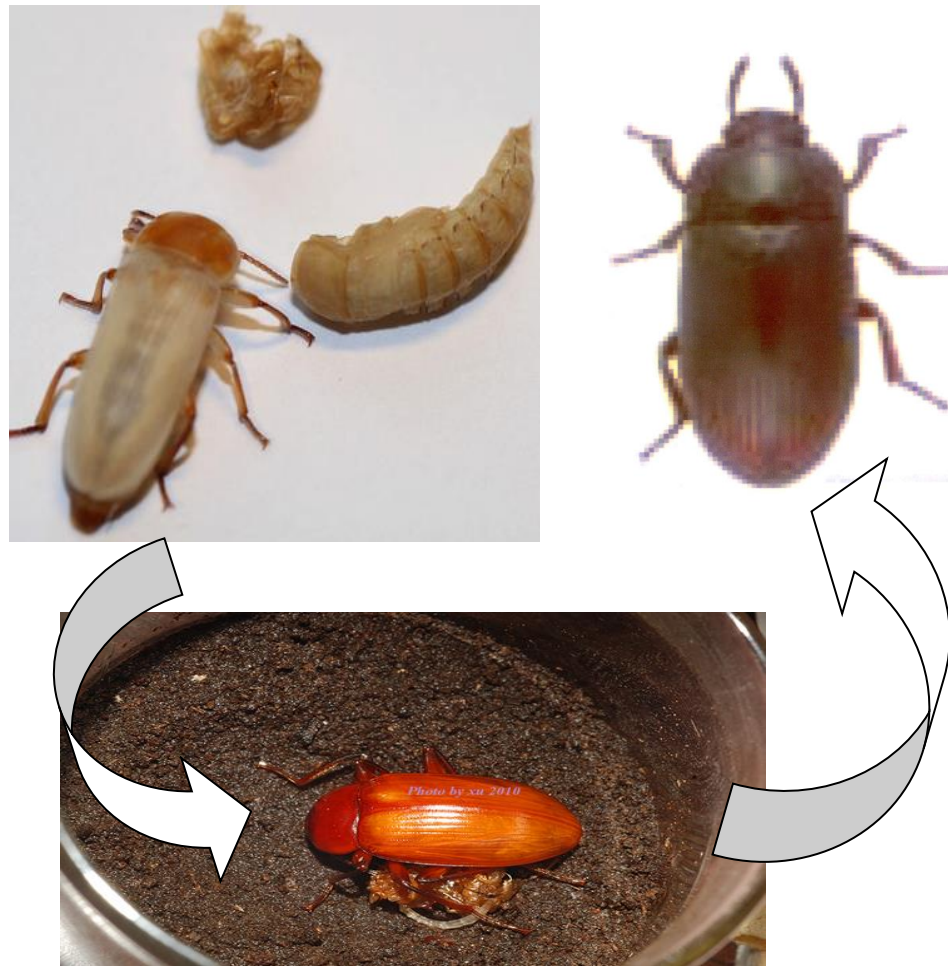


Plate 9: Newly enclosed adults to darken color

2.2. Economic importance:

While the darkling beetle is considered a pest of stored products, it does not pose a significant economic threat to this industry. The grains these beetles feed on are often already damaged. However, the darkling beetle is considered the foremost premise pest in the poultry industry (Axtell 1999). *A. diaperinus* is a major pest in commercial poultry house (Axtell and Arends, 1990). In past few years litter beetles (especially the lesser mealworm *A. diaperinus*) have become the most serious pest affecting several types of poultry production systems (Campbell and Borden 2006). This is also cosmopolitan (Salin *et al* 2003) and a minor pest of strode products (Hinton and Corbet 1975; Ichinose *et al* 1980).

The pest status of lesser mealworm arises from three causes. Firstly, the beetles are known to harbor many important avian pathogens and parasites (Despins and Axtell 1995, Despins *et al* 1994, Davis and Wray 1995, Davis *et al* 1996, Goodwin and Waltman 1996, McAllister *et al* 1995b 1996).

Secondly, adult beetles cause public nuisance problems by invading the homes and businesses of neighbors (Turner 1986, Schmitz and Wohlgemuth 1988).

Thirdly, the destruction of thermal insulation materials by beetle larvae results in increased energy consumption and costly replacement of the insulation (Ichinose *et al* 1980, LeTorc'h and Letenneur 1983, Vaughan *et al* 1984).

2.2.1. As a Poultry Pest: *A. diaperinus* is one of the key insect pest. In the poultry industry. In the past years, the beetles have become the most serious pest affecting several types of poultry production systems. They cause considerable damage to insulation in poultry facilities, and may migrate from litter disposal sites to urban housing areas where they are a nuisance. Litter beetles are known to harbor a number of disease organisms that affect poultry. These are fowl pox, *E. coli*, Salmonella sp., Marck's disease, Avian influenza, Fowl pox, botulism, coccidiosis, Newcastle disease, avian leukosis virus and infectious bursal disease virus (IBDV). The beetle has also been identified as the intermediate host of poultry tapeworms and cecal worms. The beetles are known to harbor pathogens for at least 14 days and still remain infectious to broiler chicks if eaten. The presence of abundant disease organisms is particularly troublesome in floor-litter systems such as broiler, breeder, and turkey housing where the birds can consume large quantities of beetles, especially in the first week or so after placement. Consumption of large numbers of beetles also has adverse effects on these young birds (Adams 2003).

2.2.2. As a stored pest: *A. diaperinus* is a cosmopolitan pest commonly found in stored grains and flour. The beetle is also known as the lesser meal worm due to the appearance of the larval stage and its frequent occurrence in flour and feed products (Legner and Olton 1970, Pfeffer & Axtell 1980). *A. diaperinus* is a notorious and harmful pest of a great variety of stored grains and cereal products, but the status is of minor pest (McAllester *et al* 1995a).

2.2.3. As a human health hazard: *A. diaperinus* is a known reservoir for many human being due to salmonella infected poultry caused by *A. diaperinus* (McAllister

et al., 1994). The beetle also cause human allergy (Schroeckenstein *et al.*, 1990, Hickie *et al.*, 2008). Another area of concern regarding *A. diaperinus*, produce highly reactive benzoquinones as defense against predation (Tschinkel 1975). Quinones can be hazardous to human health and cause health risks when exposed to the insect for extended periods. Reported health related ailments caused by *A. diaperinus* include symptoms of asthma, headaches, dermatitis, allergic engiodema, rhinitis, erythema (reddening) and formation of papules (Falomo 1986, Schroeckenstein *et al.* 1988, Tseng *et al.* 1971). Exposure to quinine vapors can also result in conjunctivitis and corneal ulceration (Falomo 1986, Schroeckenstein *et al.* 1988).

2.2.4. As a Predator: The potential of *A. diaperinus* as a biological control agent for stored products pests have been reported. Larvae and adults of *A. diaperinus* feed voraciously on eggs of *S. cerealella* (25eggs/day); adults also preyed on larval of *S. cerealella*, *Tribolium castaneum* and *Lasioderma serricorne* (Gautam 1989). The beetle dries up the moist manure of the poultry and makes it unfavorable for fly breeding (Propp and Morgan 1985, Wallace *et al.*, 1985). The larvae and adult of *A. diaperinus* gregariously feed on eggs and larvae of *Corcyra cephalonica* (Das *et al.* 1986). It also plays role as predator of larvae and pupae of *Musca domestica* in poultry houses (Neves *et al.* 1987). Intensive beetle activity helps to aerate and dry the manure (Despins *et al.* 1988).

2.2.5. Other economic Importance: Beetle populations in the hundreds of thousands have been found on and in caves inhabited by bats in various parts of the world. Larvae are known to feed on bat guano, mold and on sick or dead bats, chickens and pigeons. They also feed on animal parts such as feathers, and other lesser mealworm individuals (Falomo 1986).

2.3 IMIDACLOPRID

Imidacloprid (Table-1) was introduced in Europe and Japan in 1990 and first registered in the U.S. in 1992 (Matsuda *et al.* 2001). Very possibly it is used in the greatest volume globally.

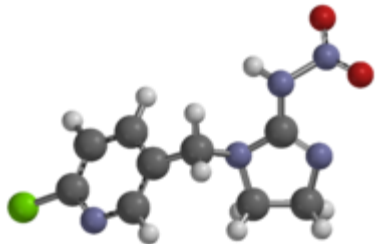
Imidacloprid is a systemic insecticide, having good root-systemic characteristics and notable contact and stomach action. It is used as a soil, seed or foliar treatment in cotton, rice cereals, peanuts, potatoes, vegetable, pome fruits, pecans and turf, for

the control of sucking insects, soil insects, whiteflies, termites, turf insects and the Colorado potato beetle, with long residual control. Imidacloprid has no effect on mites or nematodes. Imidacloprid is not very toxic to fish, amphibians, and even some aquatic invertebrates. No effects on any aquatic species are likely after either tree injection or soil injection applications to predominantly clay or loam soils.

Trade and brand name: Imidacloprid is currently the most widely used insecticide in the world (Doull *et al.*, 1991). Although it is now off patent, the primary manufacturer of this chemical is Bayer Crop Science (part of Bayer AG). It is sold under many names for many uses; it can be applied by soil injection, tree injection, application to the skin of the plant, broadcast foliar, ground application as a granular or liquid formulation, or as a pesticide-coated seed treatment (USDA 2005, National Pesticide Information Center, 2010) Imidacloprid is widely used for pest control in agriculture. Other uses include application to foundations to prevent termite damage, pest control for gardens and turf, treatment of domestic pets to control fleas (Gervais *et al.* 2010), protection of trees from boring insects and in preservative treatment of some types of lumber products (ESR, 2011). It is currently marketed as several proprietary products worldwide, e.g., Admire, Confidor, Gaucho, and Provado (Matsuda *et al.* 2001).

Metabolism: The metabolism of neonicotinoid compounds, including imidacloprid, is complex (Tomizawa and Casida 2004). Imidacloprid is a nitroguanidine molecule, composed of a pyridinyl moiety (a 6- member nitrogen-containing ring with a chloride substituent) and an imidazolidine ring (a 5- member ring with 2 nitrogens, with the =N-NO₂ nitroimine substituent on the carbon between the nitrogens). On the basis of the observed LD₅₀ values, imidacloprid and its nitrosoimine metabolite are classified by EPA as slightly to moderately toxic.

Table 1. Physical and chemical properties of imidacloprid (California Department of Pesticide Regulation, internal database).

Common name	Imidacloprid
IUPAC name	1-[(6-chloropyridin-3-yl) methyl]-N-nitro-4,5-dihydroimidazol-2-amine
CAS Number	105827-78-9
Color and odor	colorless, odorless crystal
Molecular weight	255.7
Water solubility	514 mg/L (20°C at pH 7)
Vapor pressure	1.00×10^{-7} mmHg (20°C)
Hydrolysis half-life	>30 days (25°C at pH 7)
Aqueous photolysis half-life	<1 hour (24°C at pH 7)
Anaerobic half-life	27.1 days
Aerobic half-life	997 days
Soil photolysis half-life	38.9 days
Field dissipation half-life	26.5 – 229 days
Henry's constant	6.5×10^{-11} atm m ³ /mole (20°C)
Octanol-water coefficient (K_{ow})	3.7
Chemical structure	

Mode of action:

Imidacloprid is a systemic insecticide which acts as an insect neurotoxin and belongs to a class of chemicals called the neonicotinoids which act on the central nervous system of insects with much lower toxicity to mammals. The chemical works by interfering with the transmission of stimuli in the insect nervous system. Specifically, it causes a blockage in the nicotinergeric neuronal pathway. This blockage leads to the accumulation of acetylcholine, an important neurotransmitter, resulting in the insect's paralysis, and eventually death. It is effective on contact and via stomach action. Because imidacloprid binds much more strongly to insect neuron receptors than to mammal neuron receptors, this insecticide is selectively more toxic to insects than mammals (Gervais *et al.* 2010).

Imidacloprid controls sucking insects, soil insects, termites, and some chewing insects, and is effective against all feeding stages. It is used to treat seeds, soil, crops and structures, and is a flea control treatment on domestic pets (Meister, 2000).

Toxicology

The technical grade imidacloprid is more toxic than imidacloprid formulations, and more toxic than its nitrosoimine metabolite (not the des-nitro metabolite) which is sometimes found in food commodities. The lowest LD₅₀ value for technical grade imidacloprid, 131 mg/kg body weight, was observed in male mice (Bomann 1989b). The lowest LD₅₀ value for the nitrosoimine metabolite (NTN 37571 or WAK 3839), 200 mg/kg, was observed in fasted male and female mice. Animal toxicity is moderate when ingested orally and low when applied dermally. It is not irritating to eyes or skin in rabbits and guinea pigs (although some commercial preparations contain clay as an inert ingredient, which may be an irritant). In rats, the thyroid is the organ most affected by imidacloprid (Nakazato 1988).

2.4 DELTAMETHRIN

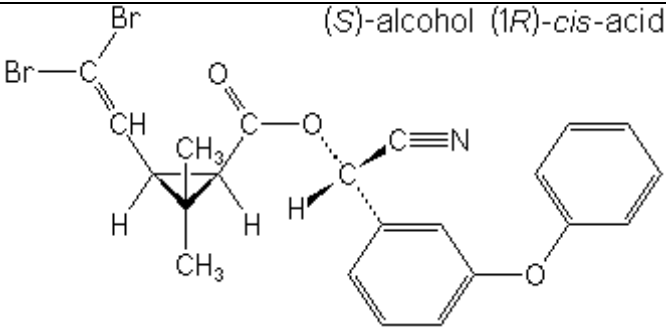
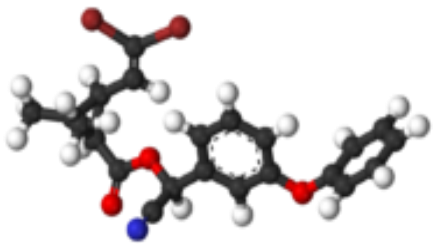
Deltamethrin (Table-2) was first synthesized in 1974. Deltamethrin belongs to the chemical class of pyrethroids, naturally occurring insecticidal compounds that are synthesized from chrysanthemum flowers. Its most common appearance is either as a colorless or slightly beige powder, both of which are odorless (Casida 1973). As a group, pyrethroids are regarded as safer for humans than the other classes of insecticide (Kolaczinski and Curtis 2004).

Deltamethrin is widely used in the agricultural sector, as it has high efficacy against a large number of insects. Its low toxicity to humans has made it one of the insecticides of choice in many countries (Aldridge 1990). Deltamethrin acts on the nervous system via its interaction with various channels and receptors, although its primary target is the voltage-dependent sodium channels (Ray and Fry 2006). Its neurotoxicity in adults is well characterized, although information regarding its developmental neurotoxicity is still limited (Shafer *et al* 2005). As a lipophilic compound, deltamethrin is not soluble in water and therefore is highly stable in the physical environment. Deltamethrin can produce a variety of acute health conditions, but these can be prevented with necessary precautions.

Toxicity:

Deltamethrin is considered low in toxicity to birds but highly toxic to several fish species and other aquatic organisms. Testing with earthworms indicates that there was no observable adverse effect at high agronomic application rates but when the concentrations were increased by 5 to 10 times the highest application rate - there was significant toxic effects (Ray and Fry 2006). In laboratory trials Deltamethrin LC50 for fish is C. 0.001 -0.01 mg/l. In normal conditions outdoors it is harmless to honeybees. Aquatic fauna, particularly Crustacea, may be affected, but fishes are not harmed under normal conditions of use. ADI for man is 0.01 mg/kg (Worthing 1987).

Table 2. Physical and chemical properties of Deltamethrin

Common name	Deltamethrin (ISO, BSI), formerly Decamethrin
Synonyms	Decamethrin; Decis ^(R) ; K-othrin ^(R) ; NRDC 161; OMS 1998; RU-22974
IUPAC Name	(S)- -cyano-3-pehoxybenzyl(1R)-cis-3-(2,2- dibromovinyl)-2,2-dimethylcyclopropane carboxylate
CA Name	(1R (S*),3)-cyano(3-phenoxybenzyl) methyl 3- (2,2-dibromovinyl)-2,2-dimethylcyclopropanecarboxylate
Empirical formula	C ₂₂ H ₁₉ Br ₂ NO ₃
Structural formula	(S)-alcohol (1R)-cis-acid 
Moller mass	505.2
Melting point	98-101°C
Appearance	Colorless
Physical state	crystalline powder
Odour	it is odourless and non-corrosive
Vapour pressure	At 25°C, 1.9996 x 10 ⁻⁹ kPa (1.5 x 10 ⁻⁸ mmHg).
Chemical structure	

Ecotoxicity

Acute oral LD50 for rat	135-5000 mg/kg
Acute oral LD50 For dog	> 300 mg/kg
Acute oral LD50 For duck	> 4640 mg/kg
Honey bees LD50	50 mg/bee

Characteristics

Deltamethrin is a pyrethroid insecticide exclusive to Roussel Uclaf. Deltamethrin is non-systemic insecticide with contact and stomach action (BCPC 1994) and with good residual activity (Hill 1990). Delta dust (a.i, deltamethrin, 05%) is an odourless and non-staining product. It is the world's only water-proof insecticide dust. It is extremely stable on exposure to air (stable 190°C). Under UV irradiation and in sunlight, a cis-trans isomerisation, splitting of the ester bond and loss of bromine occur. It is more stable in acidic than in alkaline media (BCPC 1994).

Advantages

Deltamethrin has many advantages over other insecticides. It has low mammalian toxicity. According to Jermannaud and Pochon (1994), deltamethrin is exceptionally potent insecticide which is used at very low application rates against a spectrum of stored-product pests. At the normal application rates required to give at least six months control of most stored-grain insects, the level of residues in processed and baked products will remain well below the limits accepted by national and international regulatory authorities.

Deltamethrin can either be used alone (Guizhong *et al* 1999) or combined with other insecticides (Xianjin *et al* 1999) for the protection of stored cereals. Its characteristics are different from other insecticides already marketed for the protection of grains. It has a residual activity that generally lasts more than a year. Its effect is increased by the use of piperonylbutoxide (PB) whereas organophosphorous compounds used to protect grains often have their activity showed by this synergist. Deltamethrin is not particularly susceptible to temperature variations. It has been noted that pests which have become resistant to organophosphorous insecticides remain susceptible to deltamethrin and that certain Malathion resistant species show an increased susceptibility to deltamethrin (Picollo de Villar *et al* 1987, Duguet *et al* 1990).

2.5 FENITROTHION

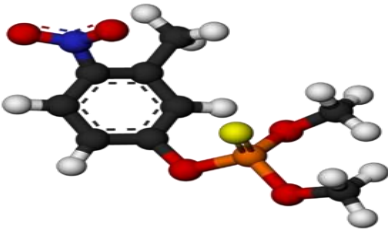
Fenitrothion was introduced in 1959 by both Sumitomo Chemical Company and Bayer Leverkusen and later by American Cyanamid Company (Worthing 1987, Gallo and Lawryk 1991, Hayes 1982). It is a general use pesticide.

Fenitrothion is a contact insecticide and selective acaricide of low ovicidal properties (Spencer 1981). It belongs to the organophosphate family of insecticides. It is considered as a cholinesterase inhibitor (Kidd and James 1991). Fenitrothion is effective against a wide range of pests, i.e. penetrating, chewing and sucking insect pests on cereals, cotton, orchard fruits, rice, vegetables, and forests. It may also be used against fly, mosquito, and cockroach residual contact spray for farms and public health programs (Worthing 1987, Thomson 1982). Fenitrothion is also effective against household insects and all of the nuisance insects listed by the World Health Organization. Its effectiveness as a vector control agent for malaria is confirmed by the World Health Organization (Worthing 1987). Fenitrothion is non-systemic and non-persistent (Spencer 1981, Hassall 1990, Briggs 1992). Fenitrothion is far less toxic than parathion.

Trade or other names: The active ingredient fenitrothion is found in a variety of commercial insecticides. Trade names for products containing fenitrothion include Accothion, Agrothion, Cyfen, Cytel, Dicofen, Fenstan, Folithion, Kaleit, Mep, Metathion, Micromite, Novathion, Nuvanol, Pestroy, Sumanone, Sumithion, and Verthion (Worthing 1987). Fenitrothion comes in dust, emulsifiable concentrate, flowable, fogging concentrate, granules, ULV, oil-based liquid spray, and wettable powder formulations. It is available as a 95% concentrate, 50% emulsifiable concentrate, 40% and 50% wettable powder and 2%, 2.5%, 3% and 5% dusts (Gallo and Lawryk 1991, Hayes 1982). It is compatible with other neutral insecticides (Kidd and James 1991).

Fate in Humans and Animals: Fenitrothion is oxidized by mono-oxygenases in animals, insects and plants and is thereby changed to derivatives containing the P=O group, which are more powerful inhibitors of cholinesterase than was the original thiophosphate. After that, further degradation occurs by rupture of a P-O-CH₃ linkage which is more quickly metabolized in the liver than the P-O phenyl linkage rupture occurring with parathion, which could contribute to fenitrothion's low mammalian toxicity (Gallo and Lawryk 1991).

Table 3. Physical and chemical properties of Fenitrothion

Appearance	Pure material forms a yellowish brown liquid with an unpleasant odor (Gallo and Lawryk 1991, Hayes, 1982)
Chemical Name	O,O-dimethyl O-4-nitro-m-tolyl phosphorothioate (IUPAC), O,O-dimethyl O-(3-methyl-4-nitrophenyl) phosphorothioate (CA), O,O-dimethyl O-(3-methyl-4-nitrophenyl) thiophosphate (Kidd and James, 1991)
Molecular Weight	277.25 (Gallo and Lawryk 1991, Hayes, 1982)
Solubility	Insoluble in water (Gallo and Lawryk 1991). Readily soluble in common organic solvents, e.g. acetone, alcohol, benzene and chlorinated hydrocarbons (Kidd and James, 1991).
Melting Point	0.3 °C (U.S. Environmental Protection Agency. July 30, 1987)
Vapor Pressure	18 m Pa at 20°C (Worthing, 1987)
Stability & Volatility	Fenitrothion is completely stable for two years if stored at temperatures between 20 and 25 °C. Storage temperature should not exceed 40°C. It is unstable in alkaline media (Gallo and Lawryk 1991, Hayes, 1982). The thermal stability of this compound is low. Volatility 0.09 mg/m ³ (Melnikov, 1971)
Boiling point	244°F (118 °C) at 0.05 mmHg (OHS Database. Occupational Health Services, Inc. 1993).
Molecular structure	

Ecological effects

Effects on Birds: Fenitrothion was found to be highly toxic to upland gamebirds and slightly toxic to waterfowl.

Effects on Arthropods (Nontarget species): There is sufficient information to characterize fenitrothion as highly toxic to honeybees (acute toxicity value = 0.383 micrograms/bee) when bees are exposed to direct treatment or to dried residues on foliage (Kidd and James 1991, Thomson 1982, U.S. Environmental Protection Agency. July, 1987). Fenitrothion is considered toxic to spider mites with long residual action (Spencer, 1981). Fenitrothion, applied to host eggs at field rates in the laboratory were found to be highly toxic to *Trichogramma orasiliensis* released on the eggs, causing 84-100% mortality in 24 hours (Elzen 1989). The long-term effects of fenitrothion and phosphamidon were evaluated on predaceous carabid beetles and lycosid spiders one year after treatment of Northwestern Ontario forests at 6 oz/A and 4 oz/A, respectively. The populations of these predators were clearly suppressed in the treated area.

Fenitrothion is a moderately toxic organophosphorus ester insecticide. However, over-exposure from handling during manufacture or use and accidental or intentional ingestion may cause serious poisoning. Despite its high toxicity for non-target arthropods, fenitrothion has been extensively used for pest control with few, or no, adverse effects on populations in the environment.

2.6 THIODICARB

Thiodicarb is a carbamate insecticide that acts by inhibiting acetylcholinesterase activity. It was evaluated in 1985 and 1986. Thiodicarb was first registered in the United States in 1984 (USEPA 2009).

In the environment, thiodicarb rapidly degrades to methomyl, which is also registered for use as an insecticide. Thiodicarb degrades within several hours to a few days with the primary degradation mechanisms thought to be biodegradation, hydrolysis, and photolysis (USEPA 1998, Jones *et al* 1989). While thiodicarb does not appear to be very persistent or highly mobile, its metabolite, methomyl, is more persistent, more mobile, and more toxic. Thiodicarb is not expected to have a high potential to contaminate

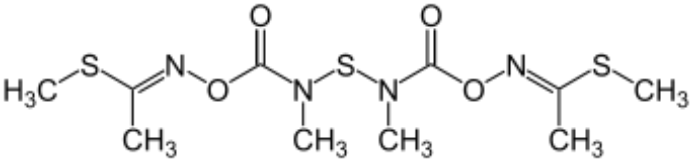
groundwater. However, methomyl has been detected infrequently in groundwater due to its moderate persistence and high mobility (USEPA 1998).

Thiodicarb is not regulated by USEPA in drinking water, but is an unregulated contaminant (USEPA Office of Ground Water and Drinking Water 2008). Thiodicarb is classified as a Group B2 agent, meaning it is a probable human carcinogen (USEPA 1998). It is also a blood toxicant, neurotoxicant and an acetylcholinesterase inhibitor.

Thiodicarb is not registered for residential use. It is registered for application via aerosol dispersal (USEPA 1998). It is primarily used for control of a number of insect pests on cotton, sweet corn, and soybeans, although it is also used to a lesser extent on leafy vegetables, cole crops, and ornamentals. It is also a molluscicide.

There have been no significant changes in the composition of technical-grade thiodicarb over time. The compound is also known as 'Larvin technical' and 'UC51762'.

Table 4. Physical and chemical properties of Thiodicarb.

Chemical Name	Thiodicarb
Synonyms	Dicarbasulf, Judge, Larvin, Lepicron, Toro
CAS Number	59669-26-0
Molecular Weight	354.4693
Molecular Formula	C ₁₀ H ₁₈ N ₄ O ₄ S ₃
Density	1.31g/cm ³
Melting Point	168-172°C
Boiling point	433.84°C at 760 mmHg
Flashpoint	216.179°C
Structural formula	Thiodicarb 

Biochemical aspects

Absorption, distribution, and excretion

Thiodicarb, which consists essentially of two methomyl moieties joined through their amino nitrogen by sulfur, is rapidly degraded to S- methyl- N [(methylcarbamoyl) oxy] thioacetamide (methomyl) in the rat stomach.

Thiodicarb is degraded in the stomach not only to methomyl but also to some other unstable intermediates, including methomyl methylol, methomyl oxime, methomyl sulfoxide, and methomyl sulfoxide oxime, which are subsequently converted to acetonitrile and carbon dioxide and eliminated primarily by respiration and in the urine. Acetonitrile is the only metabolite retained to some extent in body tissues and fluids; a small fraction of the acetonitrile is further degraded to carbon dioxide, acetic acid, and acetamide, which is suspected to be carcinogenic in mice and rats. The ultimate metabolic fate of methomyl in animals depends on its isomeric configuration. In rats, the stable and predominant form is the syn isomer, which is metabolized primarily to carbon dioxide, while partial conversion from the syn isomer to the anti isomer leads primarily to acetonitrile, most of which is respired unchanged.

Application

Thiodicarb is a high efficient insecticide similar to methomyl, but it has lower toxicity compared with methomyl. Pharmacodynamics is mainly stomach toxicity, with little contact action. It can kill lepidoptera, coleoptera and diptera pests effectively.

Thiodicarb can be used to control cotton bollworm, pink bollworm. It can also be used to prevent pests on cotton, soybeans, corn and other crops and widely used in fruit trees, cotton, vegetables, grain and other plant and disease control.

Toxicology:

Rats, LD ₅₀ , oral	50–100 mg/kg bw (depending on vehicle)
Rats, LD ₅₀ , intraperitoneal	No data
Mice, LD ₅₀ , oral	75 mg/kg bw

2.7 AZADIRACHTIN

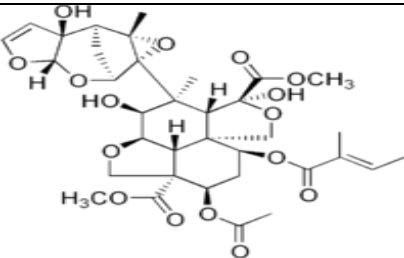
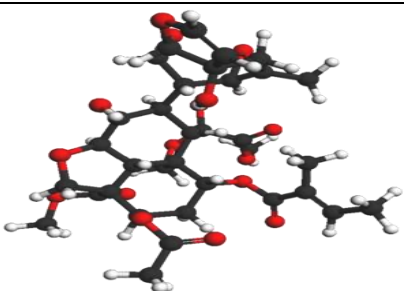
Azadirachtin - a component of the neem was first observed in 1959 when it was noticed that neem trees in Africa were undamaged during a plague of locusts (Schmutterer 1990).

The neem tree (*Azadirachta indica* A. Juss), from the Meliaceae family, has long been recognized for its properties both against insects and in improving human health. The seed consist of a shell and 1-3 kernels which contain azadirachtin and its homologues. Both the bark and leaves also contain biologically active molecules but not high levels of azadirachtin which is found mainly in the seed kernels. Azadirachtin occurs on amounts of some 4-6g/kg seeds depending upon tree ecotype and local environmental conditions. Mature trees may produce some 2 kg of seed per year. The tree is now grown in most tropical and sub-tropical areas of the world for shade, for reforestation programmes and in plantations for the production of compound which have toxic, antifeedant and repellent properties against insects (Mordue (Luntz) and Nisbet 2000).

Neem, an evergreen tree is commonly known as neem or margosa. The name Azadirachta has been derived from Farsi word Azad Dirakht meaning free tree (Ahmed and Grainge 1986) and since long time, the neem has been well known to be free of insect and nematode attacks and plant diseases (Jacobson 1985). Neem tree originated in the Indian Subcontinent (Ansari and Joshi 2004) from where it has been spread to many Asian and African countries (Anon 1983, Srivastava 2001). The trees grow on almost all kinds of soils, including clay, saline and alkaline soil. It also grows in poor dry soil and tolerates heat well, but not excessive cold or frost (Jacobson 1985). It may live for more than 200 years (Ketkar 1976). In Bangladesh, the population of neem trees is approximately half million (Mansour *et al.* 1986) and this is a household tree, widely distributed in the northwest part of the country (Board 2004).

The antifeedant effects of neem on the insect pests are well known. The desert locusts *Schistocerca gregaria* (Forsk.) has an unusually high sensitivity to azadirachtin as an antifeedant, perhaps related to the supposed co-evolutionary origins of both tree and locust in Burma. There have been at least six international conferences on neem to date, the first taking place in Germany in 1980, and there is a vast scientific literature which reveals both the antifeedant effects of neem and the more important physiological effects (as far as crop protection is concerned) (Mordue (Luntz) & Nisbet 2000).

Table 5. Physical and chemical properties of Azadirachtin.

ISO common name	Azadirachtin
IUPAC Name	dimethyl (2a <i>R</i> ,3 <i>S</i> ,4 <i>S</i> , <i>R</i> , <i>S</i> ,7a <i>S</i> ,8 <i>S</i> ,10 <i>R</i> ,10a <i>S</i> ,10b <i>R</i>)- 10-(acetyloxy)- 3,5-dihydroxy- 4-[(1 <i>S</i> ,2 <i>S</i> ,6 <i>S</i> ,8 <i>S</i> ,9 <i>R</i> ,11 <i>S</i>)- 2-hydroxy-11-methyl- 5,7,10-trioxatetracyclo[6.3.1.0 ^{2,6} .0 ^{9,11}]dodec- 3-en-9-yl]- 4-methyl- 8-{[(2 <i>E</i>)- 2-methylbut- 2-enoyl]oxy}octahydro-1 <i>H</i> -furo[3',4':4,4a]naphtho[1,8- <i>bc</i>]furan- 5,10a(8 <i>H</i>)-dicarboxylate
Empirical formula	C ₃₅ H ₄₄ O ₁₆
Structural formula	
Moller mass	720.71 g mol ⁻¹
Melting point	154-158 c Azadirachtin
Appearance and Odour	Yellow to light brown powder. Strong garlic- sulfur odor
Physical state	Liquid
Molecular structure	

Chemistry

The active ingredient azadirachtin was isolated from the seeds of *A. indica* by David Morgan (Butterworth and Morgan 1968) and its full structural determination was completed (Bilton *et al* 1987, Turner and Carter 1987). Azadirachtin, a complex tetranortriterpenoid limonoid from the neem seeds, is the main component responsible for both antifeedant and toxic effects on insects. Other limonoid and sulphur-containing compound with repellent, antiseptic, contraceptive, antipyretic and antiparasitic properties are found elsewhere in the tree, e.g. leaves, flowers, bark, roots. Azadirachtin has a complex molecular structure, and as a result the first synthesis was not published for over 22 years after the compound's discovery. The

first total synthesis was completed by Steven Ley in 2007. Both secondary and tertiary hydroxyl groups and tetrahydrofuran ether are present and the molecular structure reveals 16 stereogenic centres, 7 of which are tetrasubstituted. These characteristics explain the great difficulty encountered when trying to produce it by a synthetic approach.

The natural mixtures of azadirachtin in neem insecticides may usefully mitigate against the development of resistance compared to azadirachtin alone (Feng & Isman 1995). Azadirachtin in Neem Oil (50-2250 ppm), in Extracts: Powder (41.77 %) & Formulations (300 to 50000 ppm)

Acute toxicity hazard – Ecotoxicity

Rat (LD50 oral)	> 5000 mg/kg
Rat (LD50 dermal)	> 2000 mg/kg
Mammalian (LD50)	4,241 mg/kg
Avian (LD50)	816 mg/kg
Honey bee or insect (LD50)	2.5 ug/bee
Fish (LC50)	>4 mg/L
Crustacean (LC50)	11.6 mg/L

Single-dose toxicity testing indicates that azadirachtin is low in toxicity to mammals, moderate in toxicity to birds, fish, and other aquatic organisms, and moderate to high in toxicity to honey bees and other insects.

Mode of action:

The overall effects of azadirachtin against insects are given below:

Effects	Target	Mode of action
Primary antifeedancy	Mouthparts & other Chemoreceptor	Deterrent cell stimulation Sugar cell inhibition
Secondary antifeedancy	Gut	Peristalsis inhibition Enzyme production reduced Midgut cells not replaced
Insect Growth Regulation Sterility	Cuticle	Alternations to ecdysteroid and JH titres by blockage of release of morphogenetic peptides leading to moulting defects.
	Reproductive organs	Alternations to ecdysteroid and JH titres leading to reduction in number of viable eggs and live progeny
Cellular processes	Dividing cells	Blockage of cell division post metaphase in meiosis and mitosis.
	Muscles	Loss of muscle tone.
	Cell synthetic machinery	Blockage of digestive enzyme production in gut Inhibition of protein synthesis in various tissues.

(Mordue (Luntz) & Nisbet 2000)

Azadirachtin in Insect Control

The complexity of the molecular structure of azadirachtin precluded its synthesis for pesticide use. Extracts of neem seeds containing azadirachtin together with several structurally related molecules have formed the basis of neem usage in insect control (Isman 1997). An approach may also include the production of azadirachtin for insect control by in vitro tissue cultures of neem (Allan *et al* 1994, 1999). It is effective mainly as insect growth regulator and sterilants against a broad spectrum of pest insects.

Crude neem extracts have been used at a local, small-farm level for some time in countries where neem grows indigenously or where plantations have been established. In the major western countries of the world such as the USA and Canada and in Europe few commercial neem insecticides have reached the market place to date.

With the resolution of many of the problems of supply and standardization, the full regulatory approval of neem insecticides by the USA and now in Germany for use on potatoes, apples and tomatoes, much field data is being generated which are establishing neem insecticides as viable alternatives to more conventional approaches, particularly in integrated pest management system. Now that it is realized that disruption of growth and reproduction rather than antifeedancy are the main characteristic of pest control, neem is being used in the field at lower concentrations. The value of low concentrations of neem in pest control has generated research into combined approaches using both neem and beneficial species.

Neem pesticides may also have a useful role to play in resistance management. It has been demonstrated that the effects of neem in reducing levels of detoxification enzymes (due to its blockage of protein synthesis) may make insecticides more effective in resistant strains of insect (Lowery and Smirle 2000). Also, it has been shown in Bt resistant strains of *Leptinotarsa decemlineata* Say, the Colorado potato beetle, that 0.25% Neemix combined with *Bacillus thuringiensis* can act as a resistance breaking compound (Trisyono and Whalon 2000). In this instance depending upon the resistance mechanism, the neem effects may be due also to blockage of enzyme production, or to the reduced midgut cell turnover rate (Nasiruddin and Mordue (Luntz) 1993).

Advantages:

1. Broad spectrum of activity. Low use rates.
2. No Known insecticide resistance mechanisms
3. Compatible with many commercial insecticides and fungicides
4. New mode of action with possible multiple sites of attack.
5. Classified as a biological insecticide for registration purposes.
6. Compatible with other biological agents for IPM Programme.
7. Not persistent in the Environment.

8. Minimal impact of Non-target organisms.
9. Formulation flexibility. No re-entry restrictions
10. Supply available from pre-existing infrastructure.
11. Application flexibility - can be sprayed or drenched.
12. Non-phytotoxic formulations available.

2.7.1 NIMBECIDINE 0.03%

Nimbicidine from Neem

An effective supplement for synthetic pesticides, Nimbicidine has been proved and recognized as an ideal molecule in the Integrated Pest Management (IPM) programme.



(Source: Krishnaiah 1999)

All the goodness of neem in this product is Nimbicidine. It is a totally natural neem-oil based product with Azadirachtin as the labeled active ingredient. Nimbicidine is found to be effective against over 300 species of pests belonging to various orders of insects that infest very many cropping systems. It acts as an insect repellent, antifeedant, insect growth regulator and mating disruptor.

Chemistry of Nimbicidine

Nimbicidine, the neem-oil-based pesticide contains Azadirachtin as an active ingredient. It also contains many other active compounds like Meliantriol, Salanin, Nimbin, and the like, of which Azadirachtin is the most effective insect growth regulator molecule. The Azadirachtin content in Nimbecidine is not less than 300ppm. Neem based products are photodegradable to varying degrees. When isolated, Azadirachtin is highly photolabile and thermolabile. But Nimbicidine is an improved version of neem extract, which contains ingredients to protect Azadirachtin from photo and thermal degradation, making it highly bio-effective. Nimbicidine is also compatible with other chemical pesticides. So, it can act independently as well as in combination with pesticides.

Specification of formulation

Colour	Dark brown oil
Consistency	Oily and sticky
Specific gravity (30°C)	0.9087
Refractive index (30°C)	1.4612
PH	4.8
Flash point	+77.7°C

Contents of Nimbicidine

Azadirachtin	0.03% (300 ppm)
Neem Oil*	90.57%
Emulsifier	5.00%
Stabilizer	0.50%
Solvent and other constituents	3.90% approx. (S.Q.)

Chapter – 3

General methodology

3.1 Source of *A. diaperinus*

The insects used in the present experiments were originally collected as adults from the grain shops of the local market and poultries in Rajshahi City, Bangladesh.

3.1.1 Food medium

The beetles were reared on diet containing of wheat flour and brewer's yeast (19:1), the standard food as used for *Tribolium* (Park and Frank 1948). Few slices of fresh potatoes were kept in the foods which were changed every three to five days, to keep the food humid. The wheat flour was sterilized in an oven at 120°C for 6 hours and was allowed to cool down at room temperature, then mixed thoroughly with yeast. Both the flour and yeast before mixing were passed through a 250mm aperture sieves (Plate 10).

3.1.2 Culture of beetles

The culture was maintained in beakers and or plastic jars. About 200 of beetles were placed in 250g of standard food medium in a beaker/jar. A few small slices of potato were kept along with the food for maintaining humidity inside the culture. The mouths of the jars/beakers were covered with a piece of cloth using a rubber band. After a month the stock culture was divided into four to five sub-cultures. The cultures were reared in an incubator at $30 \pm 1^\circ\text{C}$ without controlling light and humidity. These cultures were examined regularly and only the healthy cultures were kept. Infested culture if any (observed) was readily discarded. Dead individuals were removed from the culture; old slices of potatoes were replaced from time to time by new ones (Plates 11-13).

3.1.3 Collection of eggs

About 50 unsexed adults were isolated in a petridish (6cm diameter) containing standard food medium and potato slices. If adults were kept in food without potato slices they did not oviposit regularly. After 24 hours eggs were collected by sieving the medium through 500 and 250mm aperture sieves (Khan and Selman 1981). Eggs were transferred to glass petridish and incubated at $30 \pm 1^\circ\text{C}$.

3.1.4 Transfer of larvae to food medium

The newly hatched larvae were collected with a fine camel hair brush. The neonates were transferred to fresh food medium in glass jars or beakers.

3.1.5 Determination of larval instars

The larval instars were determined by counting the exuviae (larval skin) deposited in the standard food medium as described by Mondal (1983) in case of *Tribolium castaneum*. Food and temperature have effect on moulting. Edwards and Abraham (1985) observed seven larval instars in *A. diaperinus* whereas Victor and Ogonor (1987) reported more than eight larval instars. In the present study eight larval instars of *A. diaperinus* was confirmed. At 30°C the neonates were hatched 2 to 3 days after egg laying. The 2nd, 3rd, 4th, 5th, 6th, 7th and 8th instars took 4-5, 4-5, 3-5, 3-4, 3-5, 4-6 and 4-8 days respectively to moult. The total larval period varied from 25 to 38 days.

3.1.6 Determination of sex

The pupae were sexed by examining their exogenital processes under a microscope (Halstead 1963) (Plate 14)

3.2 Precaution

All glassware's and sieves used in the experiment were cleaned after every use and sterilized in an oven at 80°C for about six hours (Mondal 1984a). Cultures having any infestation were discarded readily.

3.3 Test chemicals

In this investigation four insecticides belonged to the four different chemical groups and one botanical insecticide were used as follow –

Sl	Commercial name	Common name	Chemical family
1	Botanicals	Azadirachtin	Nimbidine 0.03%
2	Pyrethroid	Deltamethrin	Decis 2.5 EC
3	Neonectinoid	Imidacloprid	Confidor 70 WG
4	Carbamet	Thiodicarb	Larvin 75 WP
5	Organophosphet	Fenitrothion	Sumithion 50EC

Solvent: As a solvent Acetone has been chosen for all insecticides. Only for Imidacloprid (Confidder 70 WP) water has been chosen as solvent, because this insecticide was not soluble in acetone.

Active ingredient (a.i.) of Azadirachtin was used in the experiment.

3.4 Statistical analysis

For statistical analysis of the data Microsoft Excel and Minitab (12.1) software packages were used (Minitab 1997)

3.3.1 Probit analysis

The percent mortality was subjected to statistical analysis according to Finny (1947) 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) formula.

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

P = 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 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 insecticide i.e. higher the LD_{50} value lower to toxicity of the insecticide.



Plate 10: Food medium and culture of beetles.



Plate 11: Insecticides used in the experiments.



Plate 12: Culture of beetle in the laboratory.



Plate 13: Glasswares used in experiments.



Plate 14: Exogenital prolegs of pupae of *A. diaperinus*.

Chapter – 4

Toxicity of Insecticides on *A. diaperinus*

4.1 Introduction

Application of protectant insecticides is an integral part of the management of insect pests and has been considered as the most important and powerful tool in controlling insect pests both in the field and storage. Even it has many serious limitations including resistance (Pacheco *et al* 1990, Sartori *et al* 1990), outbreaks of secondary pests, adverse effects on non-target organisms, objectionable pesticide residues, and direct-hazards to the users (Smith 1970), elimination of beneficial insects and the several predators of the pests (Smith and Van den Bosch 1967). Many beetles may survive the insecticide treatment by avoidance of contact with the insecticides due to their repellent actions (Mondal and Khalifa 1990, Mondal 1984 a, c). The repellent effects of different chemical insecticides viz. malathion (Strong *et al.* 1967, Pinniger 1975), pirimphos-methyl (Kamaruzzaman 2000, Mondal 1984 a,b), ficam plus (Mondal and Khalifa 1990), Diazinon (Ali *et al* 1991, Hussain *et al* 1994), nogos (Hussain *et al* 1991a, b), fenitrothion (Pinniger 1975), sumithion (Hussain *et al.* 1991b), bioresmethrin, lindane and DDT (Prickett and Ratcliffe 1977), pyrethrins (Prickett and Ratcliffe 1977, Rajasekaran *et al.* 1996, Khatun and Mondal 2004) and phosphine (Bond and Upitis 1973) have already been reported in *T. castaneum*. Hasnat (2003) reported that both adults and larvae of *T. castaneum* were repelled by the sublethal doses of deltamethrin.

The search for alternative and environmental friendly insecticides has led to the use of botanicals for plant protection (Khalequzzaman and Nahar 2003). Azadirachtin- a botanical pesticide derived from the neem tree is one of the alternative. It is generally less harmful to the environment than other more commonly used pesticides. It is an example of natural chemical defense by plants, affecting feeding primarily through chemoreception (deterrence) and secondarily through toxic effects (Mordue (Luntz) and Blackwell 1993).

Evaluation of Azadirachtin against numerous species of insects pests have demonstrated neem's diverse biological effects viz. repellence (Sahayaraj and Paulraj 2000, Zahoor *et al.* 2002), feeding deterrence (Isman 1993), reduced growth

and abnormal development (Jbilou *et al.* 2006), oviposition deterrence (Lohra *et al.* 2001), reduced egg laying due to sterilizing effect (Sharma 1995 a,b) and also direct toxicity (Ahmed *et al.* 2001, Khanom 2004, Khalequzzaman and Nahar 2003, 2008). The technical grade of Azadirachtin were used in the previous experiments. The commercial grade of Azadirachtin was used against *Tribolium* by Khatun (2010).

4.2 Materials and Methods:

Two types of experiments were designed to study of the toxicity of these chemicals on *A. diaperinus* viz. (1) firstly, Residual Film Method (RFM) (Busvine 1971) and (2) secondly, the Treated Food Method (TFM).

4.2.1 Residual Film Method (RFM)

The chemicals were diluted in acetone and different doses were made. Using 1 ml pipette, one ml of liquid from each dose was dropped on petridish (6cm diameter) containing filter paper, covering uniformly the whole area of the petridish (Shawir and Mansee 1997, Khalequzzaman and Nahar 2001). They were then kept open for 30 minutes to evaporate the solvent. Ten test insects (larvae/ adults) were introduced inside the petridish and kept in an incubator at 30°C (Plate 15). The mortality was recorded after 24h and 48h and 72h for larvae and adults. Five replicates were used for each test, each replicate consisting of 10 test insects (N=50). Experiments were conducted for 1, 10, 20, 30 and 40 days old larvae and unsexed adults.

The doses were calculated by measuring the actual amount of active ingredient (fig) in one ml of the liquid divided by the total surface area of the petridish. A control batch was maintained in which only acetone was applied. The mortality percentage was corrected using Abbott's formula (Abbott 1925) wherever necessary and observed data was subjected to Probit analysis according to Finney (1971) and Busvine (1971). The LD50 values were expressed as $\mu\text{g}/\text{cm}^2$ (Khalequzzaman and Nahar 2001).

4.2.2 Treated Food Method (TFM)

The flour media (flour : yeast =19:1 ratio) were treated with the test chemicals dissolved in solvent - the acetone at the desired doses (concentrations) and the medium was thoroughly mixed using an electric blender. The mixed/ treated flour media were allowed to evaporate the solvent for approximately one hour.

Approximately, 1g of treated flour was poured in a petridish (6cm diameter) and spread uniformly over the whole area of the petridish. Ten test insects (larvae/adults) were introduced in the petridish and kept in an incubator at 30°C (Plate 16).

The mortality was recorded after 24h and 48h, 72h for larvae/adults. Five replicates were used for each test, each replicate consisting of 10 test insects (N=50). Experiments were conducted for 1, 10, 20, 30 and 40 days old larvae and adults.

The doses were calculated by measuring the actual amount of active ingredient (μg) in one ml of the liquid divided by the amount of flour medium used and expressed in ppm (parts per million). A control batch was maintained in the flour medium in which only acetone was applied. The mortality percentage was corrected and observed data were subjected to statistical analyses as mentioned earlier in RFM method.

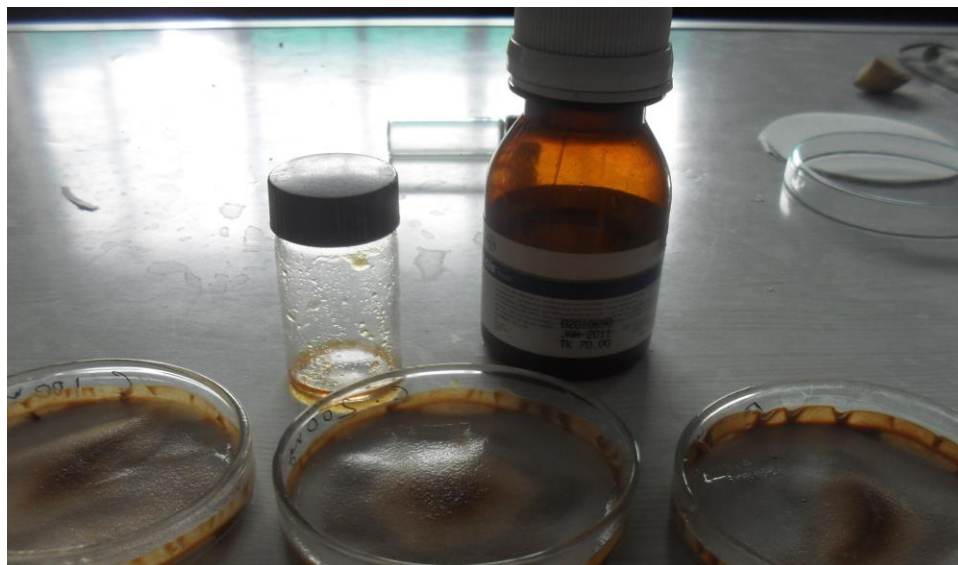


Plate 15: Prepared doses for Residual Film Method (RFM).



Plate 16: Treated Food Method (TFM).

4.3 RESULTS AND OBSERVATION

In the present experiment, Fenitrothion (Sumithion 50 EC), Deltamethrin (Desis 2.5 EC), Thiodicarb (Larvin 75WP), Imidacloprid (Confidor 70WP) and Azadirachtin (Nimbecidine 0.03%) were used to evaluate their action on *A. diaperinus*. The toxicity of the insecticides was recorded properly.

4.3.1 Toxicity of Azadirachtin (Nimbecidin 0.03%) on *A. diaperinus*

Calculation of log/ Probit regression line for the dose mortality experiment of different doses of Azadirachtin have been estimated in Appendix Table (1-36) and Fig 1 and 5 showed that LD₅₀ values were increased with ages and decreased with time.

A. RFM Test method

Effect on Larval ages

The LD₅₀ values for the 1, 10, 20, 30, and 40 days old larvae were recorded as 4.49, 4.77, 18.14, 22.22 and 25.28 µg/cm² respectively after 24 hrs exposure.

The LD₅₀ values were 3.71, 4.01, 9.34, 10.92, and 12.27 µg/cm² for the same aged larvae respectively after 48 h.

After 72 hours these values were 3.42, 3.76, 7.48, 8.99, and 10.84 µg/cm² respectively.

Effect on adults

The LD₅₀ values for the adults recorded after 24, 48 and 72 hours were 61.85, 45.83, 34.69 µg/cm² respectively.

The LD₅₀ values, 95% confidence limits, regression equation, Chi-squared values are shown in Table 6. Regression lines are shown in Fig. 2-4.

B. TFM test

Effect on Larval ages

The LD₅₀ values for 1, 10, 20, 30, and 40 days old larvae were recorded as 81.42, 106.32, 298.57, 315.46 and 340.98 ppm respectively after 24 hours.

The values after 48 hours were 59.33, 70.57, 250.35, 270.35, and 290.43 ppm respectively.

At the exposure period of 72 hours the values were 29.96, 52.38, 219.29, 260.15, and 256.69 ppm respectively.

Effect on adults

The LD₅₀ values were found to be 579.83, 488.79, 440.11 ppm after 24, 48, and 72 hours respectively.

The LD₅₀ values along with 95% confidence limits, regression equation, Chi-square values have been estimated in the Table 7 and Regression lines are shown in Fig. 6-8.

Table 6: LD₅₀ values, 95% confidence limits and regression equations for Azadirachtin against *A. diaperinus* exposed to the treated filter paper for 24, 48 and 72 hrs.

Life stage (Age)	Exposure period (Hours)	LD ₅₀ (µg/cm ²)	Regression equation	95% confidence limit		Chi-square value (3df)
				Lower (µg/cm ²)	Upper (µg/cm ²)	
Larvae (1 day)	24	4.482	Y=.6840587+.62424X	4.213	4.768	0.883
	48	3.708	Y=2.432036+.51139X	3.388	4.058	1.196
	72	3.419	Y=2.693293+4.3201X	3.122	3.744	2.641
Larvae (10 days)	24	4.762	Y=.7141719+6.3231X	4.451	5.094	0.784
	48	4.006	Y=2.352077+4.3926X	3.631	4.420	0.344
	72	3.755	Y=2.482408+4.3806X	3.422	4.122	1.160
Larvae (20 days)	24	18.135	Y=2.633149+1.8806X	14.55	22.59	1.753
	48	9.333	Y=2.99109+2.07091X	7.625	11.42	0.686
	72	7.470	Y=3.081435+2.1968X	6.174	9.037	2.469
Larvae (30 days)	24	22.211	Y=2.538315+1.8281X	17.54	28.06	0.531
	48	10.917	Y=2.980608+1.9452X	8.738	13.64	0.895
	72	8.997	Y=3.032173+2.0624X	7.347	11.01	1.266
Larvae (40 days)	24	25.271	Y=2.423959+1.8365X	19.81	32.23	0.948
	48	12.263	Y=2.87948+1.94792X	9.767	15.39	0.855
	72	10.839	Y=2.99524+1.93695X	8.707	13.49	0.667
Adults	24	61.849	Y= 1.95931+1.69744X	47.82	79.98	0.852
	48	45.824	Y=2.25797+1.65073X	35.93	58.42	1.182
	72	34.699	Y=2.093837+1.8867X	27.17	44.31	0.196

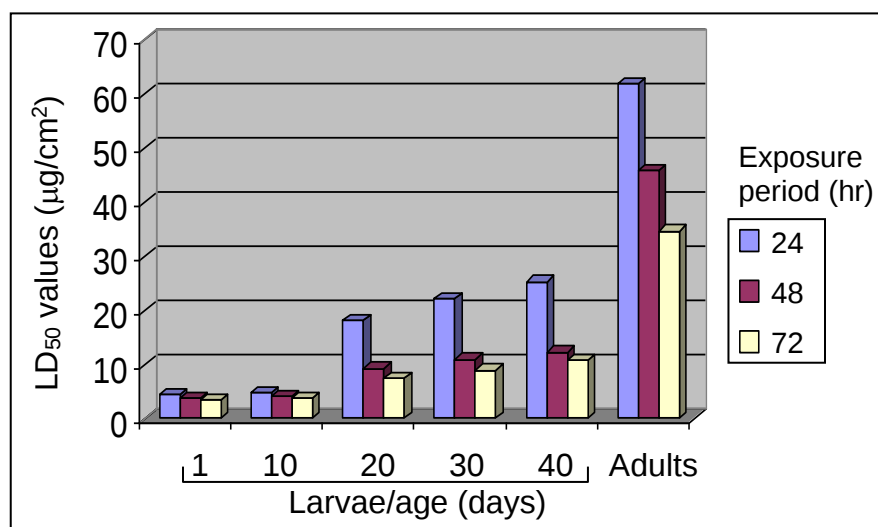


Fig.1: LD₅₀ values of Azadirachtin for both larvae and adults of *A. diaperinus* after 3 different exposure periods in RFM test.

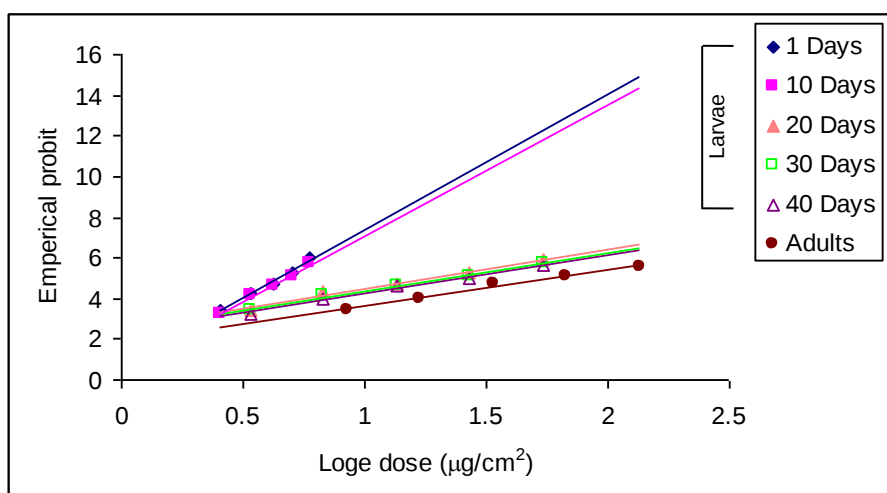


Fig.2: Regression lines of Azadirachtin against *A. diaperinus* larvae (different ages) and adults exposed to treated filter paper for 24hrs.

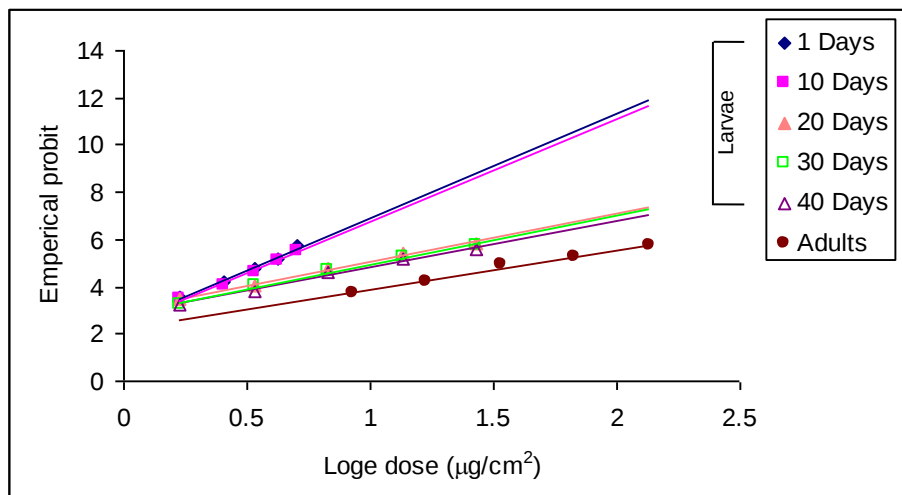


Fig.3: Regression lines of Azadirachtin against *A. diaperinus* larvae (different ages) and adults exposed to treated filter paper for 48hrs.

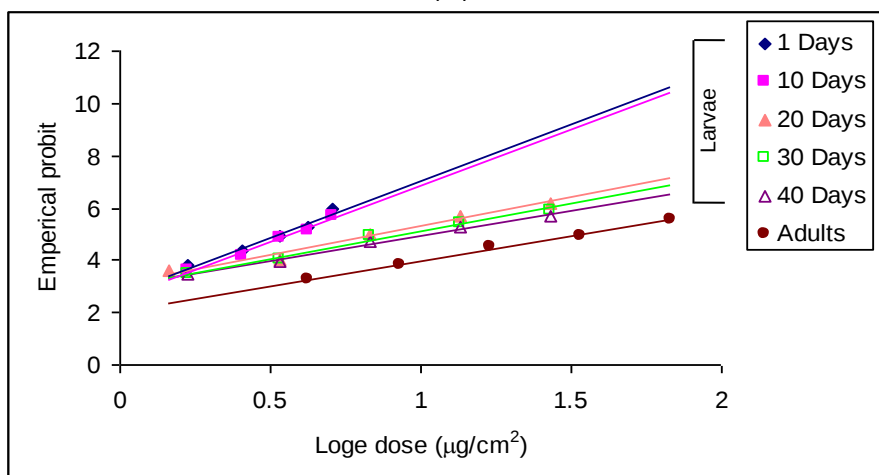


Fig.4: Regression lines of Azadirachtin against *A. diaperinus* larvae (different ages) and adults exposed to treated filter paper for 72hrs.

Table 7: LD₅₀ values, 95% confidence limits and regression equations for Azadirachtin against *A. diaperinus* exposed to the treated flour medium for 24, 48 and 72 hrs.

Life stage (Age)	Exposure period (Hours)	LD ₅₀ (ppm)	Regression equation	95% confidence limit		Chi-square value (3df)
				Lower (ppm)	Upper (ppm)	
Larvae (1 day)	24	81.412	$Y=1.254356+1.96036X$	65.906	100.560	0.284
	48	59.334	$Y=1.77451+1.818914X$	47.701	73.805	0.110
	72	29.958	$Y=2.547667+1.66088X$	23.671	37.914	0.468
Larvae (10 days)	24	106.321	$Y=.9551957+1.99582X$	85.072	132.880	0.566
	48	70.562	$Y=1.609281+1.8342X$	53.973	92.250	0.316
	72	52.372	$Y=2.17689+1.642196X$	40.275	68.104	0.281
Larvae (20 days)	24	298.565	$Y=-9.01958+5.66439X$	278.414	320.173	1.354
	48	250.345	$Y=-6.12708+4.63911X$	229.687	272.860	2.645
	72	219.293	$Y=-5.640165+4.5450X$	200.866	239.410	1.225
Larvae (30 days)	24	315.457	$Y=-8.18080+5.27455X$	292.554	340.153	1.060
	48	270.341	$Y=-6.293095+4.6437X$	247.186	295.666	0.386
	72	260.145	$Y=-3.72626+3.6130X$	232.999	290.454	3.458
Larvae (40 days)	24	340.974	$Y=-10.11186+5.9666X$	317.331	366.378	0.294
	48	290.423	$Y=-7.072156+4.9013X$	265.320	317.901	0.928
	72	256.691	$Y=-6.254833+4.6711X$	235.414	279.891	1.704
Adults	24	579.822	$Y=-15.19761+7.3092X$	546.134	615.589	0.123
	48	488.793	$Y=-9.957424+5.562X$	451.836	528.772	0.034
	72	440.107	$Y=-9.697008+5.5595X$	409.503	472.998	0.283

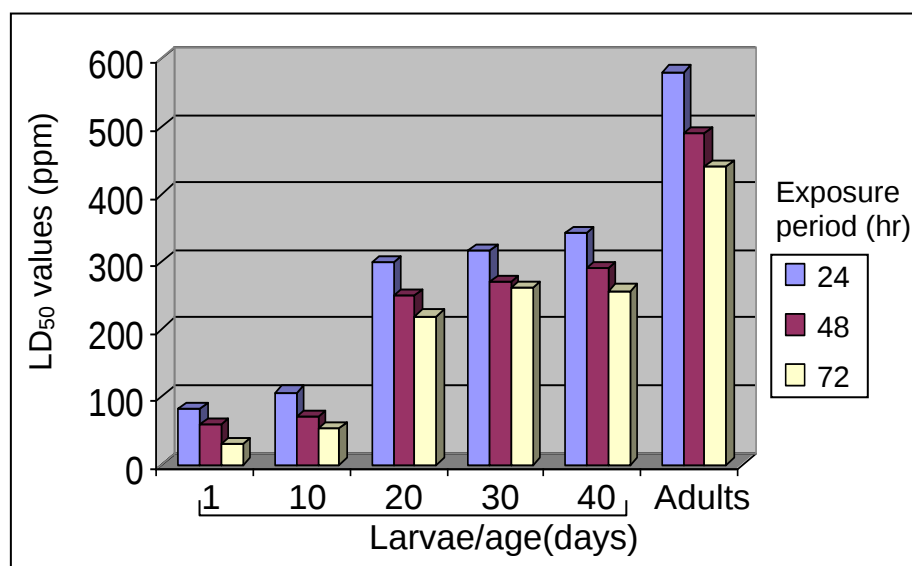


Fig.5: LD₅₀ values of Azadirachtin for both larvae and adults of *A. diaperinus* after 3 different exposure periods in TFM test.

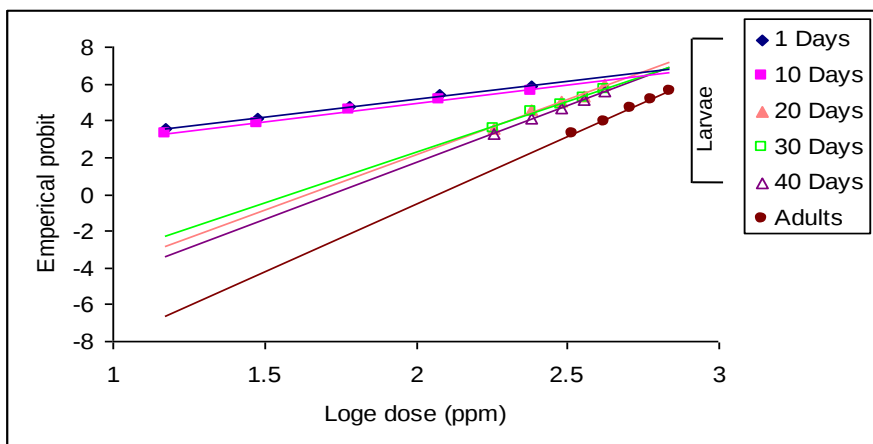


Fig.6: Regression lines of Azadirachtin against *A. diaperinus* larvae (different ages) and adults exposed to treated flour medium for 24 hours.

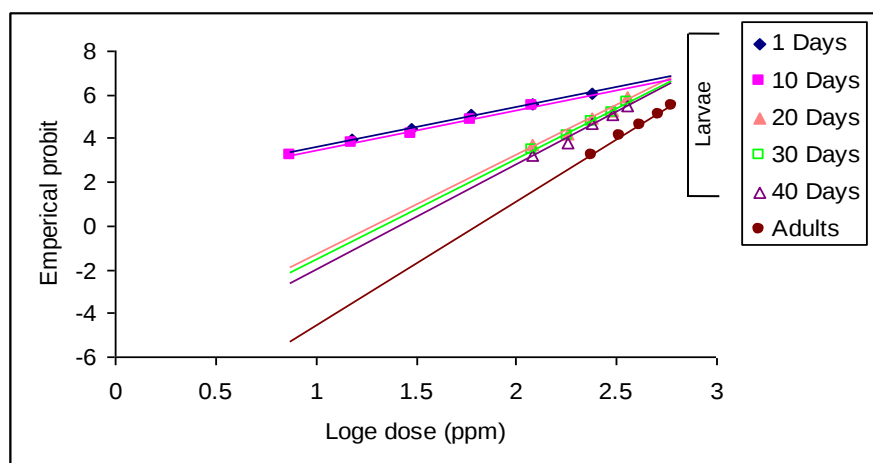


Fig.7: Regression lines of Azadirachtin against *A. diaperinus* larvae (different ages) and adults exposed to treated flour medium for 48 hours.

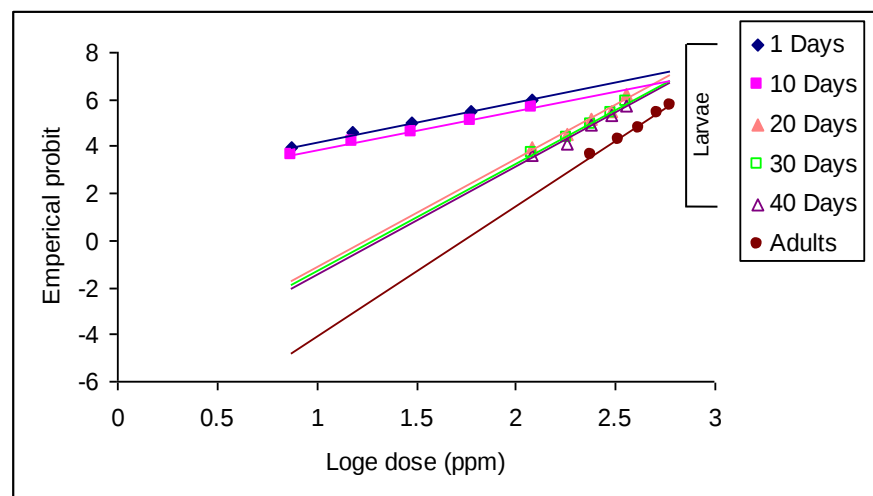


Fig.8: Regression lines of Azadirachtin against *A. diaperinus* larvae (different ages) and adults exposed to treated flour medium for 72 hours.

4.3.2 Toxicity of Deltamethrin (Decis 2.5EC) on *A. diaperinus*

Calculation of log/ Probit regression line for the dose mortality experiment of different doses of Deltamethrin have been estimated in Appendix Table (37-72) and Fig 9 and 13 showed that LD₅₀ values were increased with ages and decreased with time.

A. RFM test Method

Effect on Larval ages

The LD₅₀ values for the 1, 10, 20, 30, and 40 days old larvae were recorded as 3.05, 25.44, 142.94, 225.84, 361.78 µg/cm² respectively at the exposure period of 24 hrs.

At the exposure period of 48 hours the LD₅₀ values were 1.95, 14.37, 129.57, 196.77, and 340.81 µg/cm² for the 1, 10, 20, 30, and 40 days old larvae respectively.

After 72 hours exposure the LD₅₀ values were recorded as 1.59, 11.96, 112.01, 180.75, and 327.13 µg/cm² for the 1, 10, 20, 30, and 40 days old larvae respectively.

Effect on adults

The LD₅₀ values for the adults were recorded as 1229.48, 973.17, 854.11 µg/cm² after the exposure periods of 24, 48 and 72 hrs respectively.

The LD₅₀ values along with 95% confidence limits, regression equations, Chi-square values are shown in Table. 8 and regression lines are shown in Fig. 10-12.

B. TFM test

Effect on Larval ages

The LD₅₀ values for 1, 10, 20, 30, and 40 days old larvae were recorded as 168.90, 226.43, 956.95, 1027.41, and 1095.91 ppm respectively after 24 hours.

After 48 hours the values were 71.21, 96.87, 752.58, 799.66, and 856.59 ppm respectively.

When the exposure period was 72 hours then these values were 48.73, 61.11, 690.93, 729.67, and 778.62 ppm respectively.

Effect on adults

Food was treated with different doses of Deltamethrin, and adults were reared on the treated food. The LD₅₀ values were observed as 2044.41, 1502.81, 1243.20 ppm after 24, 48, and 72 hours respectively.

The LD₅₀ values along with 95% confidence limits, regression equations, Chi-square values are shown in the Table. 9 and Regression lines are shown in Fig. 14-16.

Table 8: LD₅₀ values, 95% confidence limits and regression equations for Deltamethrin against *A. diaperinus* exposed to the treated filter paper for 24, 48 and 72 hrs.

Life stage (Age)	Exposure period (Hours)	LD ₅₀ (µg/cm ²)	Regression equation	95% confidence limit		Chi-square value (3df)
				Lower (µg/cm ²)	Upper (µg/cm ²)	
Larvae (1 day)	24	3.041	Y=1.631496+2.27125X	2.530	3.655	2.525
	48	1.943	Y=2.288426+2.10441X	1.596	2.364	0.289
	72	1.598	Y=2.386624+2.17093X	1.317	1.939	0.595
Larvae (10 days)	24	25.431	Y=1.851606+2.24028X	21.102	30.645	2.314
	48	14.369	Y=2.668379+2.01445X	11.727	17.607	1.549
	72	11.961	Y=2.704438+2.12999X	9.861	14.504	2.032
Larvae (20 days)	24	142.938	Y=-7.38852+5.74834X	133.116	153.484	5.346
	48	129.556	Y=-5.003373+4.73541X	118.743	141.353	3.836
	72	112.009	Y=-4.227171+4.50269X	96.411	130.131	8.103
Larvae (30 days)	24	225.830	Y=-15.18247+8.57448X	215.341	236.831	3.516
	48	196.765	Y=-11.54664+7.21316X	186.026	208.124	1.389
	72	180.749	Y=-11.27852+7.21221X	170.775	191.305	2.667
Larvae (40 days)	24	361.776	Y=-33.00723+14.8556X	351.924	371.903	2.932
	48	340.805	Y=-31.88683+14.565X	331.022	350.878	1.812
	72	327.121	Y=-29.38725+13.6744X	317.376	337.166	2.068
Adults	24	1229.48	Y=-15.06803+6.49508X	1154.28	1309.58	5.156
	48	973.162	Y=-7.902562+4.31785X	887.377	1067.24	0.839
	72	854.103	Y=-7.87462+4.3918X	777.967	937.690	3.401

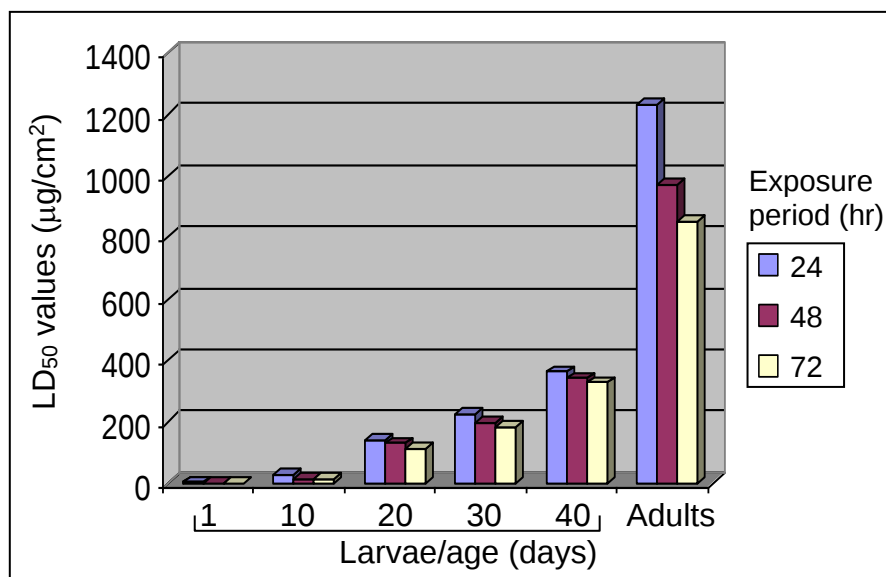


Fig.9: LD₅₀ values of Deltamethrin for both larvae and adults of *A. diaperinus* after 3 different exposure periods in RFM test.

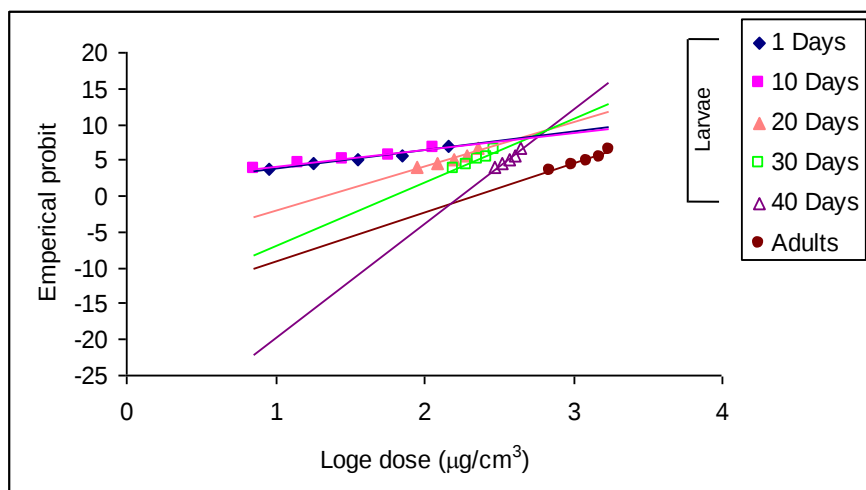


Fig.10: Regression lines of Deltamethrin against *A. diaperinus* larvae (different ages) and adults exposed to treated filter paper for 24 hours.

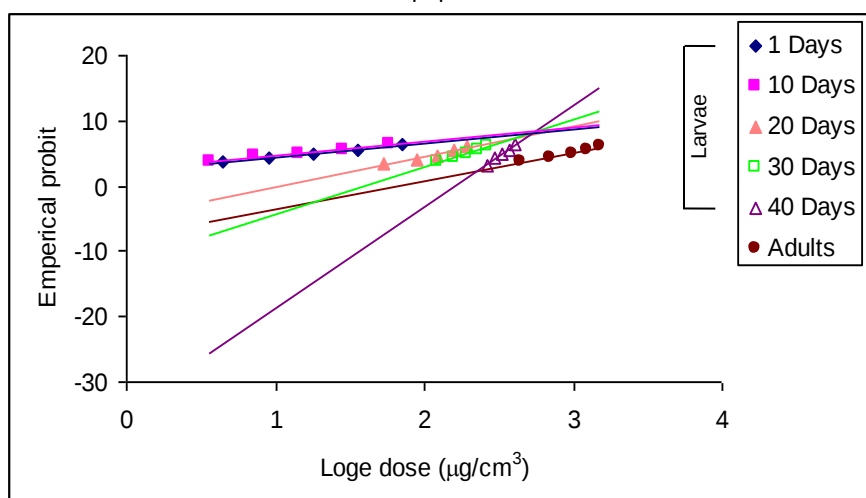


Fig.11: Regression lines of Deltamethrin against *A. diaperinus* larvae (different ages) and adults exposed to treated filter paper for 48 hours.

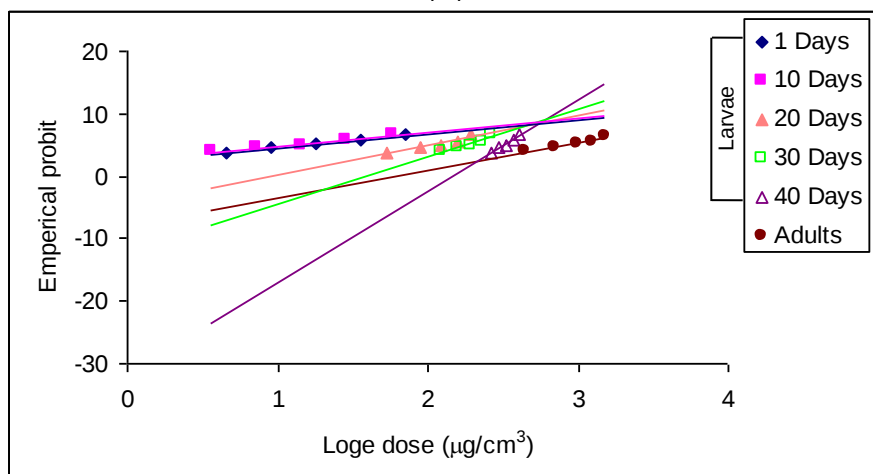


Fig.12 Regression lines of Deltamethrin against *A. diaperinus* larvae (different ages) and adults exposed to treated filter paper for 72 hours.

Table 9: LD₅₀ values, 95% confidence limits and regression equations for Deltamethrin against *A. diaperinus* exposed to the treated flour medium for 24, 48 and 72 hrs.

Life stage (Age)	Exposure period (Hours)	LD ₅₀ (ppm)	Regression equation	95% confidence limit		Chi-square value (3df)
				Lower (ppm)	Upper (ppm)	
Larvae (1 day)	24	168.901	$Y=1.046537+1.77472X$	134.234	212.534	2.078
	48	71.207	$Y=2.206673+1.50784X$	54.265	93.438	3.034
	72	48.727	$Y=2.482739+1.49146X$	36.952	64.255	4.289
Larvae (10 days)	24	226.421	$Y=.5829625+1.87566X$	180.981	283.269	1.119
	48	96.863	$Y=1.894867+1.56338X$	74.001	126.788	3.399
	72	61.101	$Y=2.306036+1.50833X$	46.645	80.036	3.856
Larvae (20 days)	24	956.946	$Y=-14.62587+6.5839X$	899.531	1018.03	5.144
	48	752.573	$Y=-8.408714+4.6613X$	690.583	820.128	3.797
	72	690.922	$Y=-9.194282+4.9989X$	600.201	795.357	8.313
Larvae (30 days)	24	1027.42	$Y=-16.33951+7.0854X$	969.601	1088.67	3.029
	48	799.650	$Y=-8.929312+4.7984X$	735.711	869.145	1.916
	72	729.662	$Y=-10.17187+5.2990X$	675.014	788.734	7.680
Larvae (40 days)	24	1095.91	$Y=-16.94525+7.2193X$	1033.89	1161.74	0.482
	48	856.594	$Y=-10.45696+5.2704X$	792.737	925.595	1.456
	72	778.612	$Y=-9.342322+4.9604X$	718.312	843.975	4.011
Adults	24	2044.42	$Y=-13.06815+5.4577X$	1896.44	2203.95	4.369
	48	1502.82	$Y=-5.953027+3.4477X$	1337.71	1688.31	5.497
	72	1243.21	$Y=-5.308184+3.3310X$	1099.59	1405.58	7.628

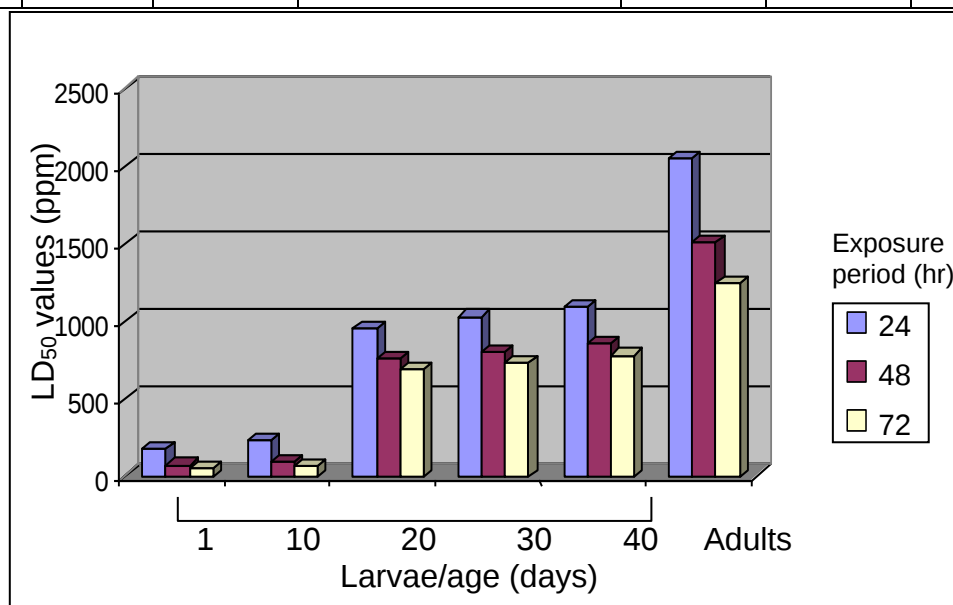


Fig.13: LD₅₀ values of Deltamethrin for both larvae and adults of *A. diaperinus* after 3 different exposure periods in TFM test.

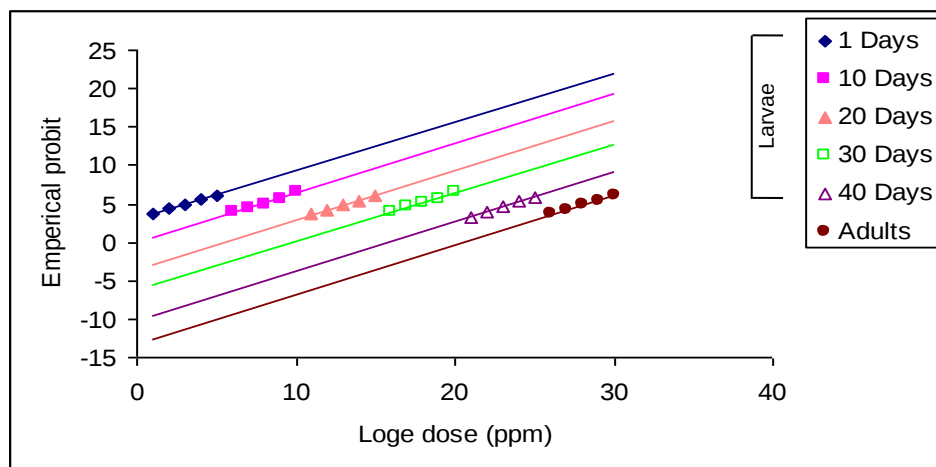


Fig.14: Regression lines of Azadirachtin against *A. diaperinus* larvae (different ages) and adults exposed to treated flour medium for 24 hours.

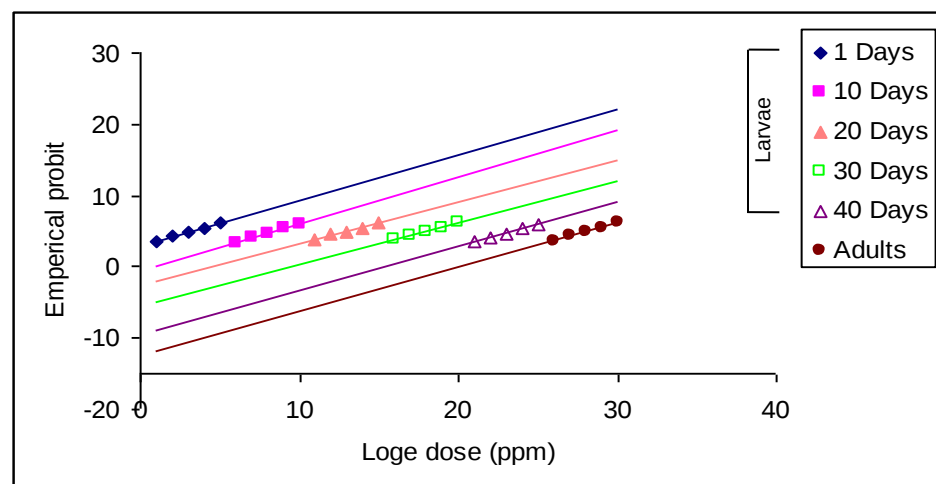


Fig.15: Regression lines of Azadirachtin against *A. diaperinus* larvae (different ages) and adults exposed to treated flour medium for 48 hours.

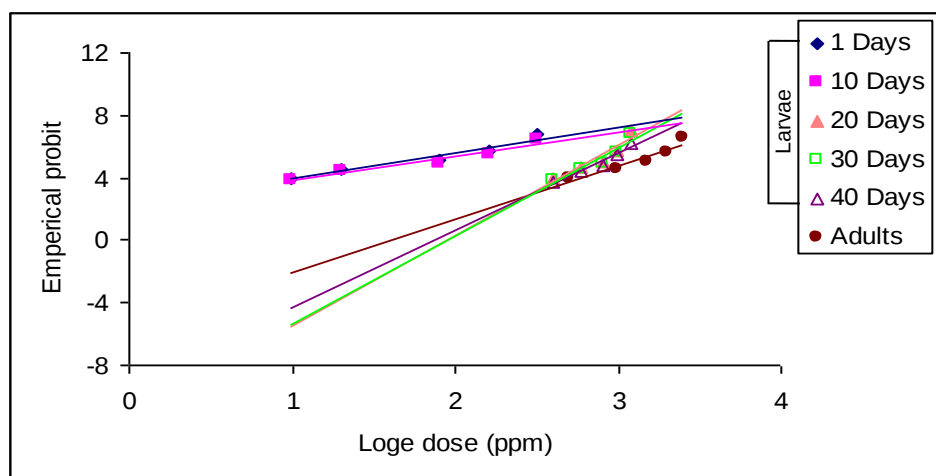


Fig.16: Regression lines of Azadirachtin against *A. diaperinus* larvae (different ages) and adults exposed to treated flour medium for 72 hours.

4.3.3 Toxicity of Imidacloprid (Confidor 70WP) on *A. diaperinus*

Calculation of log/ Probit regression line for the dose mortality experiment of different doses of Imidacloprid have been estimated in Appendix Table (89-124) and Fig 17 and 21 showed that LD₅₀ values were increased with ages and decreased with time.

A. RFM test Method

Effect on Larval ages

The LD₅₀ values for the 1, 10, 20, 30, and 40 days old larvae were recorded as 19.62, 34.16, 78.45, 188.89 and 215.48 µg/cm² respectively for the exposure period of 24 hrs.

After 48 hours exposure the LD₅₀ values were 12.59, 19.39, 59.22, 108.19, and 141.96 µg/cm² for the 1, 10, 20, 30, and 40 days old larvae respectively.

At 72 hours exposure the values were recorded as 10.32, 16.06, 43.43, 79.35, and 109.14 µg/cm² for the 1, 10, 20, 30, and 40 days old larvae respectively.

Effect on adults

The LD₅₀ values for the adults were recorded as 3082.8, 2490.8, 2215.1 µg/cm² after 24, 48 and 72 hrs respectively.

The LD₅₀ values along with 95% confidence limits, regression equations, Chi-square values are shown in the Table. 10. Regression lines are shown in Fig. 18-20.

B. TFM test/ Method

Effect on Larval ages

The LD₅₀ values for 1, 10, 20, 30, and 40 days old larvae were recorded as 22.39, 57.97, 107.13, 242.79, and 307.99 ppm respectively for 24 hours, and after 48 hours these were 22.21, 56.33, 58.73, 114.25, and 273.74ppm respectively. When the exposure period was 72 hours the LD₅₀ values were 8.29, 20.02, 47.32, 90.95, and 108.75ppm respectively.

Effects on adults

Food was treated with different doses of Imidacloprid, and adults were reared on the treated food.

The LD₅₀ values were found as 359.46, 304.02, 221.97ppm for 24, 48, and 72 hours exposure period respectively.

The LD₅₀ values along with 95% confidence limits, regression equation, Chi-square values are shown in the Table- 11 and Regression lines are shown in Fig. 22-24.

Table 10: LD₅₀ values, 95% confidence limits and regression equations for Imidacloprid against *A. diaperinus* exposed to the treated filter paper for 24, 48 and 72 hrs.

Life stage (Age)	Exposure period (Hours)	LD ₅₀ (µg/cm ²)	Regression equation	95% confidence limit		Chi-square value (3df)
				Lower (µg/cm ²)	Upper (µg/cm ²)	
Larvae (1 day)	24	19.616	Y=2.062727+2.2723X	16.321	23.575	2.523
	48	12.593	Y=2.682528+2.1065X	10.351	15.321	0.291
	72	10.317	Y=2.797208+2.1732X	8.505	12.515	0.591
Larvae (10 days)	24	34.154	Y=1.564603+2.2403X	28.341	41.158	2.315
	48	19.382	Y=2.407217+2.0139X	15.817	23.751	1.547
	72	16.059	Y=2.432563+2.1293X	13.241	19.477	2.033
Larvae (20 days)	24	78.445	Y=1.160968+2.02633X	64.135	95.947	1.831
	48	59.216	Y=1.122405+2.18771X	48.845	71.789	0.470
	72	43.422	Y=1.422481+2.18445X	35.901	52.520	2.159
Larvae (30 days)	24	188.88	Y=.5605683+1.95038X	153.133	232.973	1.417
	48	108.19	Y=1.099379+1.91751X	87.524	133.749	0.916
	72	79.343	Y=1.151554+2.02601X	64.828	97.108	1.520
Larvae (40 days)	24	215.48	Y=.1600556+2.07418X	176.606	262.931	1.787
	48	141.96	Y=.1498766+2.25358X	118.023	170.765	2.038
	72	109.14	Y=.5841641+2.16673X	89.998	132.371	2.120
Adults	24	3082.8	Y=-19.48316+7.01733X	2907.91	3268.31	4.880
	48	2490.8	Y=-11.27969+4.79329X	2292.15	2706.74	0.652
	72	2215.1	Y=-11.3863+4.8982X	2037.16	2408.42	2.923

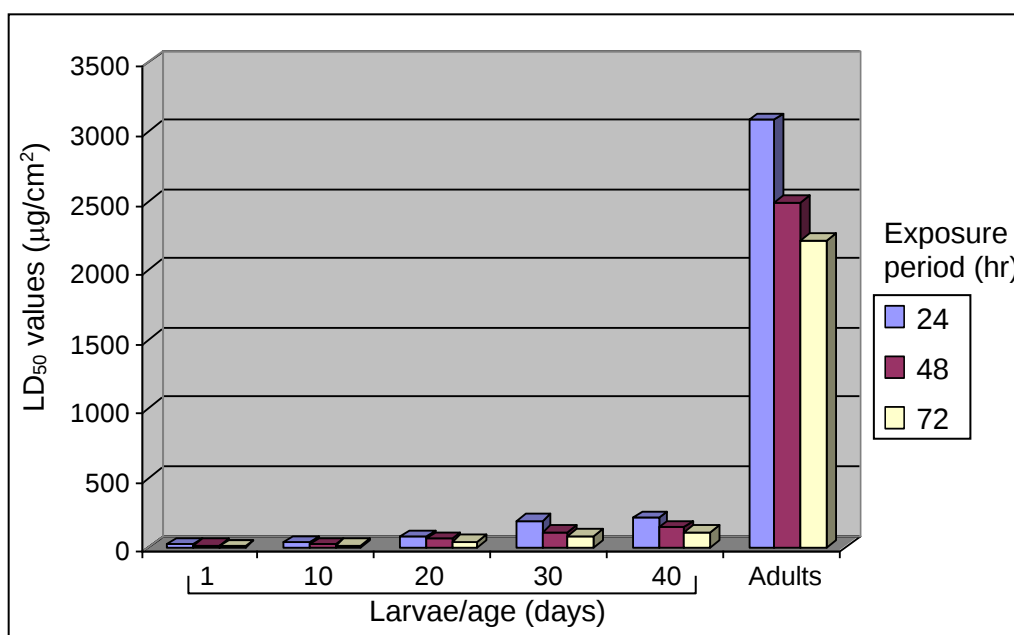


Fig.17: LD₅₀ values of Imidacloprid for both larvae and adults of *A. diaperinus* after 3 different exposure periods in RFM test.

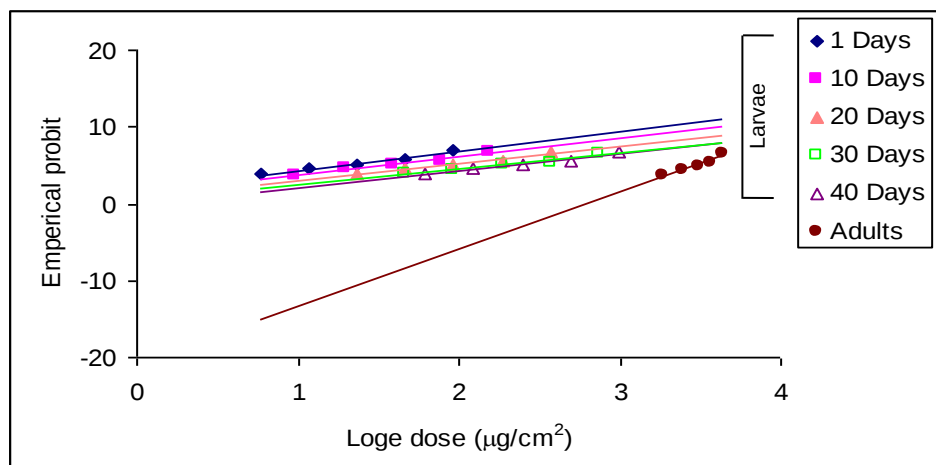


Fig.18: Regression lines of Imidacloprid against *A. diaperinus* larvae (different ages) and adults exposed to treated filter paper for 24 hours.

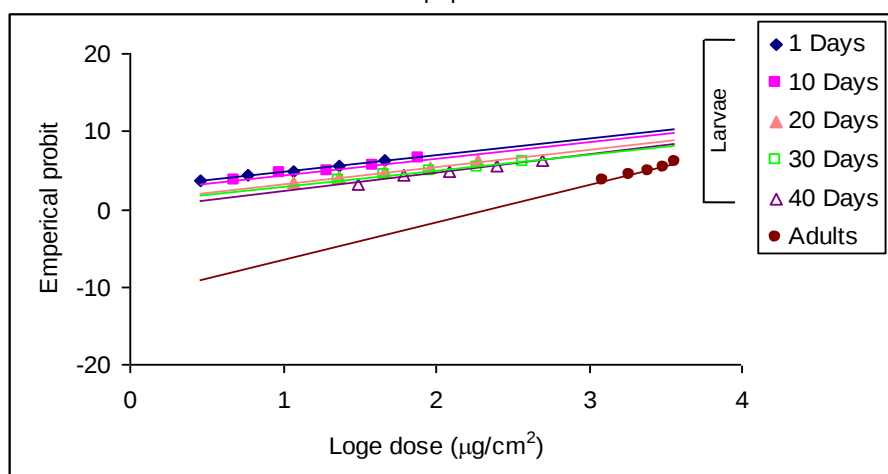


Fig.19: Regression lines of Imidacloprid against *A. diaperinus* larvae (different ages) and adults exposed to treated filter paper for 48 hours.

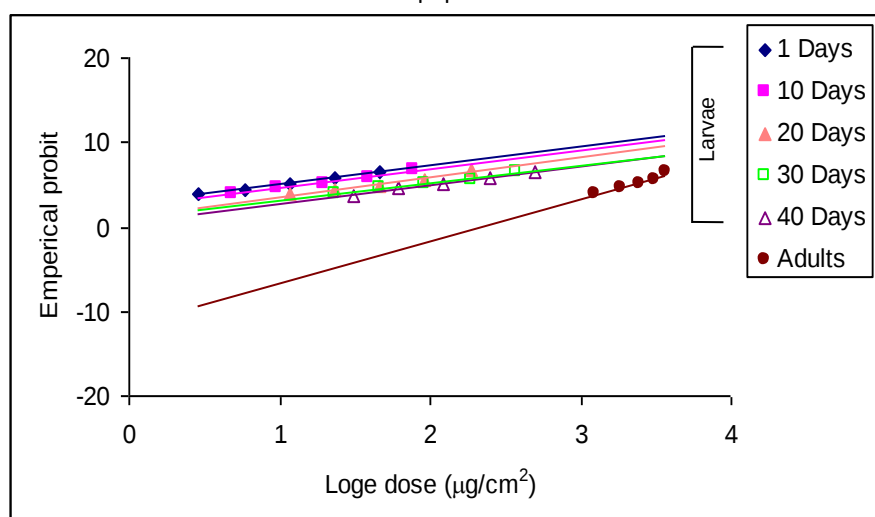


Fig.20: Regression lines of Imidacloprid against *A. diaperinus* larvae (different ages) and adults exposed to treated filter paper for 72 hours.

Table 11: LD₅₀ values, 95% confidence limits and regression equations for Imidacloprid against *A. diaperinus* exposed to the treated flour medium for 24, 48 and 72 hrs.

Life stage (Age)	Exposure period (Hours)	LD ₅₀ (ppm)	Regression equation	95% confidence limit		Chi-square value (3df)
				Lower (ppm)	Upper (ppm)	
Larvae (1 day)	24	22.381	$Y=2.116288+2.13625X$	18.466	27.126	0.241
	48	22.203	$Y=2.205209+2.07571X$	18.223	27.052	0.234
	72	8.287	$Y=3.05077+2.122316X$	6.828	10.058	1.722
Larvae (10 days)	24	57.966	$Y=1.152119+2.18236X$	47.662	70.496	0.133
	48	56.322	$Y=1.292341+2.11784X$	46.036	68.905	0.559
	72	20.019	$Y=2.389318+2.00598X$	16.323	24.551	0.617
Larvae (20 days)	24	107.127	$Y=.333674+2.298793X$	79.704	143.987	8.441
	48	58.728	$Y=1.649389+1.89423X$	47.502	72.606	0.598
	72	47.311	$Y=1.423841+2.13506X$	38.919	57.5130	3.701
Larvae (30 days)	24	242.786	$Y=-7.6571E-2+2.1283X$	199.687	295.186	0.270
	48	114.248	$Y=1.08814+1.900941X$	92.485	141.132	8.81-02
	72	90.948	$Y=.816376+2.135817X$	75.048	110.216	2.876
Larvae (40 days)	24	307.984	$Y=-.163981+2.07511X$	250.688	378.377	0.304
	48	273.730	$Y=4.4304E-2+2.0332X$	223.065	335.902	0.151
	72	108.749	$Y=1.035259+1.946911X$	88.329	133.889	1.337
Adults	24	359.452	$Y=-1.002059+2.34855X$	302.307	427.399	1.963
	48	304.017	$Y=6.2149E-02+1.9887X$	247.615	373.266	0.969
	72	221.968	$Y=.3313422+1.989803X$	180.686	272.683	1.252

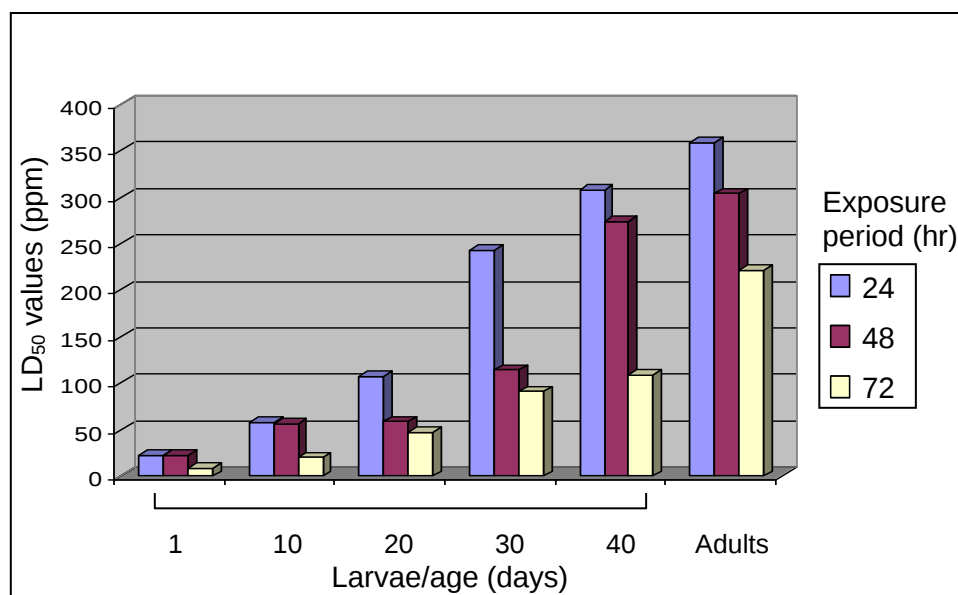


Fig.21: LD₅₀ values of Imidacloprid for both larvae and adults of *A. diaperinus* after 3 different exposure periods in TFM test.

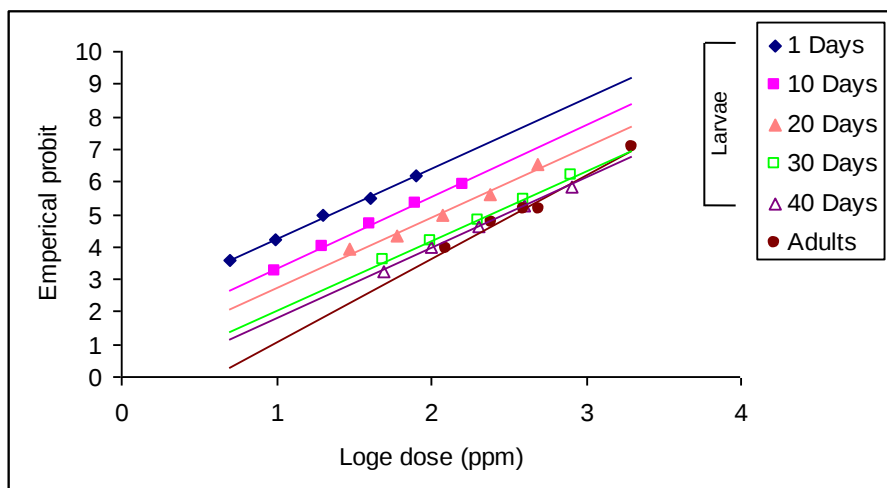


Fig.22: Regression lines of Imidacloprid against *A. diaperinus* larvae (different ages) and adults exposed to treated flour medium for 24 hours.

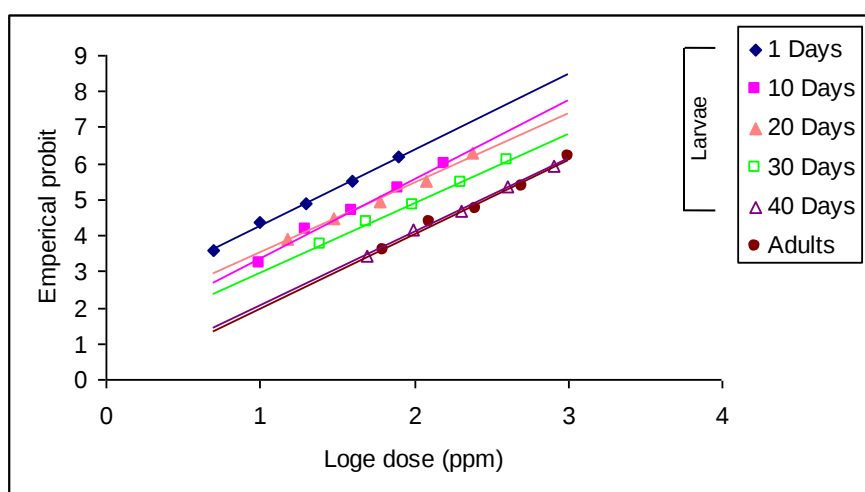


Fig.23: Regression lines of Imidacloprid against *A. diaperinus* larvae (different ages) and adults exposed to treated flour medium for 48 hours.

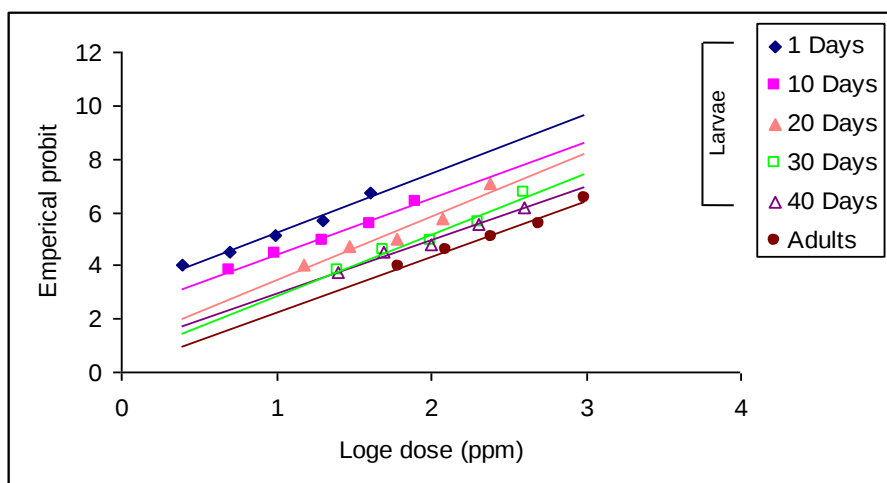


Fig.24: Regression lines of Imidacloprid against *A. diaperinus* larvae (different ages) and adults exposed to treated flour medium for 72 hours.

4.3.4 Toxicity of Thiodicarb (Larvin 75WP) on *A. diaperinus*

Calculation of log/ Probit regression line for the dose mortality experiment of different doses of Thiodicarb have been estimated in Appendix Table (141-176) and Fig 25 and 29 showed that LD₅₀ values were increased with ages and decreased with time.

A. RFM test

Effect on Larval ages

The LD₅₀ values for the 1, 10, 20, 30, and 40 days old larvae were recorded as 4.43, 34.13, 57.32, 83.52 and 78.47µg/cm² respectively, after 24 hrs exposure.

When the exposure period was 48 hours the LD₅₀ values were 2.83, 25.92, 48.75, 42.26, and 64.84µg/cm² for the 1, 10, 20, 30, and 40 days old larvae respectively.

After 72 hours the values were 2.07, 16.76, 34.11, 29.54, and 45.64µg/cm² for the 1, 10, 20, 30, and 40 days old larvae respectively.

Effect on adults

The LD₅₀ values for the adults were recorded as 376.31, 240.43, 134.34µg/cm² after the exposure period of 24, 48 and 72 hours respectively.

95% confidence limits, regression equations, Chi-squared values are shown in the Table 12 and regression lines are shown in Fig. 26-28.

B. TFM test/ Method

Effect on Larval ages

The LD₅₀ values for 1, 10, 20, 30, and 40 days old larvae were recorded as 14.62, 23.19, 23.15, 29.14, and 49.28ppm respectively, after 24 hours.

After 48 hours the values were 7.21, 11.27, 11.75, 13.72, and 21.81ppm respectively.

When the exposure period was 72 hours the LD₅₀ values were 4.98, 8.01, 9.45, 10.92, and 17.68ppm respectively.

Effect on adults

Food treated with different concentration of Thiodicarb, and adult insects reared in food. The LD₅₀ values were recorded as 194.23, 97.29, 53.31ppm after 24, 48, and 72 hours respectively.

The LD₅₀ value along with 95% confidence limits, regression equation, Chi-square values are shown in the Table 13. Regression lines are shown in Fig.30-32.

Table 12: LD₅₀ values, 95% confidence limits and regression equations for Thiodicarb against *A. diaperinus* exposed to the treated filter paper for 24, 48 and 72 hrs.

Life stage (Age)	Exposure period (Hours)	LD ₅₀ (µg/cm ²)	Regression equation	95% confidence limit		Chi-square value (3df)
				Lower (µg/cm ²)	Upper (µg/cm ²)	
Larvae (1 day)	24	4.421	Y=3.59308+2.179677X	3.654	5.347	6.741
	48	2.829	Y=2.552567+1.685872X	2.225	3.598	1.344
	72	2.062	Y=2.598434+1.82706X	1.657	2.567	0.527
Larvae (10 days)	24	34.122	Y=1.872489+2.040075X	27.917	41.705	1.001
	48	25.914	Y=2.112729+2.042577X	21.022	31.945	0.313
	72	16.754	Y=2.651138+1.918791X	13.602	20.636	4.259
Larvae (20 days)	24	57.311	Y=1.737474+1.855559X	46.151	71.172	4.023
	48	48.742	Y=1.51488+2.064753X	39.276	60.491	2.505
	72	34.109	Y=1.572203+2.236188X	28.247	41.188	2.438
Larvae (30 days)	24	83.511	Y=1.212657+1.970786X	67.831	102.814	1.978
	48	42.253	Y=2.234448+1.700978X	33.387	53.473	1.679
	72	29.531	Y=2.27656+1.852337X	23.771	36.685	0.408
Larvae (40 days)	24	78.468	Y=1.259032+1.974443X	64.023	96.172	0.369
	48	64.832	Y=1.518883+1.921369X	51.832	81.091	1.067
	72	45.631	Y=1.515381+2.100104X	37.411	55.658	2.007
Adults	24	376.394	Y=.4159141+1.92529X	194.709	296.871	1.614
	48	240.421	Y=.1488919+1.88345X	298.340	474.869	0.852
	72	134.334	Y=1.104191+1.83057X	107.749	167.485	1.884

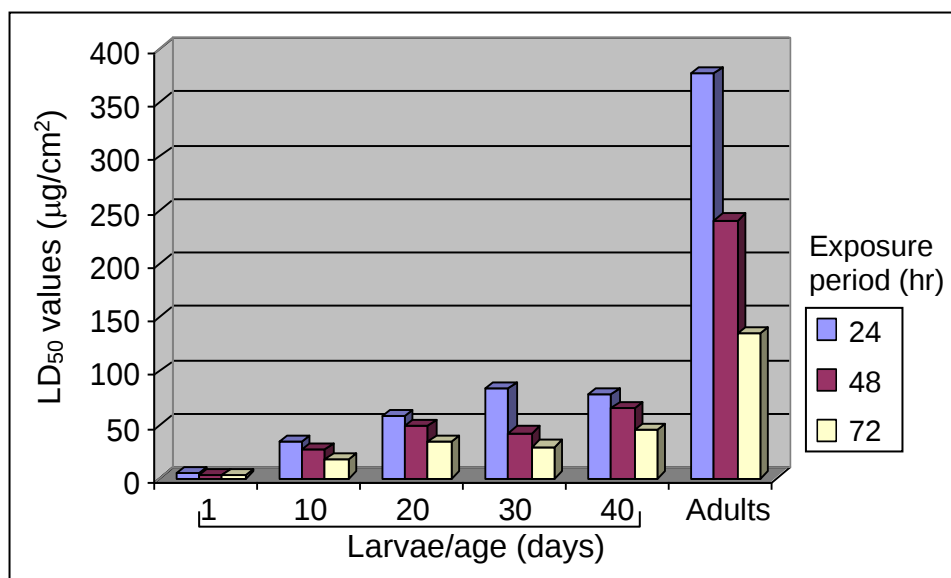


Fig.25: LD₅₀ values of Thiodicarb for both larvae and adults of *A. diaperinus* after 3 different exposure periods in RFM test.

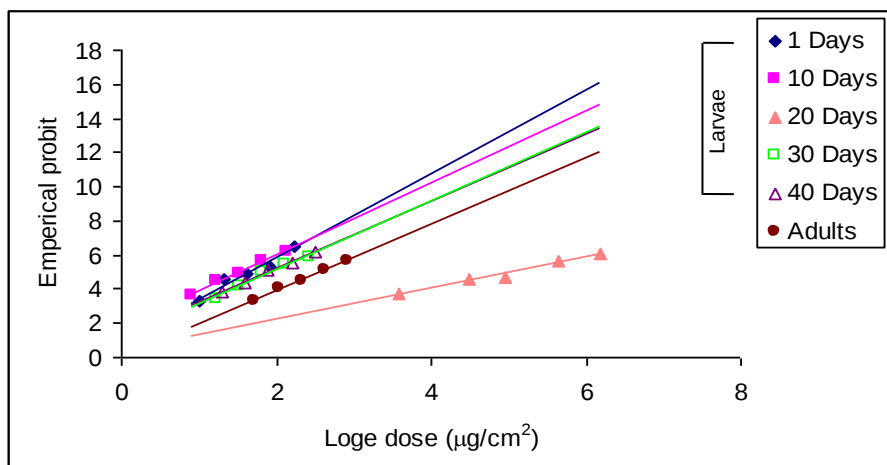


Fig.26: Regression lines of Thiodicarb against *A. diaperinus* larvae (different ages) and adults exposed to treated filter paper for 24 hours.

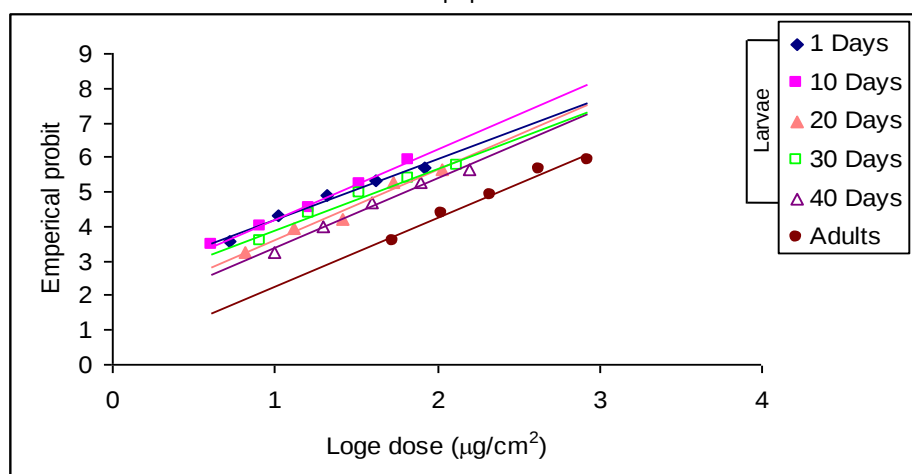


Fig.27: Regression lines of Thiodicarb against *A. diaperinus* larvae (different ages) and adults exposed to treated filter paper for 48 hours.

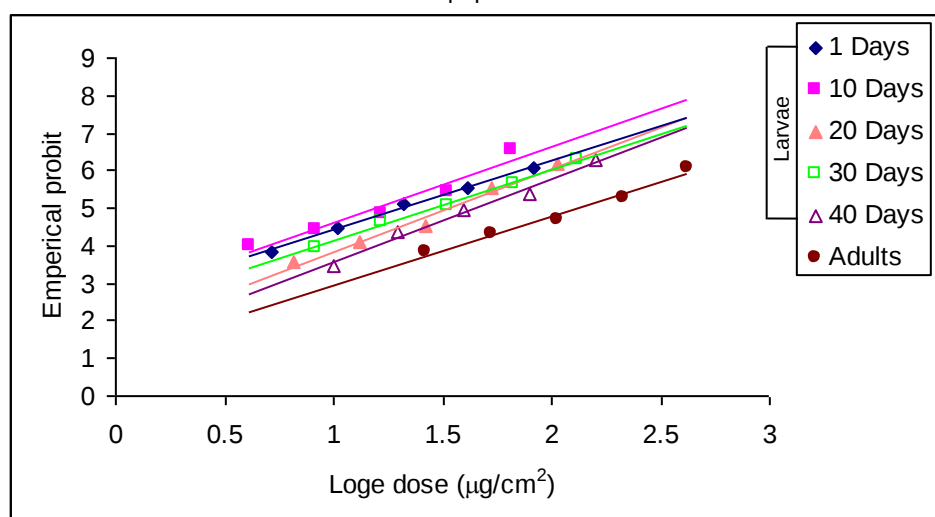


Fig.28: Regression lines of Thiodicarb against *A. diaperinus* larvae (different ages) and adults exposed to treated filter paper for 72 hours.

Table 13: LD₅₀ values, 95% confidence limits and regression equations for Thiodicarb against *A. diaperinus* exposed to the treated flour medium for 24, 48 and 72 hrs.

Life stage (Age)	Exposure period (Hours)	LD ₅₀ (ppm)	Regression equation	95% confidence limit		Chi-square value (3df)
				Lower (ppm)	Upper (ppm)	
Larvae (1 day)	24	14.611	$Y=2.679973+1.99199X$	11.903	17.933	1.913
	48	7.201	$Y=3.344418+1.93088X$	5.831	8.893	1.836
	72	4.972	$Y=3.521596+2.12231X$	4.097	6.035	1.722
Larvae (10 days)	24	23.186	$Y=2.020559+2.18236X$	19.065	28.198	0.133
	48	11.264	$Y=2.772636+2.11783X$	9.207	13.781	0.559
	72	8.007	$Y=3.18757+2.005992X$	6.529	9.821	0.617
Larvae (20 days)	24	23.144	$Y=2.164383+2.07820X$	18.979	28.223	1.616
	48	11.745	$Y=2.973384+1.89423X$	9.501	14.521	0.597
	72	9.462	$Y=2.916174+2.13505X$	7.784	11.502	3.701
Larvae (30 days)	24	29.134	$Y=1.883211+2.12835X$	23.963	35.423	0.271
	48	13.711	$Y=2.838545+1.90094X$	11.098	16.936	8.792
	72	10.914	$Y=2.783058+2.13581X$	9.006	13.226	2.876
Larvae (40 days)	24	49.278	$Y=1.487543+2.07511X$	40.111	60.541	0.304
	48	21.899	$Y=2.274583+2.03325X$	17.845	26.872	0.151
	72	17.671	$Y=2.53434+1.976849X$	14.396	21.691	0.905
Adults	24	194.229	$Y=.1296496+2.12835X$	159.75	236.149	0.269
	48	97.286	$Y=1.046289+1.98873X$	79.237	119.447	0.969
	72	53.307	$Y=1.967546+1.75612X$	42.264	67.235	2.757

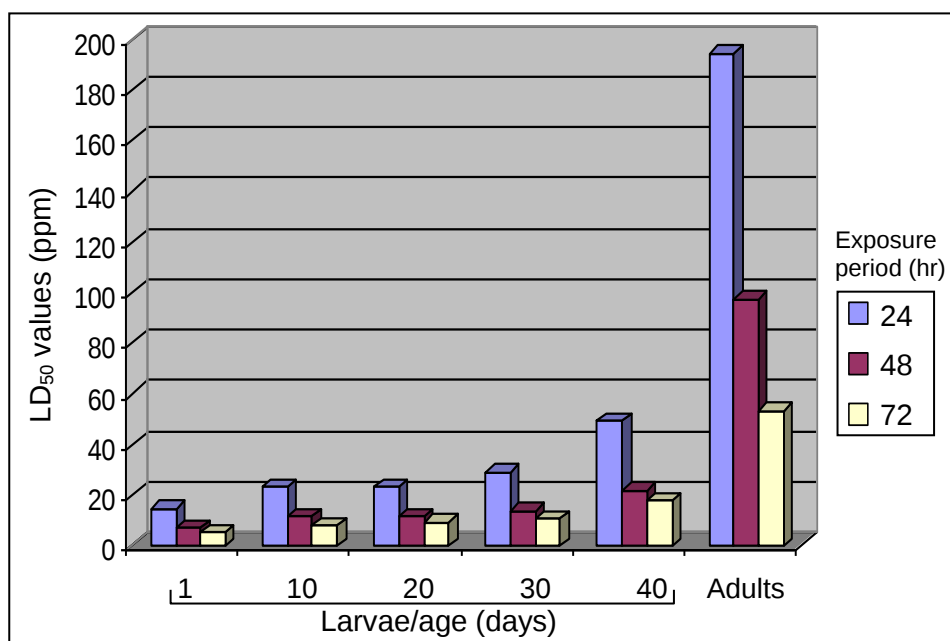


Fig.29: LD₅₀ values of Thiodicarb for both larvae and adults of *A. diaperinus* after 3 different exposure periods in TFM test.

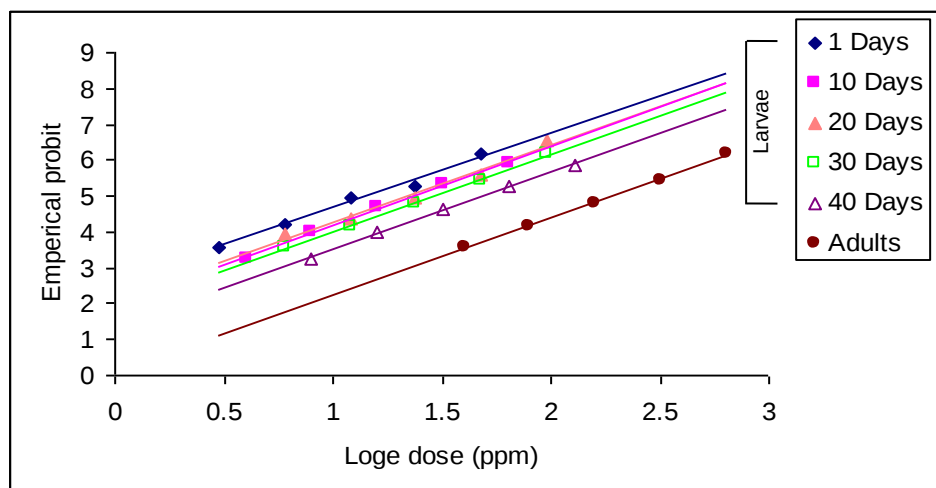


Fig.30: Regression lines of Thiodicarb against *A. diaperinus* larvae (different ages) and adults exposed to treated flour medium for 24 hours.

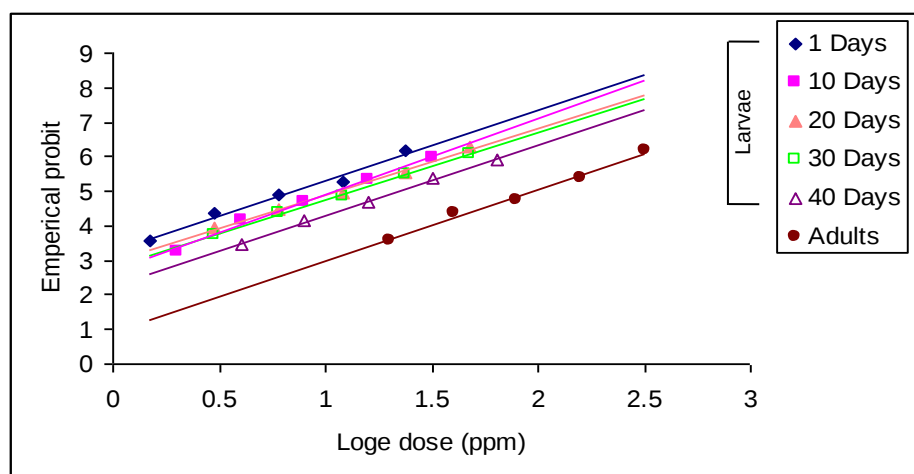


Fig.31: Regression lines of Thiodicarb against *A. diaperinus* larvae (different ages) and adults exposed to treated flour medium for 48 hours.

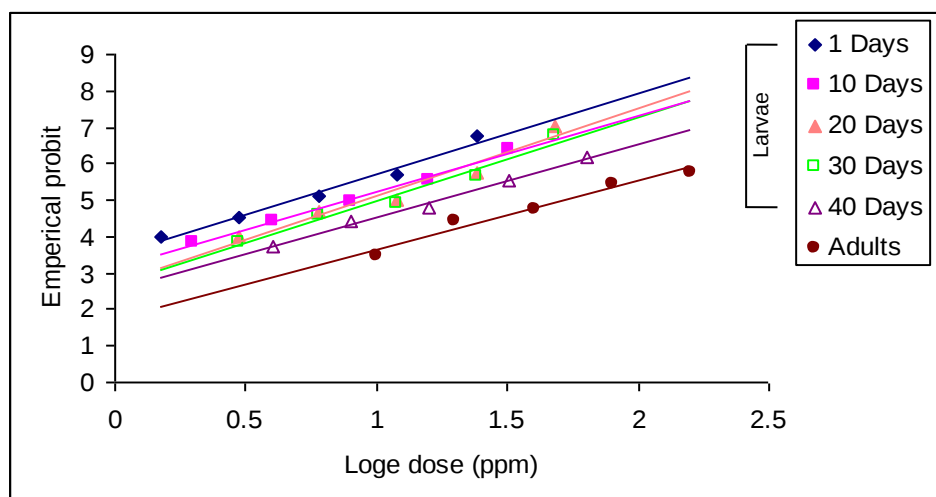


Fig.32: Regression lines of Thiodicarb against *A. diaperinus* larvae (different ages) and adults exposed to treated flour medium for 72 hours.

4.3.5 Toxicity of Fenitrothion (Sumithion 50%) on *A. diaperinus*

Calculation of log/ Probit regression line for the dose mortality experiment of different doses of Fenitrothion have been estimated in Appendix Table (193-228) and Fig 33 and 37 showed that LD₅₀ values were increased with ages and decreased with time.

A. RFM test/ Method

Effect on Larval ages

The LD₅₀ values for the 1, 10, 20, 30, and 40 days old larvae were recorded as 49.74, 54.35, 86.35, 86.35 and 257.76 µg/cm² respectively after the exposure period at 24 hours. After 48 hours the LD₅₀ values were 34.11, 39.33, 53.26, 90.03, and 200.46 µg/cm² for the 1, 10, 20, 30, and 40 days old larvae respectively.

After 72 hours the values were recorded 22.03, 26.82, 36.37, 65.75, and 107.81 µg/cm² for the 1, 10, 20, 30, and 40 days old larvae respectively.

Effect on adults

The LD₅₀ values for the adults were recorded as 3033.83, 2786.11, 2032.29 µg/cm² after the exposure periods of 24, 48 and 72 hours respectively.

95% confidence limits, regression equation Chi-squared values are shown in the Table 14 and regression lines are shown in Fig. 34-36.

B. TFM test/ Method

Effect on Larval ages

The LD₅₀ values for 1, 10, 20, 30, and 40 days old larvae were 161.33, 217.36, 281.26, 293.77, and 436.40 ppm respectively after 24 hours.

After 48 hr exposure the values were 111.02, 170.19, 190.68, 243.42, and 260.67 ppm respectively.

When the exposure period was 72 hours the values were 47.81, 81.31, 101.52, 121.61, and 170.39 ppm respectively.

Effects on adults

Food treated with different concentration of fenitrothion, and adult insects reared in food. The LD₅₀ values were 901.08, 605.91, 385.77 ppm after 24, 48, and 72 hours respectively.

The LD₅₀ values along with 95% confidence limits, regression equation, Chi-square values are shown in the Table 15. Regression lines are shown in Fig.38-40.

Table 14: LD₅₀ values, 95% confidence limits and regression equations for Fenitrothion against *A. diaperinus* exposed to the treated filter paper for 24, 48 and 72 hrs.

Life stage (Age)	Exposure period (Hours)	LD ₅₀ (µg/cm ²)	Regression equation	95% confidence limit		Chi-square value (3df)
				Lower (µg/cm ²)	Upper (µg/cm ²)	
Larvae (1 day)	24	49.731	Y=1.888578+1.8339X	39.724	62.256	1.562
	48	34.109	Y=2.439103+1.6707X	26.924	43.212	1.534
	72	22.025	Y=2.245689+2.0501X	17.997	26.954	1.001
Larvae (10 days)	24	54.314	Y=.752018+2.44853X	45.546	64.769	1.601
	48	39.321	Y=1.38607+2.26633X	32.651	47.351	1.091
	72	26.819	Y=2.09747+2.03195X	21.851	32.917	1.049
Larvae (20 days)	24	86.345	Y=1.703993+1.7023X	68.638	108.621	4.114
	48	53.253	Y=1.051785+2.2871X	44.209	64.147	3.403
	72	36.368	Y=1.644966+2.1497X	29.964	44.141	1.205
Larvae (30 days)	24	170.893	Y=.737403+1.90915X	138.261	211.226	0.981
	48	90.025	Y=.5120731+2.2964X	75.202	107.771	6.185
	72	65.741	Y=1.401039+1.9799X	53.562	80.686	1.681
Larvae (40 days)	24	257.751	Y=-.149375+2.13560X	211.098	314.714	0.849
	48	200.453	Y=.2043443+2.0833X	164.331	244.517	0.601
	72	107.806	Y=1.11699+1.91033X	87.277	133.163	1.986
Adults	24	3033.83	Y=-13.57192+5.3337X	2808.41	3277.35	2.803
	48	2786.12	Y=-12.7513+5.15278X	2574.19	3015.47	3.047
	72	2032.29	Y=-5.16509+3.07289X	1788.97	2308.69	3.073

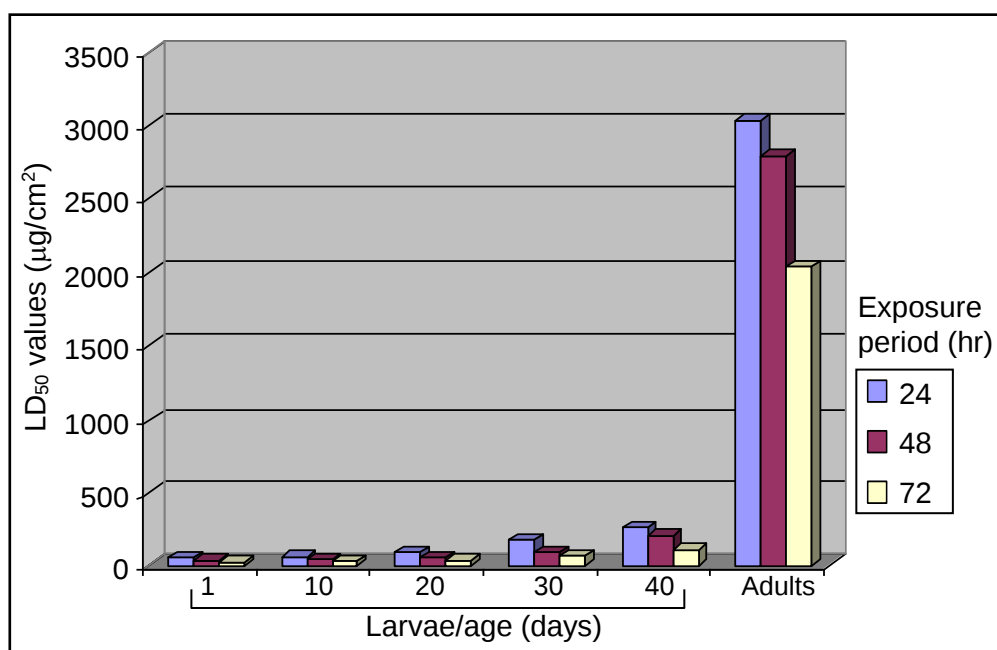


Fig.33: LD₅₀ values of Fenitrothion for both larvae and adults of *A. diaperinus* after 3 different exposure periods in RFM test.

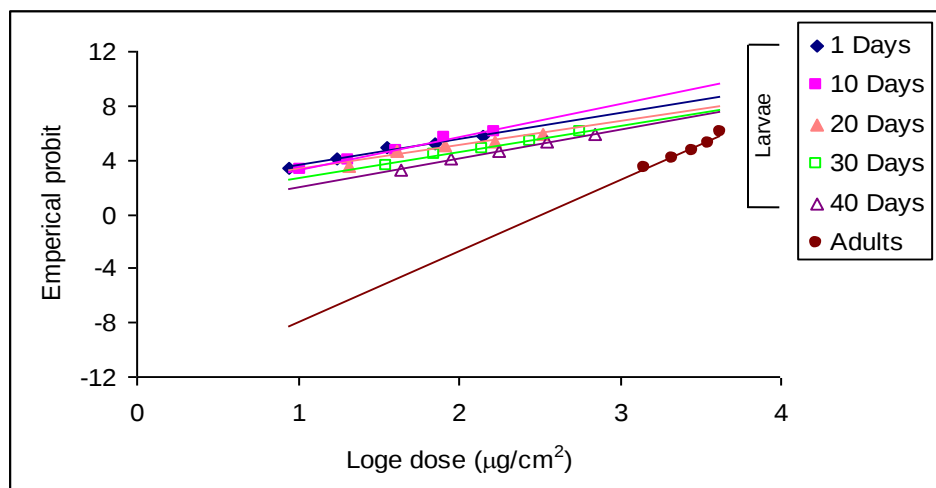


Fig.34: Regression lines of Fenitrothion against *A. diaperinus* larvae (different ages) and adults exposed to treated filter paper for 24 hours.

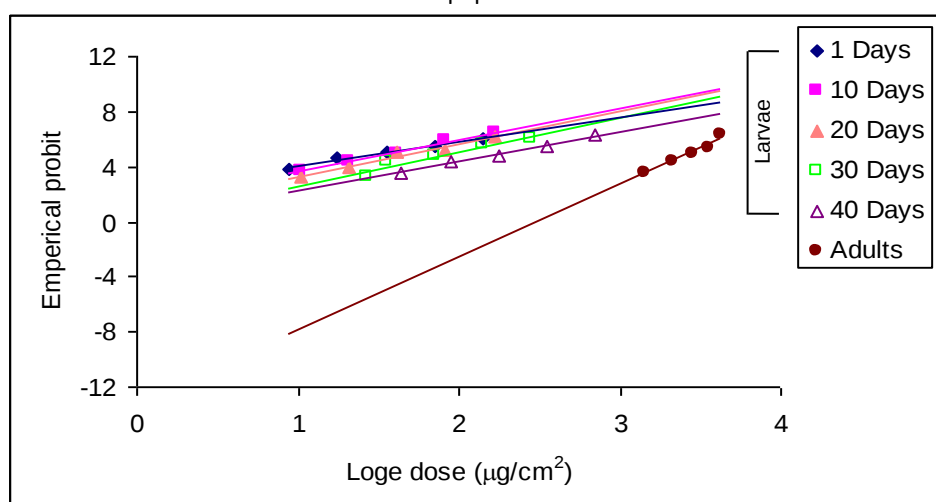


Fig.35: Regression lines of Fenitrothion against *A. diaperinus* larvae (different ages) and adults exposed to treated filter paper for 48 hours.

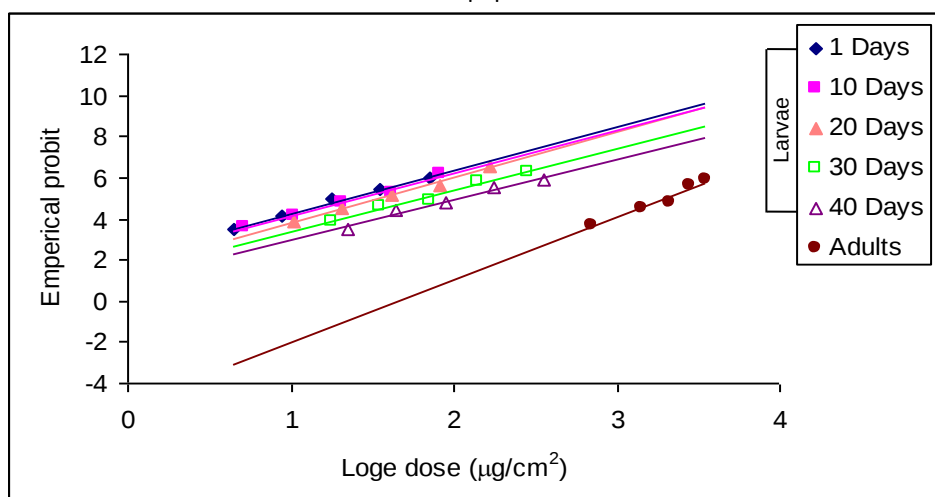


Fig.36: Regression lines of Fenitrothion against *A. diaperinus* larvae (different ages) and adults exposed to treated filter paper for 72 hours.

Table 15: LD₅₀ values, 95% confidence limits and regression equations for Fenitrothion against *A. diaperinus* exposed to the treated flour medium for 24, 48 and 72 hrs.

Life stage (Age)	Exposure period (Hours)	LD ₅₀ (ppm)	Regression equation	95% confidence limit		Chi-square value (3df)
				Lower (ppm)	Upper (ppm)	
Larvae (1 day)	24	161.331	$Y = .854304 + 1.877822X$	128.086	203.202	1.067
	48	111.015	$Y = .754371 + 2.075712X$	91.117	135.259	0.234
	72	47.897	$Y = 1.87386 + 1.860444X$	38.649	59.358	2.407
Larvae (10 days)	24	217.369	$Y = -.10059 + 2.182356X$	178.733	264.358	0.133
	48	170.181	$Y = .3805161 + 2.07067X$	139.573	207.501	0.659
	72	81.392	$Y = 1.300237 + 1.93645X$	66.036	100.318	1.866
Larvae (20 days)	24	281.266	$Y = .6652226 + 1.76993X$	223.195	354.446	1.171
	48	190.673	$Y = .3254624 + 2.04997X$	156.073	232.943	0.513
	72	101.517	$Y = 1.191671 + 1.89795X$	82.041	125.617	3.135
Larvae (30 days)	24	293.773	$Y = -.759983 + 2.33385X$	243.922	353.811	0.794
	48	243.412	$Y = .1312251 + 2.04026X$	199.092	297.599	1.560-02
	72	121.698	$Y = .8505621 + 1.98986X$	99.034	149.549	1.911
Larvae (40 days)	24	436.409	$Y = .7482176 + 2.17744X$	358.822	530.771	0.146
	48	260.664	$Y = .3787904 + 1.91268X$	210.944	322.103	0.856
	72	170.387	$Y = .3536625 + 2.08221X$	139.866	207.571	0.233
Adults	24	901.076	$Y = -.481316 + 1.85507X$	710.923	1142.09	0.636
	48	605.907	$Y = -.388651 + 1.93668X$	490.823	747.973	1.468
	72	385.769	$Y = -.266242 + 2.03618X$	313.096	475.311	0.104

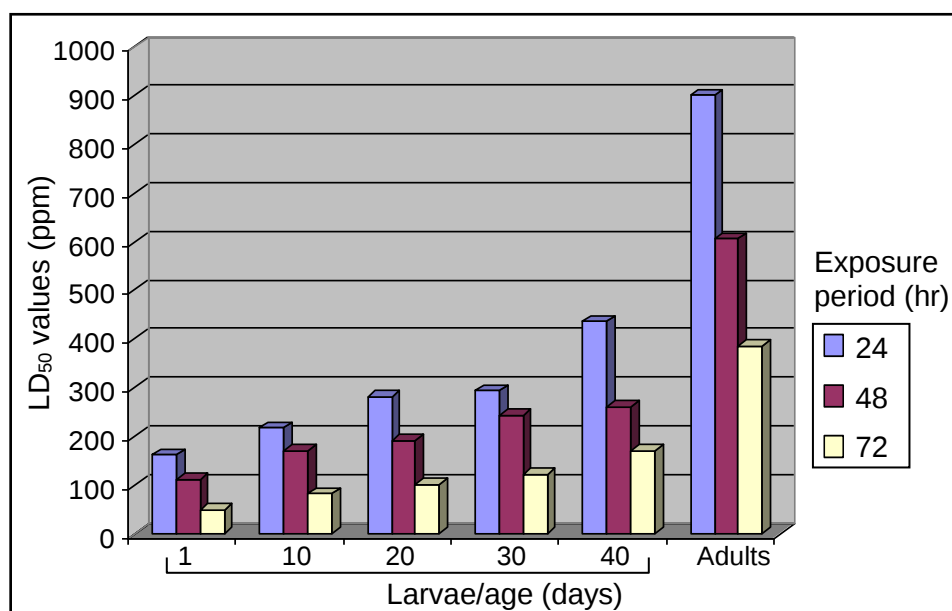


Fig.37: LD₅₀ values of Fenitrothion for both larvae and adults of *A. diaperinus* after 3 different exposure periods in TFM test.

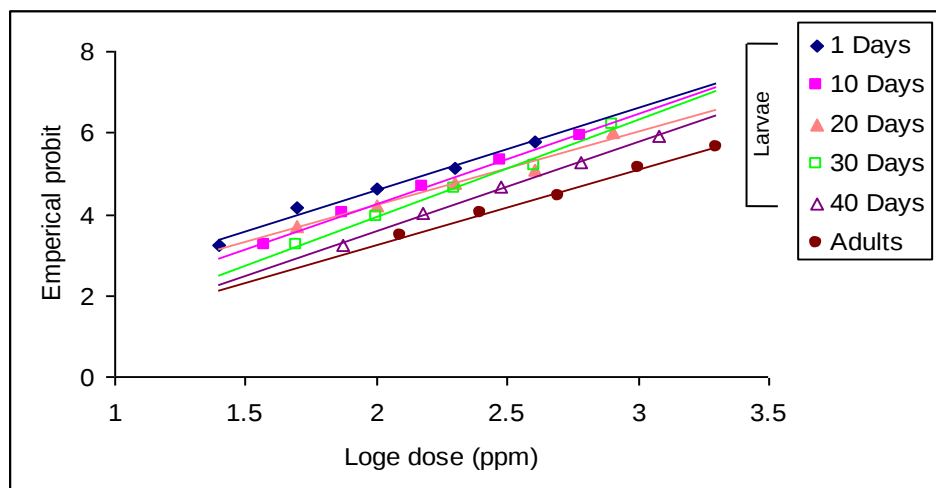


Fig.38: Regression lines of Fenitrothion against *A. diaperinus* larvae (different ages) and adults exposed to treated flour medium for 24 hours.

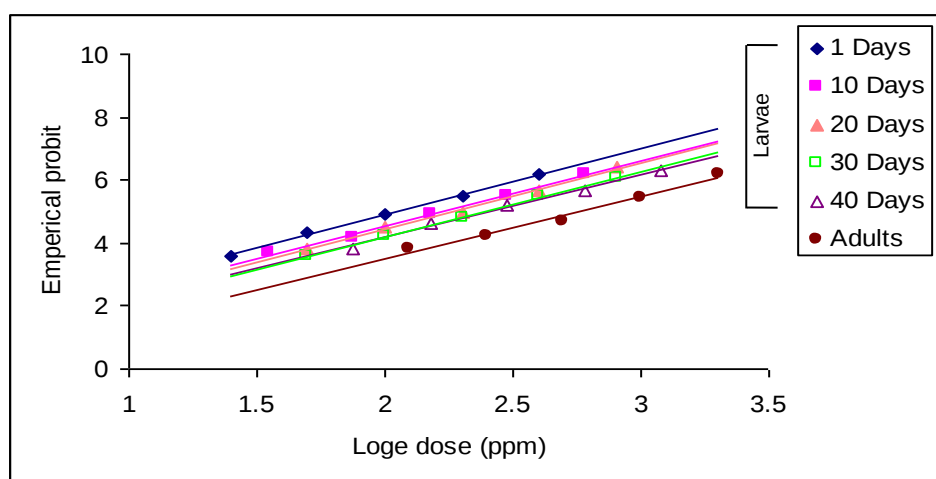


Fig.39: Regression lines of Fenitrothion against *A. diaperinus* larvae (different ages) and adults exposed to treated flour medium for 48 hours.

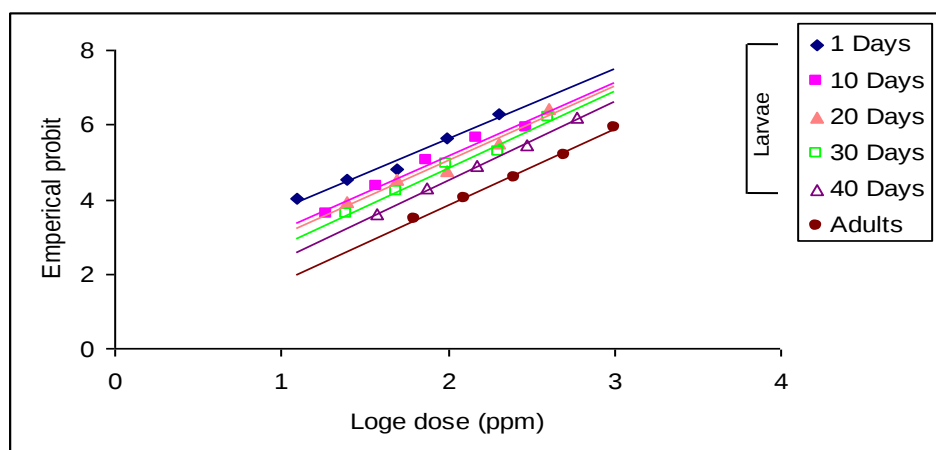


Fig.40: Regression lines of Fenitrothion against *A. diaperinus* larvae (different ages) and adults exposed to treated flour medium for 72 hours.

Chapter – 5

Combined Action of Insecticides (Deltamethrin, Imidacloprid, Thiodicarb, Fenitrothion) and Azadirachtin

5.1 Introduction

It is normally best to use a combination of pesticide applications. Because insecticide resistance is a common phenomenon (Adams 2003). To avoid this resistance, the effective insect control programs are preferably adopted by using the potential insecticides or combined insecticide treatments (Salin *et al* 2003, Acevedo *et al* 2009; Mee *et al* 2009).

Dimetry *et al* (1993), Sundaram and Solane (1995) and Sanguanpong and Schmutterer (1992) reported that different formulation of Azadirachtin including 'Margosan-O' and Neem Azal-S', affected repellency, feeding rate, oviposition, and mortality of *Tetranychus urticae* (Prostigmata) females. The lowest adult emergence was recorded in the insecticide and nimbecidine combined treatment. This was probably due to higher larval and pupal mortality because of the toxicity of cypermethrin and Deltamethrin, and also in part due to the synergistic action (Mondal 1984a, Khatun 2010) of nimbecidine or Azadirachtin.

5.2 Materials and methods

Keeping the lowest doses of individual insecticide for individual stages of insects was constant, combined with azadirachtin. Combined doses of each insecticide and azadirachtin were prepared at the different ratios. Five serial dilutions of each ratio were made and the mortality percentage was recorded after 72h. As there was no mortality or very negligible number of mortality was found after 24hr and 48hr exposure that is why the mortality was assessed after 72hr exposure. The mature larvae (40 days old) and adults of *A. diaperinus* were used for this experiment.

To study of the toxicity, here also two types of experiments were designed that were Residual Film Method (RFM) (Busvine 1971) and the Treated Food Method (TFM), which was defined in chapter-3.

5.2.1 Determination of cotoxicity coefficient

The Cotoxicity coefficient was calculated using the following formula (Sun and Johnson 1960);

$$\text{Cotoxicitycoefficient} = \frac{\text{LD}_{50} \text{ of toxicant alone}}{\text{LD}_{50} \text{ of toxicant in the mixture}} \times 100$$

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.

5.2.1 Determination of dose reduction

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

$$a - s = r \dots\dots\dots(1)$$

$$\% \text{ or redused a. i.} = r/a \times 100\dots\dots(2)$$

Where a= LD₅₀ value of the active ingredient alone

s =Share of the active ingredient in the LD₅₀ value of the mixture

r = reduced amount of the a. i. to kill 50% of the test insects (Akter 2011).

5.2.2 Construction of isobolograms

The regression lines and isoboles were drawn using the (Biosoft) package. Isobolograms for the mixtures of insecticides were constructed using the methods described by Hewlett (1960). This was done as follows: using the LD₅₀ value of individual compound, the concentration of each individual compound in the mixture was plotted. Isobole lines below the additive line indicate synergism. Isoboles were drawn by free hand curve fitting.

5.3 Results and observation

5.3.1 Deltamethrin and Azadirachtin

A. RFM test

For the mature larvae (40days old): The lowest dose of Deltamethrin was $265.20\mu\text{g}/\text{cm}^2$ for the mature larvae after 72 hours. Effects of different combined doses of Deltamethrin and Azadirachtin at the ratios of 1:0.1, 1:0.2, 1:0.4 and 1:0.5 have been estimated in Appendix Tables (73-76) where the lowest dose of Deltamethrin was constant. The dose concentration were $145.86, 72.93, 36.47, 18.24$, and $9.12\mu\text{g}/\text{cm}^2$ at ratio 1:0.1; $159.12, 79.56, 39.78, 19.89$, and $9.95\mu\text{g}/\text{cm}^2$ at ratio 1:0.2; $185.64, 92.82, 46.41, 23.21$ and $11.61\mu\text{g}/\text{cm}^2$ at ratio 1: 0.4; and $198.9, 99.45, 49.73, 24.87$ and $12.44\mu\text{g}/\text{cm}^2$ at ratio 1:0.5. The mortality percentage were recorded as 4 -58 % at 1:0.1; 4- 68 % at 1: 0.2; 6- 80 % at 1: 0.4; 10- 90 % at 1: 0.5 ratios after 72 h of exposure. Highest mortality observed at the ratio of 1:0.5.

The LD_{50} values of the mixture of Deltamethrin + Azadirachtin were recorded after 72h as $99.58, 80.36, 62.29$ and $53.54\mu\text{g}/\text{cm}^2$ at the ratios of 1:0.1, 1:0.2, 1:0.4 and 1:0.5 respectively.

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. 41.

For adults: The lowest dose of Deltamethrin was $442\mu\text{g}/\text{cm}^2$ for the adults exposed for 72 hours. Effects of different combined doses of Deltamethrin and Azadirachtin at the ratios of 1:0.1, 1:0.2, 1:0.3 and 1:0.6 have been estimated in Appendix Tables (77-80) where the lowest dose of Deltamethrin was constant. The dose concentrations were $243.1, 121.55, 60.78, 30.39$ and $15.11\mu\text{g}/\text{cm}^2$ at ratio 1:0.1; $265.2, 132.6, 66.3, 33.15$ and $16.58\mu\text{g}/\text{cm}^2$ at ratio 1:0.2; $298.35, 149.17, 74.59, 37.21$ and $18.65\mu\text{g}/\text{cm}^2$ at ratio 1: 0.3; and $353.6, 176.8, 88.4, 44.2$ and $22.1\mu\text{g}/\text{cm}^2$ at ratio 1:0.6. The mortality percentage were recorded as 2 -60 % at 1:0.1; 4- 68 % at 1: 0.2; 8-78 % at 1: 0.3; 12- 96 % at 1: 0.6 after 72 h exposure. The highest mortality was recorded at the ratio of 1:0.6.

The LD_{50} values of the mixture (Deltamethrin + Azadirachtin) were recorded after 72h as $171.01, 140.45, 121.46$ and $81.67\mu\text{g}/\text{cm}^2$ at the ratio 1:0.1, 1:0.2, 1:0.3 and 1:0.6 respectively.

LD_{50} values along with 95% confidence limits, regression equations and chi-squared values have been estimated in the Table 17. Regression lines of different ratios on log probit mortality and the log dose concentrations have been plotted as in the Fig. 42.

B. TFM test

For the mature larvae (40days old): The lowest dose of Deltamethrin was 400ppm for the mature larvae after 72 hours exposure. Effects of different combined doses of Deltamethrin and Azadirachtin at the ratios of 1:1, 1:1.5, 1:2 and 1:2.5 have been estimated in Appendix Tables (81-84) where the lowest dose of Deltamethrin was constant. The dose concentrations were 400, 200, 100, 50 and 25 ppm at ratio 1:1; 500, 250, 125, 62.5 and 31.25 ppm at ratio 1:1.5; 600, 300, 150, 75 and 37.5 ppm at ratio 1: 2; and 700, 350, 175, 87.5 and 43.75 ppm at ratio 1:2.5. The mortality percentage were recorded as 4 -60 % at 1:1; 4- 70 % at 1: 1.5; 8- 78 % at 1: 2; 14- 94 % at 1: 2.5 after 72 h of exposure. The highest mortality was found at the ratio of 1:2.5.

The LD₅₀ values of the mixture (Deltamethrin + Azadirachtin) were recorded after 72h as 204.52, 190.46, 181.48 and 170.84 ppm at the ratios of 1:1, 1:1.5, 1:2 and 1:2.5 respectively.

LD₅₀ values along with 95% confidence limits, regression equations and chi-squared values have been estimated in the Table 18. Regression lines of different ratios on log probit mortality and the log dose concentrations have been plotted as in the Fig. 43.

For adults: The lowest dose of Deltamethrin was 500 ppm for the adults after 72 hours. Effects of different combined doses of Deltamethrin and Azadirachtin at the ratios of 1:1.5, 1:2, 1:2.5 and 1:3 have been estimated in Appendix Tables (85-88) where the lowest dose of Deltamethrin was constant. The dose concentrations were 625, 312.5, 156.25, 78.13 and 39.07ppm at ratio1:1.5; 750, 375, 187.5, 93.75 and 46.88 ppm at ratio1: 2; 875, 437.5, 218.75, 109.38 and 54.69 ppm at ratio 1:2.5 and 1000, 500, 250, 125 and 62.5ppm at ratio1:3. The mortality percentage were recorded as 6 -64 % at 1: 1.5; 6-74 % at 1: 2; 10- 80 % at 1:2.5; 12- 88 % at 1:3after 72 h of exposure. The highest mortality was observed at the ratio of 1: 3.

The LD₅₀ values of the mixture (Deltamethrin + Azadirachtin) were recorded after 72h as 319.61, 298.66, 280.23 and 269.42 ppm at the ratio 1:1.5, 1:2, 1:2.5 and 1:3 respectively.

LD₅₀ values along with 95% confidence limits, regression equations and chi-squared values have been estimated in the Table 19. Regression lines of different ratios on log probit mortality and the log dose concentrations have been plotted as in the Fig. 44.

Table 16: LD₅₀ , 95% confidence limits and regression equations for combined action of Deltamethrin with Azadirachtin against the mature larvae (40 days old) of *A. diaperinus* exposed to the treated filter paper for 72 hours.

Insecticide	Life stage (Age)	Ratio of combination	LD ₅₀ of combination (µg/cm ²)	Regression equation	95% confidence limit		Chi-square value (3df)
					Lower (µg/cm ²)	Upper (µg/cm ²)	
Delta (RFM)	40day old larvae	1:0.1	99.574	Y=1.8968+1.55305X	70.9455	139.755	1.0561
		1:0.2	80.350	Y=1.824119+1.667X	61.0486	105.754	1.5103
		1:0.4	62.287	Y=1.572869+1.909X	50.1647	77.3393	0.7765
		1:0.5	53.530	Y=1.71106+1.9213X	41.7422	63.6152	2.0384

Table 17: LD₅₀ , 95% confidence limits and regression equations for combined action of Deltamethrin with Azadirachtin against the adults of *A. diaperinus* exposed to the treated filter paper for 72 hours.

Insecticide	Life stage (Age)	Ratio of combination	LD ₅₀ of combination (µg/cm ²)	Regression equation	95% confidence limit		Chi-square value (3df)
					Lower (µg/cm ²)	Upper (µg/cm ²)	
Delta (RFM)	Adults	1:0.1	171.097	Y=0.84569+1.8601X	128.03	228.640	0.2578
		1:0.2	140.445	Y=1.11397+1.8095X	108.38	181.997	0.3474
		1:0.3	121.458	Y=1.40624+1.7242X	95.144	155.049	0.6921
		1:0.6	81.6685	Y=0.87501+2.1573X	67.424	98.9211	1.7673

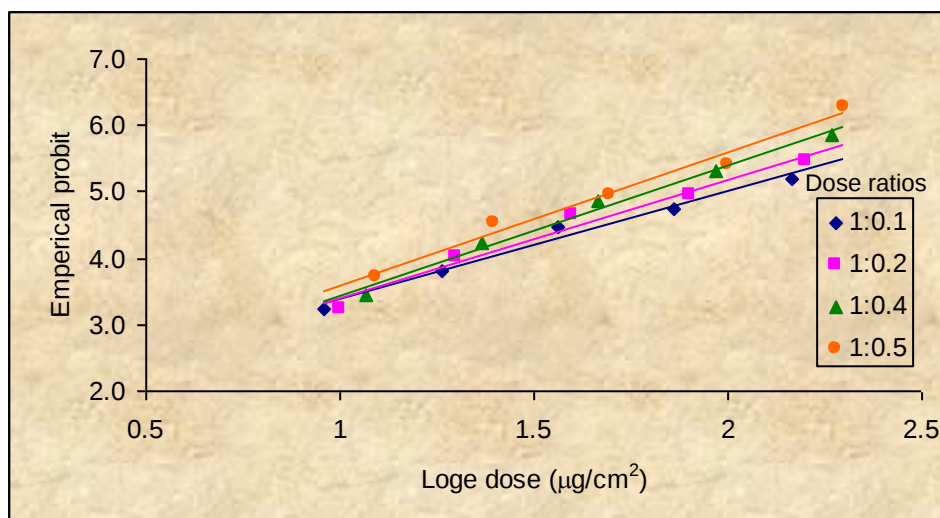


Fig 41: Regression lines of different combined doses Deltamethrin and Azadirachtin against the mature larvae (40 days old) of *A. diaperinus* exposed to the treated filter paper for 72 hours

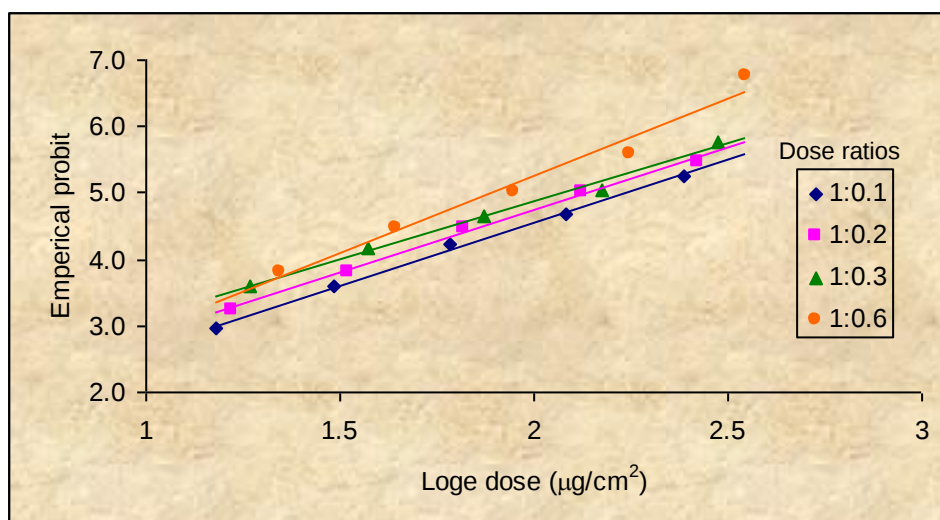


Fig 42: Regression lines of different combined doses Deltamethrin and Azadirachtin against the adults of *A. diaperinus* exposed to the treated filter paper for 72 hours.

Table 18: LD₅₀, 95% confidence limits and regression equations for combined action of Deltamethrin with Azadirachtin against the mature larvae (40 days old) of *A. diaperinus* exposed to the treated flour medium for 72 hours.

insecticide	Life stage (Age)	Ratio of combination	LD ₅₀ of combination (µg/cm ²)	Regression equation	95% confidence limit		Chi-square value (3df)
					Lower (µg/cm ²)	Upper (µg/cm ²)	
Delta (TFM)	40day old larvae	1:1	204.516	Y=1.57689+1.48139X	150.415	278.076	4.2541
		1:1.5	190.458	Y=1.22379+1.65637 X	148.508	244.259	4.6190
		1:2	181.473	Y=1.31109+1.63311X	142.372	231.312	2.7556
		1:2.5	170.833	Y=0.718322+1.9660X	122.417	185.225	1.3526

Table 19: LD₅₀ , 95% confidence limits and regression equations for combined action of Deltamethrin with Azadirachtin against the adults of *A. diaperinus* exposed to the treated flour medium for 72 hours.

Insecticide	Life stage (Age)	Ratio of combination	LD ₅₀ of combination (µg/cm ²)	Regression equation	95% confidence limit		Chi-square value (3df)
					Lower (µg/cm ²)	Upper (µg/cm ²)	
Delta (TFM)	Adults	1:1.5	319.605	Y=0.874465+1.6258X	257.65	461.296	0.5652
		1:2	298.659	Y=0.63248+1.76168X	236.893	383.529	0.4883
		1:2.5	280.222	Y=0.800675+1.7187X	219.809	350.231	0.4434
		1:3	269.419	Y=0.419982+1.9209X	196.519	298.681	0.1058

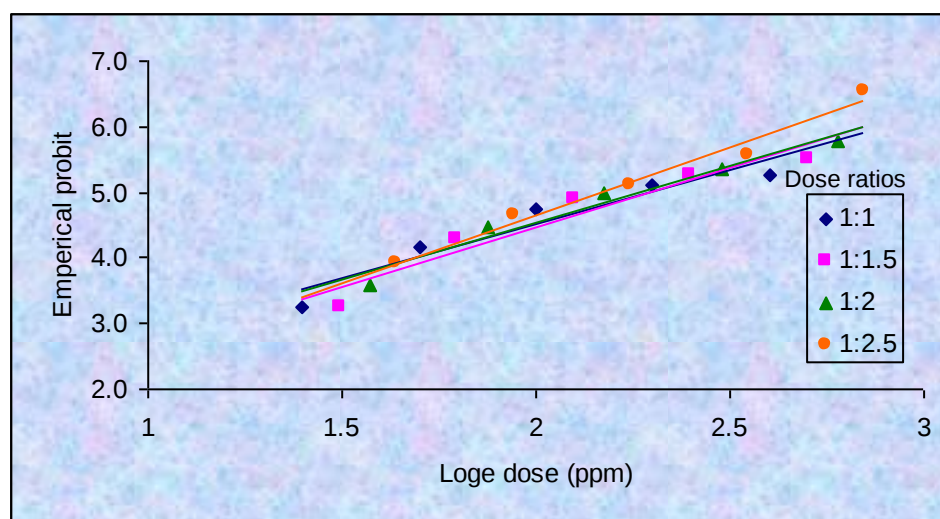


Fig 43: Regression lines of different combined doses Deltamethrin and Azadirachtin against the mature larvae (40 days old) of *A. diaperinus* exposed to the treated flour medium for 72 hours.

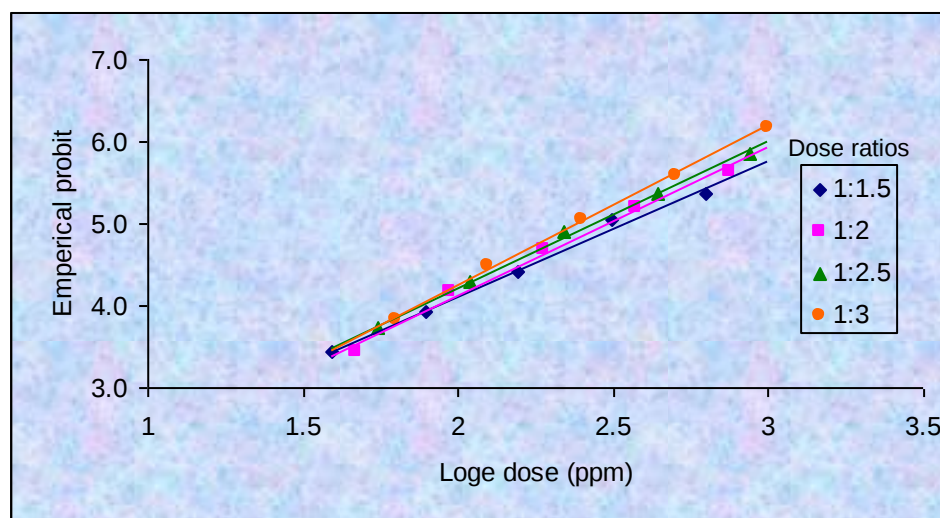


Fig 44: Regression lines of different combined doses Deltamethrin and Azadirachtin against the adults of *A. diaperinus* exposed to the treated flour medium for 72 hours.

5.3.1.1 Synergistic action of Deltamethrin + Azadirachtin

The LD₅₀ values of Deltamethrin alone has been calculated as 327.13µg/cm² for larvae (40 days old) and 854.11µg/cm² for adults exposed to treated filter paper for 72 hours. Incase of treated food media the LD₅₀ values were 778.62ppm and 1243.21ppm for larvae and adults respectively. The co-toxicity coefficient was determined as 361.37, 407.12, 735.26, 916.62 for larvae at the ratios of 1:0.1, 1:0.2, 1:0.4, 1:0.5 and 549.12, 729.77, 914.26 and 1673.31 for adults at the ratios of 1:0.1, 1:0.2, 1:0.3, 1:0.6 in RFM test respectively. Incase of TFM test the co-toxicity coefficient were 754.58, 1022.03, 1287.16 and 1595.23 for larvae at the ratios of 1:1, 1:1.5, 1:2 and 1:2.5 respectively and 972.45, 1248.79, 1552.76 and 1845.75 for adults at the ratios of 1:1.5, 1:2, 1:2.5 and 1:3 respectively.

The co-toxicity coefficients of mixtures of each ratio are greater than 100, following the principle of Sun and Johnson (1960b). These values indicated that Deltamethrin + Azadirachtin offered synergistic action to the adults and larvae in the exposure period of 72 hours in both RFM and TFM tests. Larvae were more susceptible than adults in both TFM and RFM tests.

The free hand curve fitting of isobolograms (Fig 45-48) has run below when the exposure period was 72 hours in both RFM and TFM test.

In the mixture the LD₅₀ value of Deltamethrin and Azadirachtin have been separated (Table 20-23).

RFM test

The LD₅₀ values of Deltamethrin for mature larvae were recorded as 327.121µg/cm² after 72 hours exposure on treated filter paper, but in the mixture the LD₅₀ values of Deltamethrin was 90.53, 80.36, 44.41 and 35.69 µg/cm² while those of azadirachtin was 9.06, 16.08, 17.78 and 17.85µg/cm² which gave the ratios of 1:0.1, 1:0.2, 1:0.4 and 1:0.5 respectively. Azadirachtin when combined with Deltamethrin reduced the doses of the insecticides at the levels of 72.33%, 75.44%, 86.31% and 89.01% respectively (Table-20).

The LD₅₀ value for adults has been recorded 854.11µg/cm² after 72 hours of exposure on treated filter paper, but in the mixture, the LD₅₀ value of Deltamethrin was 155.55, 117.04, 93.43 and 51.05µg/cm² while those of azadirachtin was 15.56,

23.41, 28.03 and 30.63 $\mu\text{g}/\text{cm}^2$ which gave the ratios of 1:0.1, 1:0.2, 1:0.3 and 1:0.6 respectively. Azadirachtin when combined with Deltamethrin reduced the doses of the insecticides at the levels of 81.79%, 86.21%, 89.08% and 94.03% respectively (Table-21).

17.85 $\mu\text{g}/\text{cm}^2$ azadirachtin was needed for 89% dose reduction of Deltamethrin, which was highest for larvae but in case of adults 28.03 $\mu\text{g}/\text{cm}^2$ azadirachtin was needed for same percentage dose reduction. So, larvae were more susceptible than adults in the RFM test.

TFM test

LD₅₀ value of Deltamethrin for mature larvae (40 days old larvae) after 72 hours of exposure on treated food media was 778.62ppm but in the mixture, the LD₅₀ value of Deltamethrin was 102.26, 76.19, 60.41 and 48.81ppm while those of azadirachtin was 102.26, 114.28, 120.99 and 122.03ppm which gave the ratios of 1:1, 1:1.5, 1:2 and 1:2.5 respectively. Azadirachtin when combined with Deltamethrin reduced the doses of the insecticides at the levels of 86.87%, 90.22%, 92.24%, and 93.732% respectively (Table-22).

LD₅₀ value of Deltamethrin for adults after 72 hours of exposure on treated food media was 1243.21ppm, but in the mixture, the LD₅₀ value of Deltamethrin were 127.85, 99.56, 80.07 and 67.36ppm while those of azadirachtin was 191.77, 199.11, 200.16 and 202.07 ppm which gave the ratios of 1:1.5, 1:2, 1:2.5 and 1:3 respectively. Azadirachtin when combined with Deltamethrin reduced the doses of the insecticides at the levels of 89.72%, 91.91%, 93.56%, and 94.59% respectively (Table-23).

122.03ppm Azadirachtin was needed for 93% dose reduction, which was highest for larvae but 202.07 ppm Azadirachtin was needed for 94% dose reduction, which was highest for adults. So larvae were more susceptible than adults in the TFM test.

Table 20: Effect of Deltamethrin with Azadirachtin ($\mu\text{g}/\text{cm}^2$) on *A. diaperinus* 40 days old larvae exposed to the treated filter paper for 72 hours.

Insecticide	Life stage (Age)	Ratio of combination	LD ₅₀ of combination ($\mu\text{g}/\text{cm}^2$)	LD ₅₀ of insecticide ($\mu\text{g}/\text{cm}^2$)	LD ₅₀ of Azadirachtin ($\mu\text{g}/\text{cm}^2$)	Co-toxicity coefficient	Reduction of a. i. %
Delta (RFM)	40day old larvae	1:0.1	99.5743	90.523	9.053	361.369	72.326
		1:0.2	80.3503	80.35035	16.071	407.119	75.438
		1:0.4	62.2873	44.491	17.797	735.254	86.391
		1:0.5	53.5309	35.688	17.844	916.615	89.091

Table 21: Effect of Deltamethrin with Azadirachtin ($\mu\text{g}/\text{cm}^2$) on *A. diaperinus* adults of exposed to the treated filter paper for 72 hours.

Insecticide	Life stage (Age)	Ratio of combination	LD ₅₀ of combination ($\mu\text{g}/\text{cm}^2$)	LD ₅₀ of insecticide ($\mu\text{g}/\text{cm}^2$)	LD ₅₀ of Azadirachtin ($\mu\text{g}/\text{cm}^2$)	Co-toxicity coefficient	Reduction of a. i. %
Delta (RFM)	Adults	1:0.1	171.097	155.543	15.554	549.111	81.789
		1:0.2	140.445	117.038	23.4076	729.765	86.297
		1:0.3	121.458	93.421	28.029	914.252	89.07
		1:0.6	81.6685	51.043	30.626	1673.302	94.025

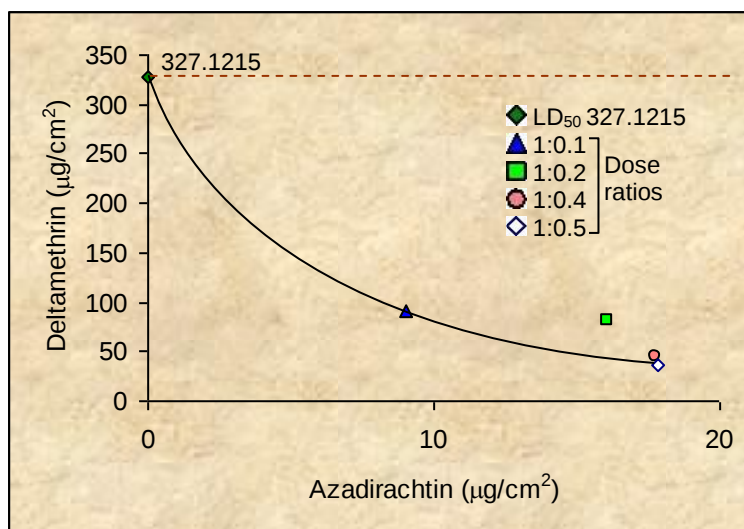


Fig 45: Isobologram of Deltamethrin with Azadirachtin against the mature larvae of *A. diaperinus* for 72 h exposure in RFM test.

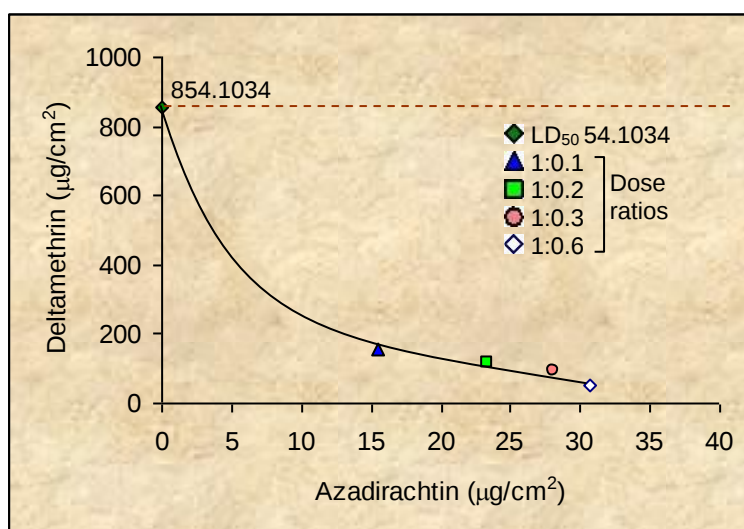


Fig 46: Isobologram of Deltamethrin with Azadirachtin against the adults of *A. diaperinus* for 72 h exposure in RFM test.

Table 22: Effect of Deltamethrin with Azadirachtin (ppm) on *A. diaperinus* 40 days old larvae exposed to treated food media for 72 hours.

Insecticide	Life stage (Age)	Ratio of combination	LD ₅₀ of combination (µg/cm ²)	LD ₅₀ of insecticide (µg/cm ²)	LD ₅₀ of Azadirachtin (µg/cm ²)	Co-toxicity coefficient	Reduction of a. i. %
Delta (RFM)	40days old larvae	1:1	204.516	102.258	102.258	754.574	86.867
		1:1.5	190.458	76.18336	114.275	1022.028	90.216
		1:2	181.473	60.4911	120.982	1287.152	92.231
		1:2.5	170.833	48.809	122.024	1595.223	93.732

Table 23: Effect of Deltamethrin with Azadirachtin (ppm) on *A. diaperinus* adults exposed to treated food media for 72 hours.

Insecticide	Life stage (Age)	Ratio of combination	LD ₅₀ of combination (µg/cm ²)	LD ₅₀ of insecticide (µg/cm ²)	LD ₅₀ of Azadirachtin (µg/cm ²)	Co-toxicity coefficient	Reduction of a. i. %
Delta (RFM)	Adults	1:1.5	319.605	127.842	191.763	972.444	89.717
		1:2	298.659	99.553	199.106	1248.784	91.993
		1:2.5	280.222	80.064	200.159	1552.759	93.559
		1:3	269.419	67.354	202.064	1845.745	94.583

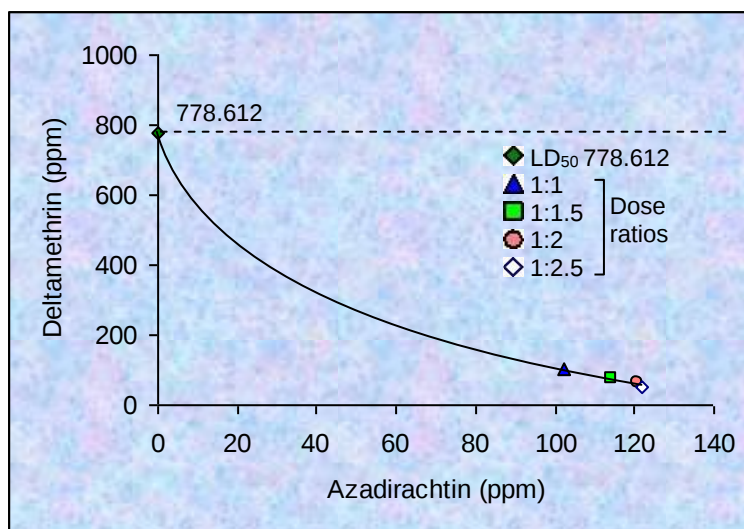


Fig 47: Isobologram of Deltamethrin with Azadirachtin against the mature larvae of *A. diaperinus* after 24 h exposure in TFM test.

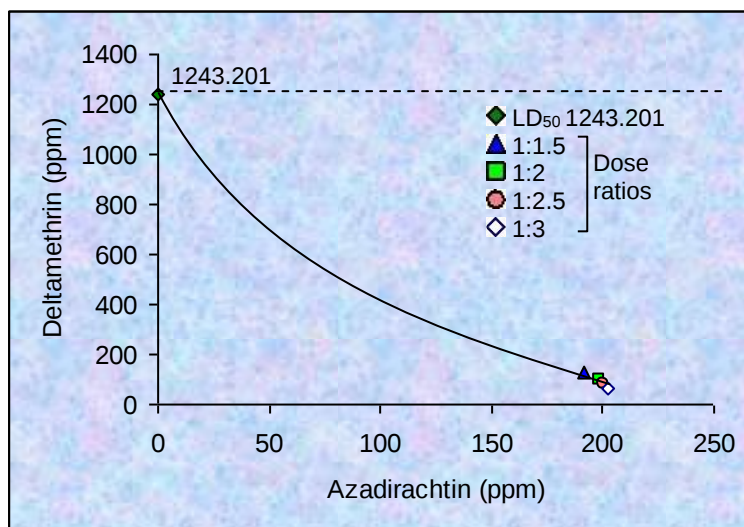


Fig 48: Isobologram of Deltamethrin with Azadirachtin against the adults of *A. diaperinus* after 72 h exposure in TFM test.

5.3.2 Imidacloprid and Azadirachtin

RFM test

For the mature larvae (40days old): The lowest dose of Imidacloprid was $30.94\mu\text{g}/\text{cm}^2$ for the mature larvae after 72 hours. Effects of different combined doses of Imidacloprid and Azadirachtin at the ratios of 1:1, 1:2, 1:3.5 and 1:5 have been estimated in Appendix Tables (126- 129) where the lowest dose of Imidacloprid was constant.

The dose concentrations were $30.94, 15.47, 7.73, 3.87$ and $1.93\mu\text{g}/\text{cm}^2$ at ratio 1:1; $46.41, 23.21, 11.61, 5.81$ and $2.91\mu\text{g}/\text{cm}^2$ at ratios of 1:2; $69.61, 34.8, 17.4, 8.71$ and $4.36\mu\text{g}/\text{cm}^2$ at ratio 1: 3.5; and $92.82, 46.41, 23.2, 11.6$ and $5.81\mu\text{g}/\text{cm}^2$ at ratio 1:5. The mortality percentage were recorded as 4 -52 % at 1:1; 4- 68 % at 1: 2; 8- 80 % at 1: 3.5; 12- 94 % at 1: 5 after 72 h of exposure. Highest mortality was recorded at the ratio of 1:5.

The LD_{50} values of the mixture (Imidacloprid + Azadirachtin) were recorded after 72h as $27.63, 22.63, 23.08$ and $23.43\mu\text{g}/\text{cm}^2$ at the ratio 1:1, 1:2, 1:3.5 and 1:5 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 as in the Fig. 49.

For adults: The lowest dose of Imidacloprid was $1237.62\mu\text{g}/\text{cm}^2$ for the adults after 72 hours. Effects of different combined doses of Imidacloprid and Azadirachtin at the ratios of 1:0.02, 1:0.05, 1:0.1 and 1:0.2 have been estimated in Appendix Tables (130- 133) where the lowest dose of Imidacloprid was constant.

The dose concentration were $631.19, 315.51, 157.72, 78.81$ and $39.45\mu\text{g}/\text{cm}^2$ at ratio 1:0.02; $649.76, 324.88, 162.44, 81.22$ and $40.61\mu\text{g}/\text{cm}^2$ at ratio 1:0.05; $680.61, 340.35, 170.18, 85.089$ and $42.55\mu\text{g}/\text{cm}^2$ at ratio 1: 0.1; and $742.572, 371.286, 185.643, 92.822$ and $46.42\mu\text{g}/\text{cm}^2$ at ratio 1:0.2. The mortality percentage were recorded as 4 -50 % at 1:0.02; 4- 62 % at 1: 0.05; 6-76 % at 1: 0.1; 10- 90 % at 1: 0.2 after 72 h of exposure. Highest mortality was recorded at the ratio of 1:0.2.

The LD_{50} values of the mixture (Imidacloprid + Azadirachtin) were recorded after 72h as $565.929, 386.025, 291.578$ and $187.428\mu\text{g}/\text{cm}^2$ at the ratio 1:0.02, 1:0.05, 1:0.1 and 1:0.2 respectively.

LD_{50} values along with 95% confidence limits, regression equations and chi-squared values have been estimated in the Table 25. Regression lines of different ratios on log probit mortality and the log dose concentrations have been plotted as in the Fig. 50.

TFM test

For the mature larvae (40 days old): The lowest dose of Imidacloprid was 25ppm for the mature larvae after 72 hours exposure. Effects of different combined doses of Imidacloprid and Azadirachtin at the ratios of 1:20, 1:25, 1:30 and 1:35 have been estimated in Appendix Tables (134-137) where the lowest dose of Imidacloprid was constant. The dose concentrations were 262.5, 131.25, 65.63, 32.82 and 16.41ppm at ratio 1:20; 325, 162.5, 81.25, 40.63 and 20.32ppm at ratio 1:25; 387.5, 193.75, 96.88, 48.44 and 24.22ppm at ratio 1: 30; and 512.5, 256.25, 128.125, 64.063 and 32.032ppm at ratio 1:35. The mortality percentage were recorded as 6 -58 % at 1:20; 6- 62 % at 1: 25; 8- 60 % at 1: 30; 12- 82 % at 1:35 after 72 h of exposure. Highest mortality was found at the ratio of 1:35.

The LD₅₀ values of the mixture (Imidacloprid + Azadirachtin) were recorded after 72h as 154.075, 173.401, 199.078 and 206.221ppm at the ratio 1:20, 1:25, 1:30 and 1:35 respectively.

LD₅₀ values along with 95% confidence limits, regression equations and chi-squared values have been estimated in the Table 26. Regression lines of different ratios on log probit mortality and the log dose concentrations have been plotted as in the Fig. 51.

For adults: The lowest dose of Imidacloprid was 62.5ppm for the adults after 72 hours exposure. Effects of different combined doses of Imidacloprid and Azadirachtin at the ratios of 1:10, 1:12, 1:15 and 1:20 have been estimated in Appendix Tables (138-141) where the lowest dose of Imidacloprid was constant. The dose concentration was 343.75, 171.88, 85.93, 42.96 and 21.48 ppm at ratio 1:10; 406.25, 203.13, 101.57, 50.79 and 25.31 ppm at ratio 1:12; 500, 250, 125, 62.5 and 31.25 ppm at ratio 1:15; and 656.25, 328.13, 164.07, 82.04 and 41.02ppm at ratio 1:20. The mortality percentage were recorded as 6-58 % at 1:10; 6- 64 % at 1:12; 10- 78 % at 1: 15; 14- 88 % at 1:20 after 72 h of exposure. Then the highest mortality was recorded at the ratio of 1:0.2.

The LD₅₀ values of the mixture (Imidacloprid + Azadirachtin) were recorded after 72h as 192.31, 195.07, 202.54 and 213.92ppm at the ratios of 1:10, 1:12, 1:15 and 1:20 respectively.

LD₅₀ values along with 95% confidence limits, regression equations and chi-squared values have been estimated in the Table 27. Regression lines of different ratios on log probit mortality and the log dose concentrations have been plotted as in the Fig. 52.

Table 24: LD₅₀ , 95% confidence limits and regression equations for combined action of Imidacloprid with Azadirachtin against the mature larvae (40 days old) of *A. diaperinus* exposed to the treated filter paper for 72 hours.

Insecticide	Life stage (Age)	Ratio of combination	LD ₅₀ of combination (µg/cm ²)	Regression equation	95% confidence limit		Chi-square value (3df)
					Lower (µg/cm ²)	Upper (µg/cm ²)	
Imida (RFM)	40days old larvae	1:1	27.6276	Y=3.06716+1.3409X	17.4984	43.6201	1.0177
		1:2	22.6298	Y=2.67535+1.7161X	17.4190	29.3994	1.1164
		1:3.5	23.0778	Y=2.69867+1.6882X	18.1648	29.3196	1.1714
		1:5	23.4284	Y=2.20223+2.1352X	16.8281	24.8016	1.4074

Table 25: LD₅₀ , 95% confidence limits and regression equations for combined action of Imidacloprid with Azadirachtin against the adults of *A. diaperinus* exposed to the treated filter paper for 72 hours.

Insecticide	Life stage (Age)	Ratio of combination	LD ₅₀ of combination (µg/cm ²)	Regression equation	95% confidence limit		Chi-square value (3df)
					Lower (µg/cm ²)	Upper (µg/cm ²)	
Imida (RFM)	Adults	1:0.02	565.929	Y=1.0771+1.42507X	367.363	871.823	0.6698
		1:0.05	386.025	Y=0.81953+1.6161X	285.004	522.854	1.9419
		1:0.1	291.578	Y=0.35236+1.8856X	231.325	367.526	0.2822
		1:0.2	187.428	Y=0.36995+2.0372X	153.296	229.161	0.1145

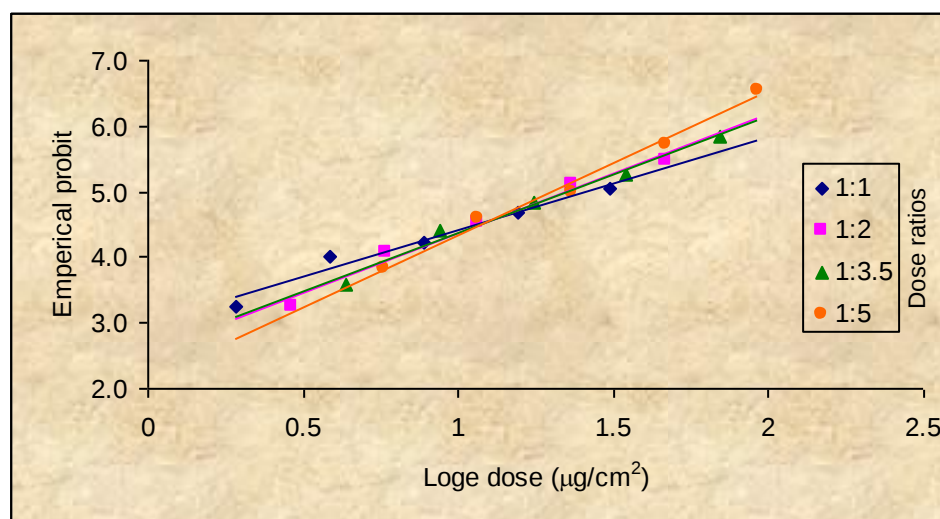


Fig 49: Regression lines of different combined doses of Imidacloprid and Azadirachtin against the mature larvae (40 days old) of *A. diaperinus* exposed to the treated filter paper for 72 hours.

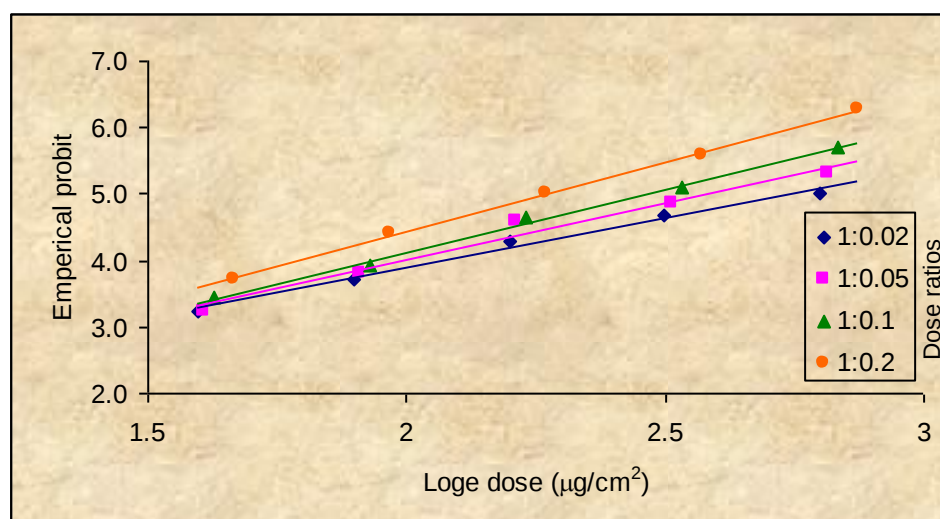


Fig 50: Regression lines of different combined doses of Imidacloprid and Azadirachtin against the adults of *A. diaperinus* exposed to the treated filter paper for 72 hours.

Table 26: LD₅₀ , 95% confidence limits and regression equations for combined action of Imidacloprid with Azadirachtin against the mature larvae (40 days old) of *A. diaperinus* exposed to the treated flour medium for 72 hours.

Insecticide	Life stage (Age)	Ratio of combination	LD ₅₀ of combination (µg/cm ²)	Regression equation	95% confidence limit		Chi-square value (3df)
					Lower (µg/cm ²)	Upper (µg/cm ²)	
Imida (TFM)	40days old larvae	1:20	154.075	Y=1.8619+1.43437X	109.829	216.145	1.9620
		1:25	173.401	Y=1.73566+1.4579X	126.101	238.447	1.2874
		1:30	199.078	Y=1.97909+1.3139X	140.733	281.612	2.3854
		1:35	206.221	Y=1.42719+1.6014X	132.813	218.164	1.4694

Table 27: LD₅₀ , 95% confidence limits and regression equations for combined action of Imidacloprid with Azadirachtin against the adults of *A. diaperinus* exposed to the treated flour medium for 72 hours.

Insecticide	Life stage (Age)	Ratio of combination	LD ₅₀ of combination (µg/cm ²)	Regression equation	95% confidence limit		Chi-square value (3df)
					Lower (µg/cm ²)	Upper (µg/cm ²)	
Imida (TFM)	Adults	1:10	192.397	Y=1.5269+1.4612X	165.689	342.338	1.144E-02
		1:12	195.061	Y=1.5717+1.45264X	165.179	317.677	0.7829
		1:15	202.539	Y=1.39435+1.5632X	154.979	264.694	2.0904
		1:20	213.915	Y=1.16248+1.6468X	167.861	272.604	4.4933

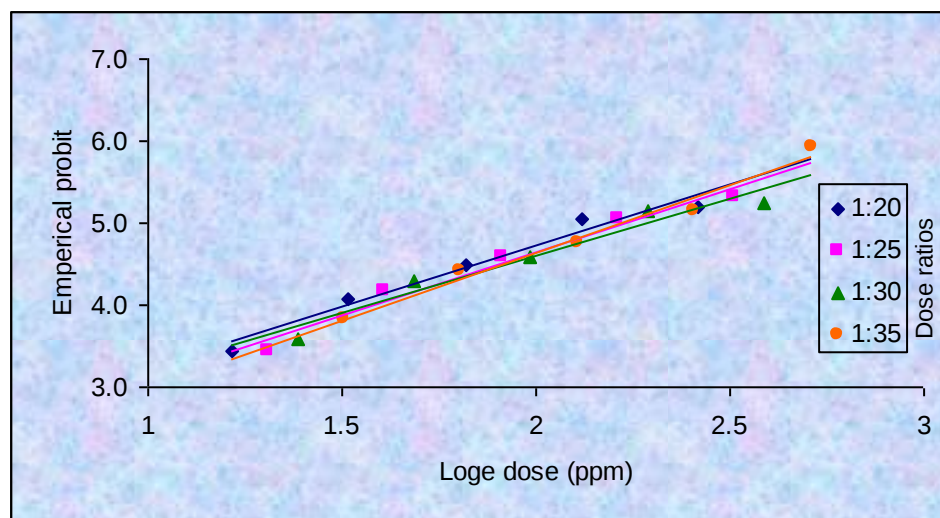


Fig 51: Regression lines of different combined doses Imidacloprid and Azadirachtin against the mature larvae (40 days old) of *A. diaperinus* exposed to the treated flour medium for 72 hours.

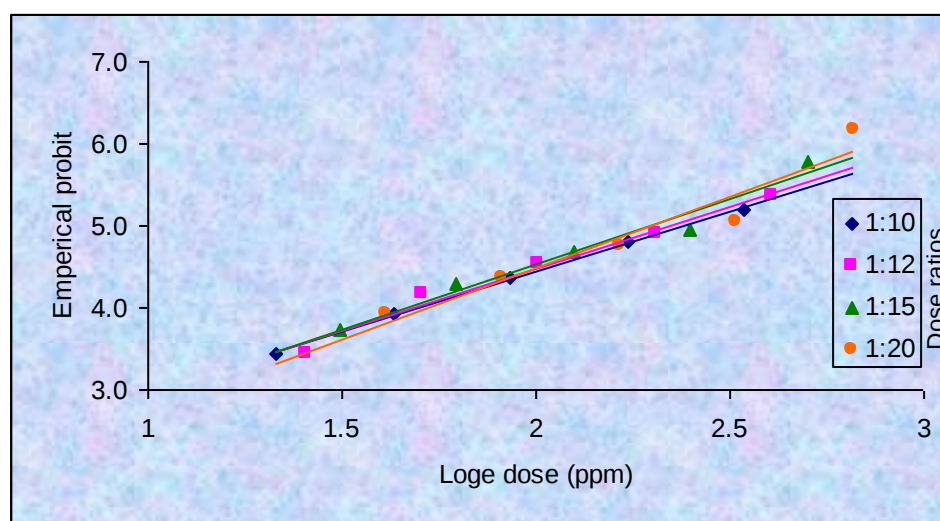


Fig 52: Regression lines of different combined doses Imidacloprid and Azadirachtin against the adults of *A. diaperinus* exposed to the treated flour medium for 72 hours.

5.3.2.1 Synergistic action of Imidacloprid+ Azadirachtin:

The LD₅₀ values of Imidacloprid alone was calculated as 109.14µg/cm² for larvae (40 days old), and 2215.1µg/cm² for adults of exposure on treated filter paper. In case of treated food media the LD₅₀ values were 108.75ppm and 221.97ppm for larvae and adults respectively after 72 hours exposure.

The shares of Imidacloprid in the LD₅₀ values of mixture have been separated (Table 28-31). The co-toxicity coefficient was determined as 790.13, 1446.82, 2128.46 and 2795.79 for larvae at the ratios 1:1, 1:2, 1:3.5, 1:5 and 399.23, 602.41, 835.64 and 1418.16 for adults at the ratio 1:0.02, 1:0.05, 1:0.1 and 1:0.2 in RFM test respectively. And in case of TFM test the co-toxicity coefficient were 1482.23, 1630.67, 1693.42 and 1898.56 for larvae at the ratios 1:20, 1:25, 1:30 and 1:35 and 1269.05, 1479.31, 1753.59 and 2179.07 for adults at the ratios of 1:10, 1:12, 1:15 and 1:20 respectively.

The co-toxicity coefficients of mixtures of each ratio were greater than 100, following the principle of Sun and Johnson (1960). These values indicated that Imidacloprid + Azadirachtin offered synergistic action to the adults and larvae in the exposure period 72 hours in both RFM and TFM tests. Larvae were found more susceptible than adults in the TFM test. But in RFM test adults were more susceptible than larvae.

The free hand curve fitting of isobolograms (Fig 53-56) have run below when the exposure period was 72 hours in both RFM and TFM tests.

RFM test

The LD₅₀ values of Imidacloprid for mature larvae was recorded 109.14µg/cm² for 72 hours exposure to the treated filter paper, but in the mixture, the LD₅₀ value of Imidacloprid was 13.82, 7.55, 5.13 and 3.91µg/cm² while that of azadirachtin were 13.82, 15.09, 17.94 and 19.53µg/cm² which gave the ratios of 1:1, 1:2, 1:3.5 and 1:5 respectively. Azadirachtin when combined with Imidacloprid reduced the doses of the insecticide at the levels of 87.35%, 93.09%, 95.31% and 96.43% respectively (Table-28).

The LD₅₀ value for adults has been recorded 2215.1µg/cm² after 72 hours of exposure to the treated filter paper, but in the mixture, the LD₅₀ value of Imidacloprid was 554.84, 367.65, 265.08 and 156.11µg/cm² while that of azadirachtin were 11.01, 18.39, 26.51 and 31.24µg/cm² which gave the ratios of

1:0.02, 1:0.05, 1:0.1 and 1:0.2 respectively. Azadirachtin when combined with Imidacloprid reduced the doses of the insecticide at the levels of 74.96%, 83.41%, 88.04% and 92.95% respectively (Table-29).

15.09 $\mu\text{g}/\text{cm}^2$ azadirachtin was needed for 93% dose reduction, but 31.24 $\mu\text{g}/\text{cm}^2$ azadirachtin was needed for 92% percentage dose reduction, which was highest for adults. So larvae were more susceptible than adults at the RFM test.

TFM test

LD₅₀ value of Imidacloprid for mature larvae (40 days old larvae) after 72 hours of exposure on treated food media was 108.75ppm but in the mixture, the LD₅₀ values of Imidacloprid was 7.34, 6.67, 6.43 and 5.73ppm at the ratio 1:1, 1:1.5, 1:2 and 1:2.5, when azadirachtin caused the reduction of the doses at the levels of 93.26%, 93.87%, 94.01% and 94.73% respectively (Table-30). The share of azadirachtin were 146.74, 166.74, 192.66 and 200.41ppm.

LD₅₀ value of Imidacloprid for adults after 72 hours of exposure on treated food media was 221.97ppm, but in the mixture, the LD₅₀ values of Imidacloprid were 17.41, 15.01, 12.66 and 10.19ppm at the ratio 1:1.5, 1:2, 1:2.5 and 1:3, when Azadirachtin caused reduction of the dose at the level of 92.13%, 93.25%, 94.21% and 95.42% respectively (Table-31). The share of azadirachtin were 174.91, 180.06, 189.89 and 203.73ppm.

200.41ppm Azadirachtin was required for 94% reduction of the doses, which was the highest for larvae but 189.89 ppm Azadirachtin was needed for 94% dose reduction for adults. So adults were more susceptible than larvae in the TFM test.

Table 28: Effect of Imidacloprid with the Azadirachtin ($\mu\text{g}/\text{cm}^2$) on *A. diaperinus* 40 days old larvae exposed to treated filter paper for 72 hours.

Insecticide	Life stage (Age)	Ratio of combination	LD ₅₀ of combination ($\mu\text{g}/\text{cm}^2$)	LD ₅₀ of insecticide ($\mu\text{g}/\text{cm}^2$)	LD ₅₀ of Azadirachtin ($\mu\text{g}/\text{cm}^2$)	Co-toxicity coefficient	Reduction of a. i. %
Imida (RFM)	40days old larvae	1:1	27.6276	13.8138	13.813	790.122	87.344
		1:2	22.6298	7.5432	15.086	1446.811	93.089
		1:3.5	23.0778	5.128	17.94	2128.459	95.302
		1:5	23.4284	3.904	19.523	2795.784	96.424

Table 29: Effect of Imidacloprid with Azadirachtin ($\mu\text{g}/\text{cm}^2$) on *A. diaperinus* adults exposed to treated filter paper for 72 hours.

Insecticide	Life stage (Age)	Ratio of combination	LD ₅₀ of combination ($\mu\text{g}/\text{cm}^2$)	LD ₅₀ of insecticide ($\mu\text{g}/\text{cm}^2$)	LD ₅₀ of Azadirachtin ($\mu\text{g}/\text{cm}^2$)	Co-toxicity coefficient	Reduction of a. i. %
Imida (RFM)	Adults	1:0.02	565.929	554.832	11.096	399.226	74.952
		1:0.05	386.025	367.644	18.3821	602.494	83.403
		1:0.1	291.578	265.072	26.508	835.635	88.034
		1:0.2	187.428	156.191	31.239	1418.156	92.949

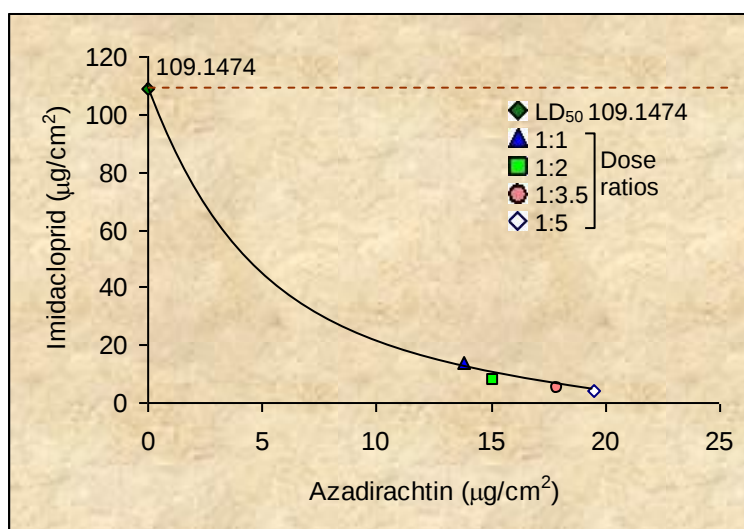


Fig 53: Isobologram of Imidacloprid with Azadirachtin against the mature larvae of *A. diaperinus* after 72 h exposure in RFM test.

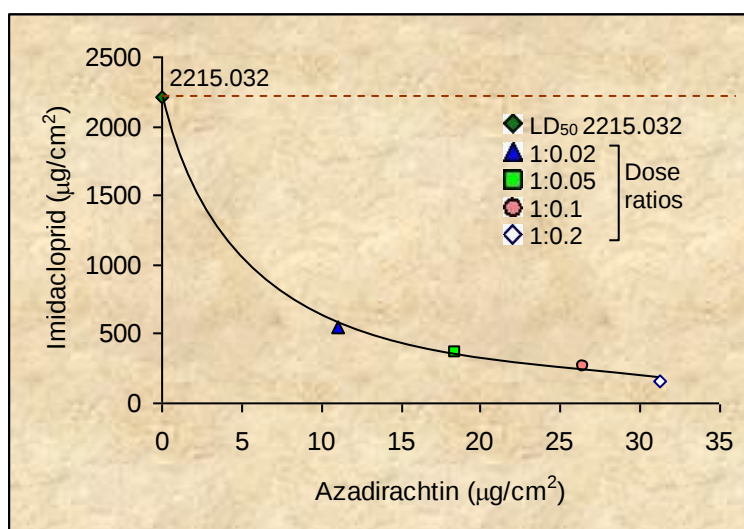


Fig 54: Isobologram of Imidacloprid with Azadirachtin against the adults of *A. diaperinus* after 72 h exposure in RFM test.

Table 30: Effect of Imidacloprid with Azadirachtin (ppm) on *A. diaperinus* 40 days old larvae after 72 hours' exposure to treated flour medium.

Insecticide	Life stage (Age)	Ratio of combination	LD ₅₀ of combination (ppm)	LD ₅₀ of insecticide (ppm)	LD ₅₀ of Azadirachtin (ppm)	Co-toxicity coefficient	Reduction of a. i. %
Imida (TFM)	40days old larvae	1:20	154.075	7.33692	146.738	1482.221	93.254
		1:25	173.401	6.669	166.732	1630.669	93.867
		1:30	199.078	6.4219	192.657	1693.414	94.094
		1:35	206.220	5.7283	200.492	1898.557	94.733

Table 31: Effect of Imidacloprid with Azadirachtin (ppm) on *A. diaperinus* adults after 72 hours' exposure to treated flour medium.

Insecticide	Life stage (Age)	Ratio of combination	LD ₅₀ of combination (ppm)	LD ₅₀ of insecticide (ppm)	LD ₅₀ of Azadirachtin (ppm)	Co-toxicity coefficient	Reduction of a. i. %
Imida (TFM)	Adults	1:10	192.397	17.491	174.908	1269.046	92.121
		1:12	195.061	15.004	180.056	1479.397	93.241
		1:15	202.539	12.658	189.881	1753.585	94.298
		1:20	213.915	10.1864	203.721	2179.069	95.411

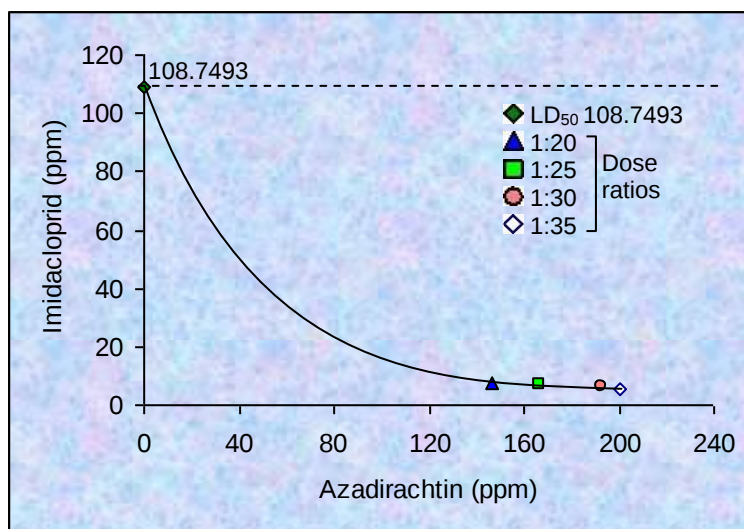


Fig 55: Isobologram of Imidacloprid with Azadirachtin against the mature larvae of *A. diaperinus* after 72 h exposure in TFM test.

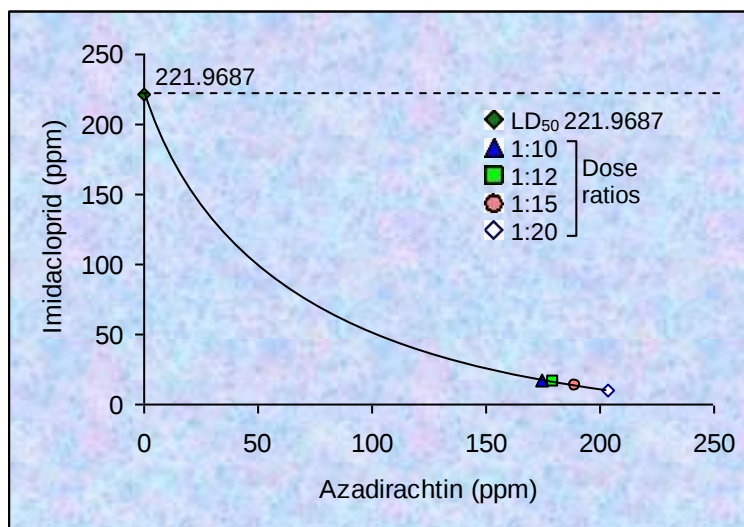


Fig 56: Isobologram of Imidacloprid with Azadirachtin against the adults of *A. diaperinus* after 72 h exposure in TFM test.

5.3.3 Thiodicarb and Azadirachtin

A. RFM test

For the mature larvae (40days old): The lowest dose of Thiodicarb was $9.95\mu\text{g}/\text{cm}^2$ for the mature larvae after 72 hours exposure. Effects of different combined doses of Thiodicarb and Azadirachtin viz., the ratios of 1:3, 1:7, 1:10 and 1:15 have been estimated in Appendix Tables (179-182) where the lowest dose of Thiodicarb was constant. The dose concentrations were 19.9, 9.95, 4.98, 2.49, and $1.25\mu\text{g}/\text{cm}^2$ at ratio 1:3; 39.8, 19.9, 9.95, 4.98, and $2.49\mu\text{g}/\text{cm}^2$ at ratio 1:7; 54.73, 27.37, 13.69, 6.85 and $3.43\mu\text{g}/\text{cm}^2$ at ratio 1: 10; and 79.6, 39.8, 19.9, 9.95 and $4.98\mu\text{g}/\text{cm}^2$ at ratio 1:15. The mortality percentage were recorded as 2-38 % at 1:3; 4- 54 % at 1: 7; 6- 54 % at 1: 10; 10- 64 % at 1: 15 after 72 h of exposure. Highest mortality was recorded at the ratio of 1:15.

The LD_{50} values of the mixture (Thiodicarb + Azadirachtin) were recorded after 72h as 30.94, 34.31, 37.36 and $44.49\mu\text{g}/\text{cm}^2$ at the ratio 1:3, 1:7, 1:10 and 1:15 respectively.

LD_{50} values along with 95% confidence limits, regression equations and chi-squared values have been estimated in the Table 32. Regression lines of different ratios on log probit mortality and the log dose concentrations have been plotted as in the Fig. 57.

For adults: The lowest dose of Thiodicarb was $26.52\mu\text{g}/\text{cm}^2$ for the adults after 72 hours exposure. Effects of different combined doses of Thiodicarb and Azadirachtin at the ratios of 1:2, 1:4, 1:6 and 1:10 have been estimated in Appendix Tables (183-186) where the lowest dose of Thiodicarb was constant. The dose concentrations were 39.78, 19.89, 9.95, 4.98 and $2.49\mu\text{g}/\text{cm}^2$ at ratio 1:2; 66.3, 33.15, 16.58, 8.29 and $4.15\mu\text{g}/\text{cm}^2$ at ratio 1:4; 680.61, 340.35, 170.18, 85.09 and $42.55\mu\text{g}/\text{cm}^2$ at ratio 1: 6; and , 92.82, 46.41, 23.21, 11.61 and, $5.81\mu\text{g}/\text{cm}^2$ at ratio 1:10. The mortality percentage were recorded as 4 -54 % at 1:2; 4- 60 % at 1: 4; 6-68 % at 1: 6; 12- 94 % at 1: 10 after 72 h of exposure. Highest mortality was found at the ratio of 1:10.

The LD_{50} values of the mixture (Thiodicarb + Azadirachtin) were recorded after 72h as 36.89, 46.93, 47.18 and $50.23\mu\text{g}/\text{cm}^2$ at the ratio 1:2, 1:4, 1:6 and 1:10 respectively.

LD_{50} values along with 95% confidence limits, regression equations and chi-squared values have been estimated in the Table 33. Regression lines of different ratios on log probit mortality and the log dose concentrations have been plotted as in the Fig. 58.

B. TFM test

For the mature larvae (40days old): The lowest dose of Thiodicarb was 8ppm for the mature larvae after 72 hours exposure. Effects of different combined doses of Thiodicarb and Azadirachtin at the ratios of 1:20, 1:40, 1:60 and 1:80 have been estimated in Appendix Tables (187-190) where the lowest dose of Thiodicarb was constant. The dose concentrations were 84, 42, 21, 10.5 and 5.25ppm at ratio 1:20; 164, 82, 41, 20.5 and 10.25ppm at ratio 1:40; 244, 122, 61, 30.5 and 15.25ppm at ratio 1: 60; and 324, 162, 81, 40.5 and 20.25ppm at ratio 1:80. The mortality percentage were recorded as 2-34 % at 1:20; 4- 52 % at 1:40; 4- 52 % at 1: 60; 8- 54 % at 1: 80 after 72 h of exposure. Then the highest mortality observed at the ratio of 1:15.

The LD₅₀ values of the mixture (Thiodicarb + Azadirachtin) were recorded after 72h as 131.21, 165.26, 222.81 and 238.41 ppm at the ratios 1:20, 1:40, 1:60 and 1:80 respectively.

LD₅₀ values along with 95% confidence limits, regression equations and chi-squared values have been estimated in the Table 34. Regression lines of different ratios on log probit mortality and the log dose concentrations have been plotted as in the Fig. 59.

For adults: The lowest dose of Thiodicarb was 40ppm for the adults after 72 hours exposure. Effects of different combined doses of Thiodicarb and Azadirachtin at the ratios of 1:10, 1:20, 1:25 and 1:30 have been estimated in Appendix Tables (191-194) where the lowest dose of Thiodicarb was constant. The dose concentrations was 220, 110, 55, 27.5 and 13.75ppm at ratio 1:10; 420, 210, 105, 52.5 and 26.25ppm at ratio 1:20; 520, 260, 130, 65 and 32.5ppm at ratio 1: 25; and 620, 310, 155, 77.5 and 38.75ppm at ratio 1:30. The mortality percentage were recorded as 2 -54 % at 1:10; 6- 64 % at 1: 20; 10-76 % at 1: 25; 14- 86 % at 1: 30 after 72 h of exposure. Then the highest mortality observed at the ratio of 1:30.

The LD₅₀ values of the mixture (Thiodicarb + Azadirachtin) were recorded after 72h as 193.75, 245.89, 251.91 and 262.96ppm at the ratio 1:10, 1:20, 1:25 and 1:30 respectively.

LD₅₀ values along with 95% confidence limits, regression equations and chi-squared values have been estimated in the Table 35. Regression lines of different ratios on log probit mortality and the log dose concentrations have been plotted as in the Fig. 60.

Table 32: The LD₅₀ values, 95% confidence limits and regression equations for combined action of Thiodicarb with Azadirachtin against the mature larvae (40 days old) of *A. diaperinus* exposed to the treated filter paper for 72 hours.

Insecticide	Life stage (Age)	Ratio of combination	LD ₅₀ of combination (µg/cm ²)	Regression equation	95% confidence limit		Chi-square value (3df)
					Lower (µg/cm ²)	Upper (µg/cm ²)	
Thiodi (RFM)	40days old larvae	1:3	30.938	Y=2.91112+1.4014X	16.528	57.911	0.2131
		1:7	34.399	Y=2.6438+1.53339X	23.214	50.973	2.28E-03
		1:10	37.352	Y=2.5691+1.43837X	31.996	74.974	0.31664
		1:15	44.489	Y=2.5582+1.4814X	32.354	61.175	0.72232

Table 33: LD₅₀ , 95% confidence limits and regression equations for combined action of Thiodicarb with Azadirachtin against the adults of *A. diaperinus* exposed to the treated filter paper for 72 hours.

Insecticide	Life stage (Age)	Ratio of combination	LD ₅₀ of combination (µg/cm ²)	Regression equation	95% confidence limit		Chi-square value (3df)
					Lower (µg/cm ²)	Upper (µg/cm ²)	
Thiodi (RFM)	Adults	1:2	36.8874	Y=2.52848+1.5773X	24.6973	55.094	0.30118
		1:4	46.9241	Y=2.19847+1.6761X	34.0269	64.709	0.18756
		1:6	47.1742	Y=2.22991+1.6550X	35.7342	62.276	0.50551
		1:10	50.2256	Y=1.70922+1.9879X	36.8131	55.561	6.52993

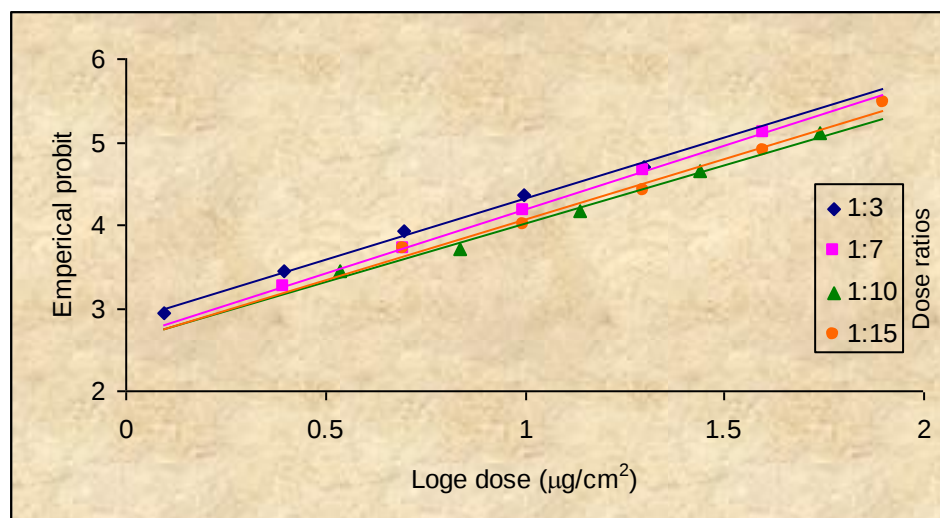


Fig 57: Regression lines of different combined doses Thiodicarb and Azadirachtin against the mature larvae (40 days old) of *A. diaperinus* exposed to the treated filter paper for 72 hours.

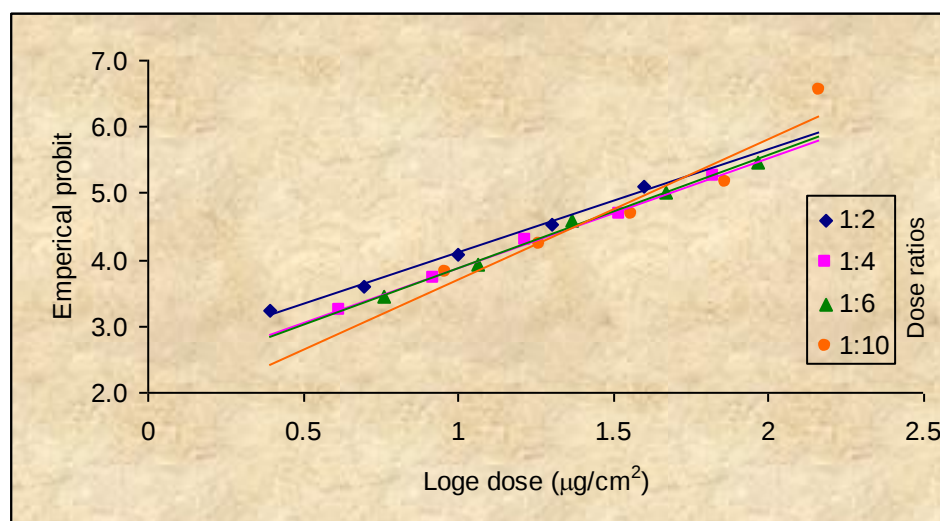


Fig 58: Regression lines of different combined doses Thiodicarb and Azadirachtin against the adults of *A. diaperinus* exposed to the treated filter paper for 72 hours.

Table 34: LD₅₀ , 95% confidence limits and regression equations for combined action of Thiodicarb with Azadirachtin against the mature larvae (40 days old) of *A. diaperinus* exposed to the treated flour medium for 72 hours.

Insecticide	Life stage (Age)	Ratio of combination	LD ₅₀ of combination (ppm)	Regression equation	95% confidence limit		Chi-square value (3df)
					Lower (ppm)	Upper (ppm)	
Thiodi (TFM)	40days old larvae	1:20	131.202	$Y=2.2167+1.23253X$	78.1799	419.982	0.8781
		1:40	165.254	$Y=1.9328+1.43279X$	91.3928	209.145	0.488
		1:60	222.803	$Y=1.6316+1.43462X$	144.402	343.771	.16888
		1:80	238.401	$Y=2.06356+1.2172X$	161.955	412.277	.37974

Table 35: LD₅₀ , 95% confidence limits and regression equations for combined action of Thiodicarb with Azadirachtin against the adults of *A. diaperinus* exposed to the treated flour medium for 72 hours.

Insecticide	Life stage (Age)	Ratio of combination	LD ₅₀ of combination (ppm)	Regression equation	95% confidence limit		Chi-square value (3df)
					Lower (ppm)	Upper (ppm)	
Thiodi (TFM)	Adults	1:10	193.748	$Y=1.0883+1.71022X$	134.993	278.076	8.2256 32E-02
		1:20	245.889	$Y=1.2619+1.56353X$	180.381	335.187	0.1316
		1:25	251.999	$Y=1.3067+1.56132X$	176.023	305.774	1.1306
		1:30	262.954	$Y=1.40099+1.5526X$	160.545	269.362	4.7118

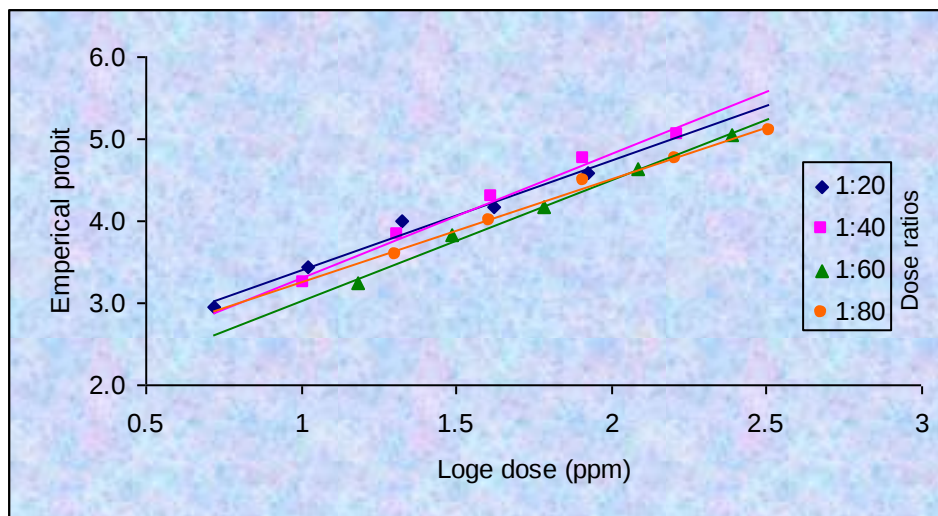


Fig 59: Regression lines of different combined doses Thiodicarb and Azadirachtin against the mature larvae (40 days old) of *A. diaperinus* exposed to the treated flour medium for 72 hours.

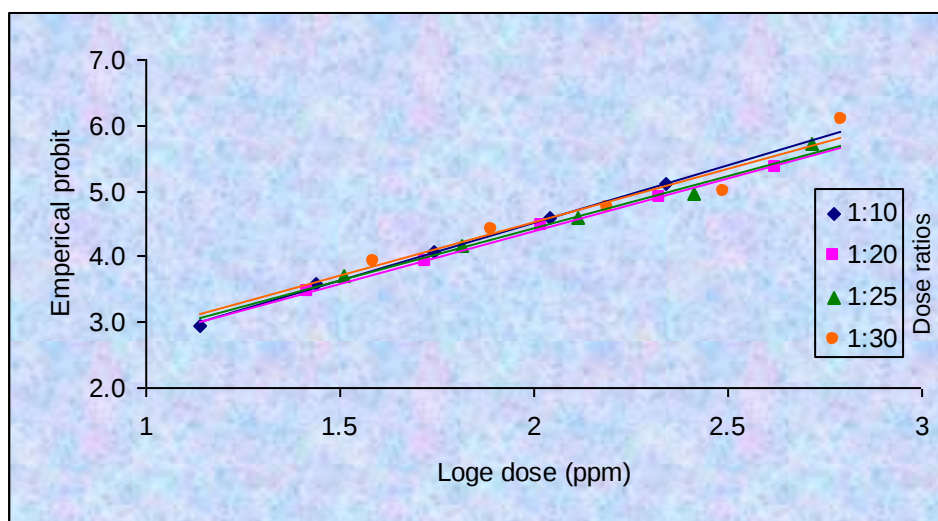


Fig 60: Regression lines of different combined doses Thiodicarb and Azadirachtin against the adults of *A. diaperinus* exposed to the treated flour medium for 72 hours.

5.3.3.1 Synergistic action of Thiodicarb + Azadirachtin

The LD₅₀ value of Thiodicarb alone was calculated as 45.631µg/cm² for larvae (40 days old), and 134.34µg/cm² for adults of exposure on treated filter paper. The LD₅₀ values were 17.68ppm and 53.31ppm for larvae and adults respectively after 72 hours exposure to the treated food media.

The shares of Thiodicarb in the LD₅₀ value of mixture have been separated (Table 36-39). The co-toxicity coefficient was determined as 589.93, 1061.44, 1344.07 and 1641.41 for larvae at the ratios of 1:3, 1:7, 1:10 and 1:15 and 1092.51, 1431.54, 1996.06 and 2945.94 for adults at the ratios of 1:2, 1:4, 1:6 and 1:10 on RFM test respectively. In TFM test the co-toxicity coefficient were 282.84, 438.41, 483.88 and 600.25 for larvae at the ratios of 1:20, 1:40, 1:60 and 1:80 and 302.66, 455.27, 550.11 and 628.48 for adults at the ratios of 1:10, 1:20, 1:25 and 1:30 respectively.

The co-toxicity coefficients of mixtures of each ratio are greater than 100, following the principle of Sun and Johnson (1960). These values indicated that Thiodicarb + Azadirachtin offered synergistic action on both adults and larvae in the exposure period of 72 hours in both RFM and TFM tests. Adults were more susceptible than larvae in TFM test. But in RFM test larvae were more susceptible than adults.

The free hand curve fitting of isobolograms (Fig 61-64) has run below when the exposure period was 72 hours in both RFM and TFM tests.

RFM test

The LD₅₀ value of Thiodicarb for mature larvae was recorded 45.64µg/cm² after 72 hours exposure to treated filter paper, but in the mixture, the LD₅₀ value of Thiodicarb was 7.74, 4.21, 3.31 and 2.78µg/cm² while those of azadirachtin were 23.21, 30.01, 33.96 and 41.71µg/cm² which gave the ratios of 1:1, 1:2, 1:3.5 and 1:5 respectively. Azadirachtin when combined with Thiodicarb reduced the doses of the insecticide at the levels of 83.05%, 90.58%, 92.56% and 93.91% respectively (table-36).

The LD₅₀ value for adults has been recorded 134.34µg/cm² after 72 hours of exposure on treated filter paper, but in the mixture, the LD₅₀ values of Thiodicarb were 12.21, 9.39, 6.73 and 4.56µg/cm² while those of azadirachtin were 24.591, 37.539, 40.435 and 45.659µg/cm² at the ratios of 1:2, 1:4, 1:6 and 1:10 respectively.

Azadirachtin when combined with Thiodicarb reduced the doses of the insecticide at the levels of 90.85%, 93.11% 94.99% and 96.61% respectively (table-37).

41.71 $\mu\text{g}/\text{cm}^2$ azadirachtin was required for 93% dose reduction which was the highest for larvae. On the otherhand, 40.44 $\mu\text{g}/\text{cm}^2$ azadirachtin was needed for 94.99% dose reduction for adults. So, adults were more susceptible than larvae in the RFM test.

TFM test

LD₅₀ value of Thiodicarb for mature larvae (40 days old larvae) after 72 hours of exposure to treated food media was 17.68ppm but in the mixture, the LD₅₀ values of Thiodicarb were 6.25, 4.03, 3.66 and 2.95ppm while those of azadirachtin were 124.96, 161.23, 219.16 and 235.46ppm which gave the ratios of 1:20, 1:40, 1:60 and 1:80 respectively. Azadirachtin when combined with Thiodicarb, reduced the doses of the insecticide at the levels of 64.65%, 77.11%, 79.34% and 83.95% respectively (Table-38).

The LD₅₀ value of Thiodicarb for adults after 72 hours of exposure on treated food media was 53.308ppm, but in the mixture, the LD₅₀ values of Thiodicarb were 17.62, 11.71, 9.61 and 8.49ppm while those of azadirachtin were 176.14, 234.18, 242.31 and 254.48ppm which gave the ratios of 1:10, 1:20, 1:25 and 1:30 respectively. Azadirachtin when combined with Thiodicarb, reduced the doses of the insecticide at the levels of 66.66%, 78.04%, 81.82% and 84.47% respectively (Table- 39).

235.46ppm Azadirachtin was required for 83.95% dose reduction, which was the highest for larvae, but 254.48ppm Azadirachtin was needed for 84.47% dose reduction which was highest for adults. So, larvae were more susceptible than adults in the TFM test.

Table 36: Effect of Thiodicarb with the lethal dose of Azadirachtin ($\mu\text{g}/\text{cm}^2$) on *A. diaperinus* 40 days old larvae after 72 hours of exposure to treated filter paper.

Insecticide	Life stage (Age)	Ratio of combination	LD ₅₀ of combination ($\mu\text{g}/\text{cm}^2$)	LD ₅₀ of insecticide ($\mu\text{g}/\text{cm}^2$)	LD ₅₀ of Azadirachtin ($\mu\text{g}/\text{cm}^2$)	Co-toxicity coefficient	Reduction of a. i. %
Thiodi (RFM)	40day old larvae	1:3	30.9384	7.734	23.203	589.929	83.049
		1:7	34.3991	4.299	30.099	1061.435	90.579
		1:10	37.3526	3.395	33.956	1344.067	92.559
		1:15	44.4892	2.78	41.708	1641.406	93.908

Table 37: Effect of Thiodicarb with Azadirachtin ($\mu\text{g}/\text{cm}^2$) on *A. diaperinus* adults of exposed to treated filter paper for 72 hours.

Insecticide	Life stage (Age)	Ratio of combination	LD ₅₀ of combination ($\mu\text{g}/\text{cm}^2$)	LD ₅₀ of insecticide ($\mu\text{g}/\text{cm}^2$)	LD ₅₀ of Azadirachtin ($\mu\text{g}/\text{cm}^2$)	Co-toxicity coefficient	Reduction of a. i. %
Thiodi (RFM)	Adults	1:2	36.8874	12.295	24.591	1092.509	90.847
		1:4	46.9241	9.384	37.539	1431.531	93.104
		1:6	47.1742	6.73	40.435	1996.059	94.991
		1:10	50.2256	4.56	45.659	2945.939	96.606

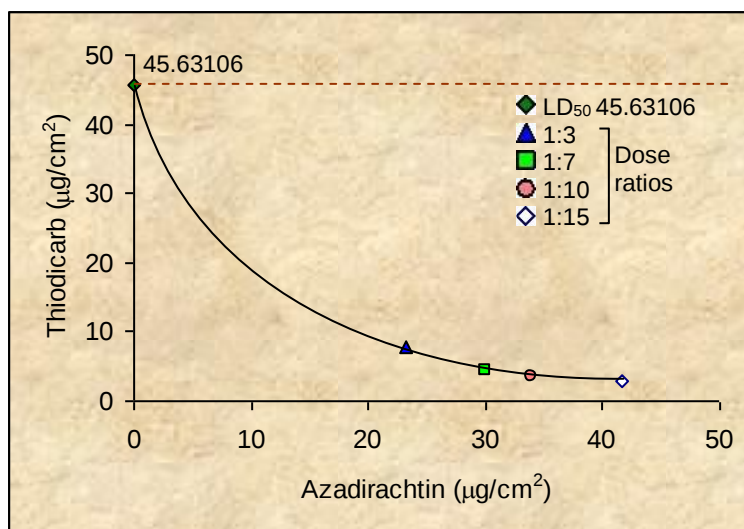


Fig 61: Isobologram of Thiodicarb with Azadirachtin against the mature larvae of *A. diaperinus* after 72 h exposure in RFM test.

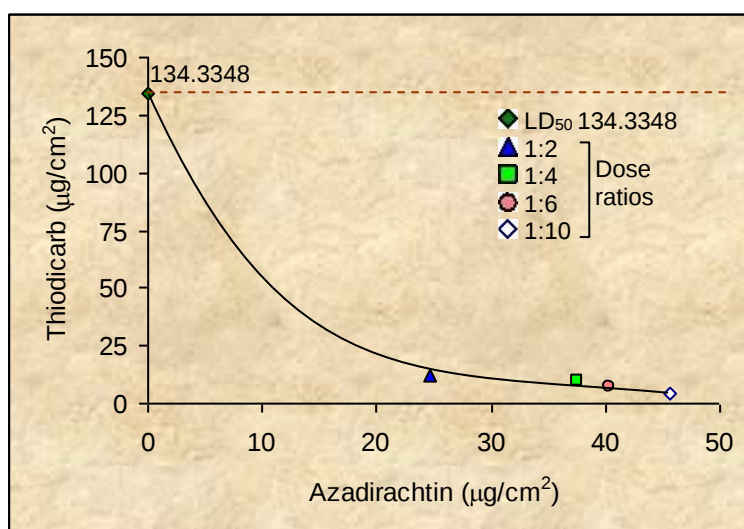


Fig 62: Isobologram of Thiodicarb with Azadirachtin against the adults of *A. diaperinus* after 72 h exposure in RFM test.

Table 38: Effect of Thiodicarb with Azadirachtin (ppm) on *A. diaperinus* 40 days old larvae exposed to treated flour medium for 72 hours.

Insecticide	Life stage (Age)	Ratio of combination	LD ₅₀ of combination (ppm)	LD ₅₀ of insecticide (ppm)	LD ₅₀ of Azadirachtin (ppm)	Co-toxicity coefficient	Reduction of a. i. %
Thiodi (TFM)	40days old larvae	1:20	131.202	6.248	124.954	282.831	64.644
		1:40	165.254	4.03	161.224	438.493	77.195
		1:60	222.803	3.652	219.151	483.879	79.334
		1:80	238.401	2.944	235.457	600.246	83.941

Table 39: Effect of Thiodicarb with Azadirachtin (ppm) on *A. diaperinus* adults exposed to treated flour medium for 72 hours.

Insecticide	Life stage (Age)	Ratio of combination	LD ₅₀ of combination (ppm)	LD ₅₀ of insecticide (ppm)	LD ₅₀ of Azadirachtin (ppm)	Co-toxicity coefficient	Reduction of a. i. %
Thiodi (TFM)	Adults	1:10	193.748	17.613	176.134	302.659	66.659
		1:20	245.889	11.709	234.18	455.268	78.035
		1:25	251.999	9.692	242.306	550.105	81.819
		1:30	262.954	8.4824	254.472	628.477	84.463

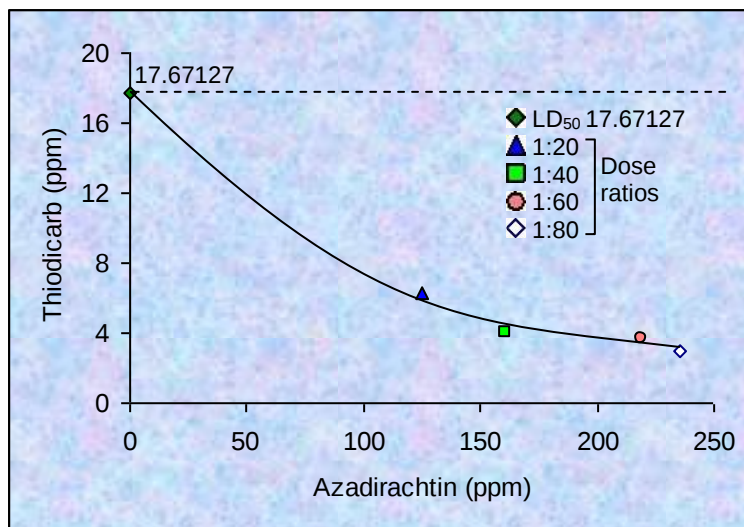


Fig 63: Isobologram of Thiodicarb with Azadirachtin against the mature larvae of *A. diaperinus* after 72 h exposure in TFM test.

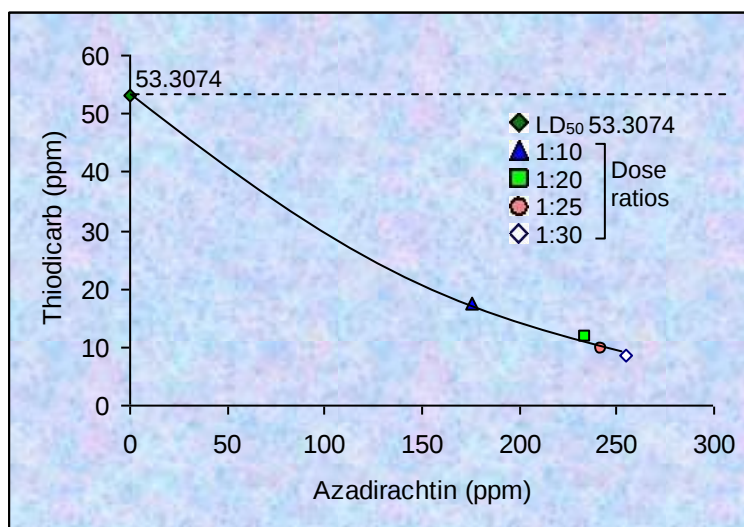


Fig 64: Isobologram of Thiodicarb with Azadirachtin against the adults of *A. diaperinus* after 72 h exposure in TFM test.

5.3.4 Fenitrothion and Azadirachtin

A. RFM test

For the mature larvae (40days old): The lowest dose of Fenitrothion was $22.1\mu\text{g}/\text{cm}^2$ for the mature larvae after 72 hours exposure. Effects of different combined doses of Fenitrothion and Azadirachtin at the ratios of 1:1, 1:3, 1:5 and 1:8 have been estimated in Appendix Tables (231-234) where the lowest dose of Fenitrothion was constant. The dose concentrations were 1.38, 2.76, 5.52, 11.05 and $22.1\mu\text{g}/\text{cm}^2$ at ratio of 1:1; 2.76, 5.53, 11.05, 22.1 and $44.2\mu\text{g}/\text{cm}^2$ at ratio of 1:3; 4.14, 8.28, 16.57, 33.15 and $66.3\mu\text{g}/\text{cm}^2$ at ratio of 1:5; and 6.21, 12.43, 24.86, 49.72 and $99.45\mu\text{g}/\text{cm}^2$ at ratio of 1:8. The mortality percentage were recorded as 4-38 % at 1:1; 2- 44 % at 1: 3; 6- 44 % at 1: 5; 10- 66 % at 1: 8 after 72 h exposure. The highest mortality was observed at the ratio of 1:8.

The LD_{50} values of the mixture (Fenitrothion + Azadirachtin) were recorded after 72h as 40.33, 49.24, 69.67 and $69.61\mu\text{g}/\text{cm}^2$ at the ratios of 1:1, 1:3, 1:5 and 1:8 respectively.

LD_{50} values along with 95% confidence limits, regression equations and chi-squared values have been estimated in the Table 40. Regression lines of different ratios on log probit mortality and the log dose concentrations have been plotted as in the Fig. 65.

For adults: The lowest dose of Fenitrothion was $707.33\mu\text{g}/\text{cm}^2$ for the adults after 72 hours exposure. Effects of different combined doses of Fenitrothion and Azadirachtin at the ratios of 1:0.05, 1:0.1, 1:0.2 and 1:0.5 have been estimated in Appendix Tables (235-238) where the lowest dose of Fenitrothion was constant. The dose concentrations were 23.21, 46.41, 92.83, 185.67 and $371.34\mu\text{g}/\text{cm}^2$ at ratio of 1:0.05; 24.31, 48.62, 97.25, 194.51 and $389.03\mu\text{g}/\text{cm}^2$ at ratio of 1:0.1; 26.52, 53.04, 106.09, 212.19 and $424.39\mu\text{g}/\text{cm}^2$ at ratio of 1: 0.2; and , 33.15, 66.31, 132.62, 265.24 and $530.49\mu\text{g}/\text{cm}^2$ at ratio of 1:0.5. The mortality percentage were recorded as 2 -32 % at 1:0.05; 4- 42 % at 1:0.1; 6-48 % at 1:0.2; 10- 60 % at 1:0.5 after 72 h exposure. The highest mortality was observed at the ratio of 1:10.

The LD_{50} values of the mixture (Fenitrothion + Azadirachtin) were recorded after 72h as 842.49, 495.74, 388.11 and $307.69\mu\text{g}/\text{cm}^2$ at the ratio 1:0.05, 1:0.1, 1:0.2 and 1:0.5 respectively.

LD_{50} values along with 95% confidence limits, regression equations and chi-squared values have been estimated in the Table 41. Regression lines of different ratios on log probit mortality and the log dose concentrations have been plotted as in the Fig. 66.

B. TFM test

For the mature larvae (40days old): The lowest dose of Fenitrothion was 37.5ppm for the mature larvae after 72 hours exposure. Effects of different combined doses of Fenitrothion and Azadirachtin at the ratios of 1:10, 1:15, 1:20 and 1:25 have been estimated in Appendix Tables (239-242) where the lowest dose of Fenitrothion was constant. The dose concentrations were 12.81, 25.79, 51.57, 103.13 and 206.25ppm at ratio 1:10; 18.75, 37.5, 75, 150 and 300ppm at ratio 1:15; 24.61, 49.22, 98.44, 196.88 and 393.75 at ratio 1:20; and 30.47, 60.94, 121.88, 243.75 and 487.5 at ratio 1: 15. The mortality percentage were recorded as 2- 44 % at 1:10; 4-60 % at 1:15; 8- 64 % at 1:20; 12- 68 % at 1:25 after 72 h exposure. The highest mortality was observed at the ratio of 1:25.

The LD₅₀ values of the mixture (Fenitrothion + Azadirachtin) were recorded after 72h as 210.72, 229.51, 242.11 and 253.63ppm at the ratio 1:10, 1:15, 1:20 and 1:25 respectively.

LD₅₀ values along with 95% confidence limits, regression equations and chi-squared values have been estimated in the Table 42. Regression lines of different ratios on log probit mortality and the log dose concentrations have been plotted as in the Fig. 67.

For adults: The lowest dose of Fenitrothion was 62.5ppm for the adults after 72 hours exposure. Effects of different combined doses of Fenitrothion and Azadirachtin at the ratios of 1:10, 1:15, 1:20 and 1:25 have been estimated in Appendix Tables (243- 246) where the lowest dose of Fenitrothion was constant. The dose concentration were 21.49, 42.97, 85.94, 171.88 and 343.75ppm at ratio 1:10; 31.25, 62.5, 125, 250 and 500ppm at ratio 1:15; 41.02, 82.04, 164.07, 328.13 and 656.25ppm at ratio 1:20; and 50.79, 101.57, 203.13, 406.25 and 812.5ppm at ratio 1:25. The mortality percentage were recorded as 4-44 % at 1:10; 6-56 % at 1:15; 10-70 % at 1:20; 16- 80 % at 1:25 after 72 h exposure. The highest mortality was observed at the ratio of 1:25.

The LD₅₀ values of the mixture (Fenitrothion + Azadirachtin) were recorded after 72h as 375.37, 385.92, 396.69 and 398.68ppm at the ratio 1:10, 1:15, 1:20 and 1:25 respectively.

LD₅₀ values along with 95% confidence limits, regression equations and chi-squared values have been estimated in the Table 43. Regression lines of different ratios on log probit mortality and the log dose concentrations have been plotted as in the Fig. 68. The lines were increased with the increase of doses gradually.

Table 40: LD₅₀ , 95% confidence limits and regression equations for combined action of Fenitrothion with Azadirachtin against the mature larvae (40 days old) of *A. diaperinus* exposed to the treated filter paper for 72 hours.

Insecticide	Life stage (Age)	Ratio of combination	LD ₅₀ of combination (µg/cm ²)	Regression equation	95% confidence limit		Chi-square value (3df)
					Lower (µg/cm ²)	Upper (µg/cm ²)	
Fenitro (RFM)	40days old larvae	1:1	40.3245	Y=2.93536+1.2859X	19.3719	83.9391	0.3292
		1:3	49.2392	Y=2.49209+1.4819X	30.4506	79.6206	0.9174
		1:5	69.6691	Y=2.73545+1.2288X	40.4786	119.909	2.318
		1:8	69.6011	Y=2.77458+1.2535X	40.4195	87.8854	0.1639

Table 41: LD₅₀ , 95% confidence limits and regression equations for combined action of Fenitrothion with Azadirachtin against the adults of *A. diaperinus* exposed to the treated filter paper for 72 hours.

Insecticide	Life stage (Age)	Ratio of combination	LD ₅₀ of combination (µg/cm ²)	Regression equation	95% confidence limit		Chi-square value (3df)
					Lower (µg/cm ²)	Upper (µg/cm ²)	
Fenitro (RFM)	Adults	1:0.05	842.485	Y=1.50166+1.1957X	347.049	2045.18	0.8677
		1:0.1	495.736	Y=1.59003+1.2651X	273.311	899.173	0.3251
		1:0.2	388.106	Y=2.0687+1.13221X	226.208	665.876	1.6301
		1:0.5	307.688	Y=1.9096+1.24204X	208.993	452.991	0.3991

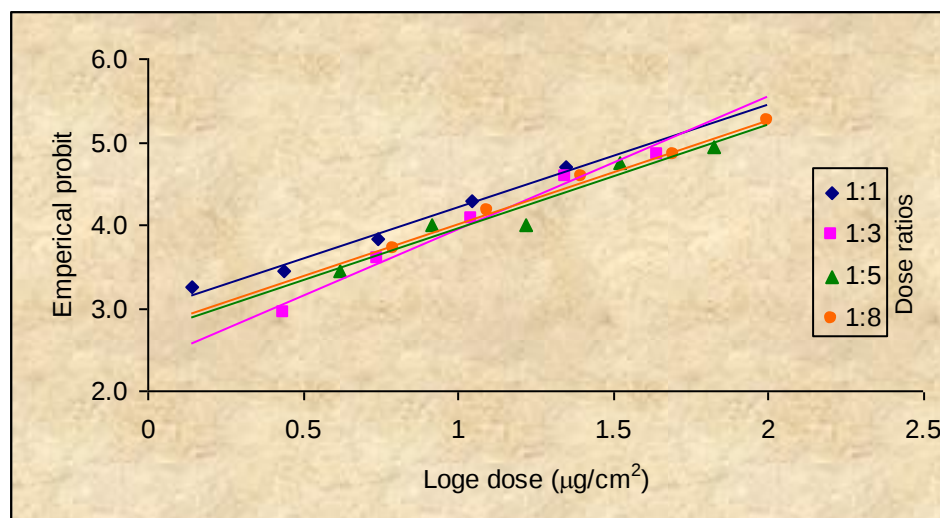


Fig 65: Regression lines of different combined doses Fenitrothion and Azadirachtin against the mature larvae (40 days old) of *A. diaperinus* exposed to the treated filter paper for 72 hours.

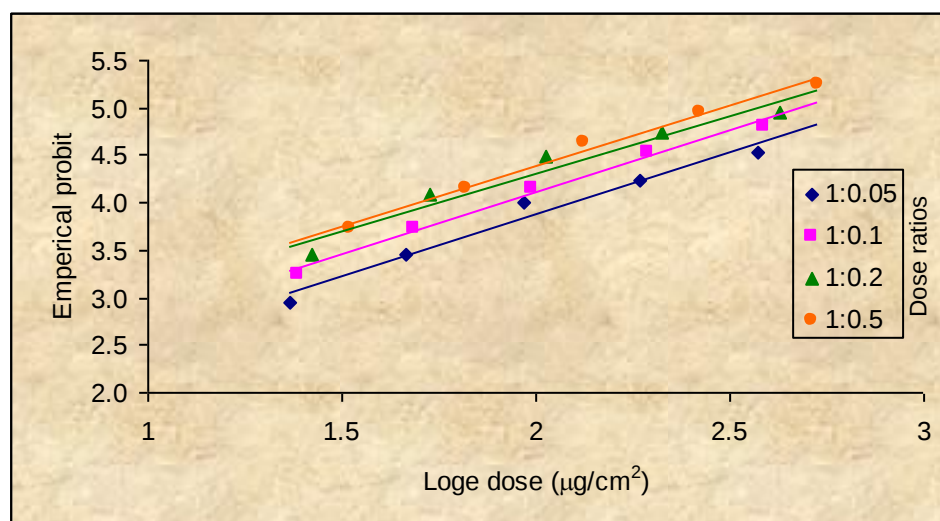


Fig 66: Regression lines of different combined doses Fenitrothion and Azadirachtin against the adults of *A. diaperinus* exposed to the treated filter paper for 72 hours.

Table 42: LD₅₀ , 95% confidence limits and regression equations for combined action of Fenitrothion with Azadirachtin against the mature larvae (40 days old) *A. diaperinus* exposed to the treated flour medium for 72 hours.

Insecticide	Life stage (Age)	Ratio of combination	LD ₅₀ of combination (ppm)	Regression equation	95% confidence limit		Chi-square value (3df)
					Lower (ppm)	Upper (ppm)	
Fenitro (TFM)	40days old larvae	1:10	210.717	$Y=1.5588+1.24302X$	187.532	581.841	7.7339 18E-02
		1:15	229.597	$Y=1.54568+1.4211X$	174.948	415.452	0.1927
		1:20	242.105	$Y=1.60278+1.4251X$	171.085	342.606	0.7155
		1:25	253.621	$Y=1.8131+1.32554X$	179.873	357.604	0.4552

Table 43: LD₅₀ , 95% confidence limits and regression equations for combined action of Fenitrothion with Azadirachtin against the adults of *A. diaperinus* exposed to the treated flour medium for 72 hours.

Insecticide	Life stage (Age)	Ratio of combination	LD ₅₀ of combination (ppm)	Regression equation	95% confidence limit		Chi-square value (3df)
					Lower (ppm)	Upper (ppm)	
Fenitro (TFM)	Adults	1:10	375.365	$Y=1.89338+1.0474X$	317.242	694.92	0.3991
		1:15	385.913	$Y=1.46488+1.3596X$	261.669	605.494	0.1322
		1:20	396.683	$Y=1.17961+1.5082X$	251.688	462.387	0.8431
		1:25	398.674	$Y=1.46677+1.4585X$	281.967	446.508	0.9347

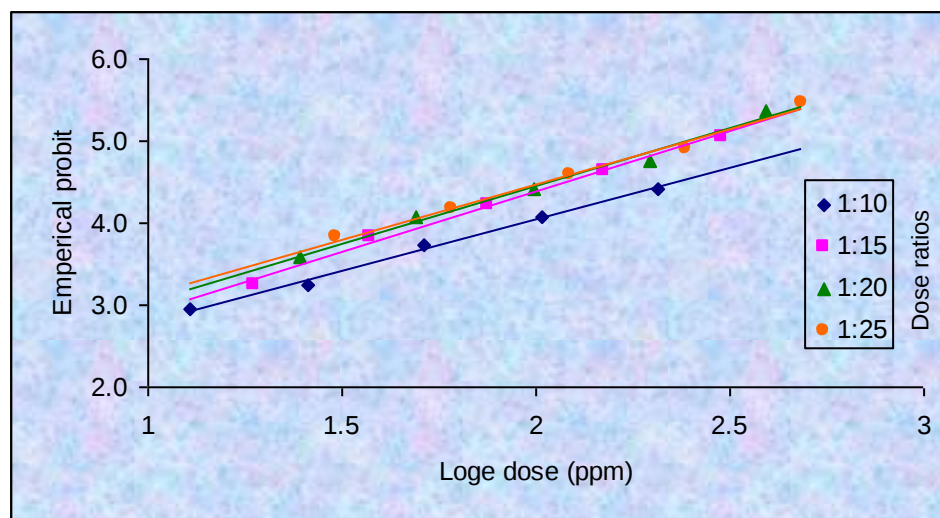


Fig 67: Regression lines of different combined doses Fenitrothion and Azadirachtin against the mature larvae (40 days old) of *A. diaperinus* after 72 hours' exposure to the treated flour medium.

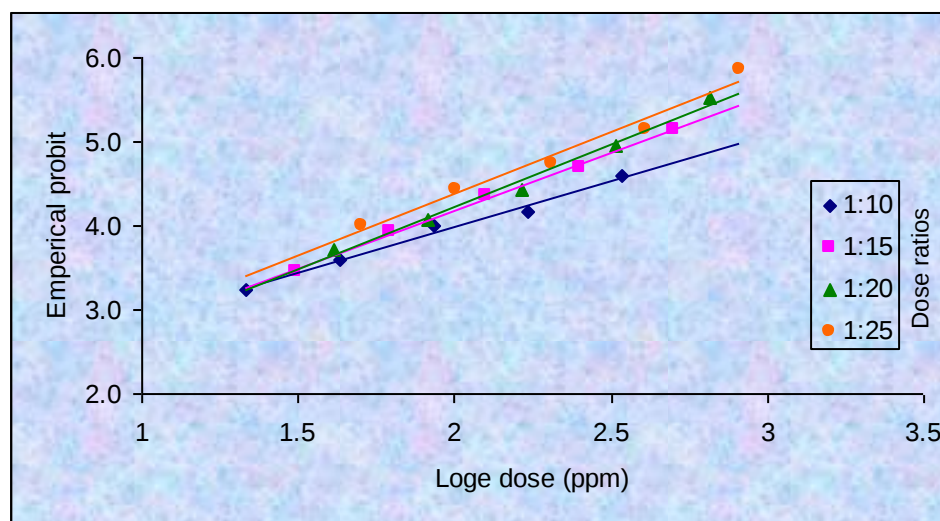


Fig 68: Regression lines of different combined doses Fenitrothion and Azadirachtin against the adults of *A. diaperinus* after 72 hours' exposure to the treated flour medium.

5.3.4.1 Synergistic action of Fenitrothion + Azadirachtin

The LD₅₀ value of Fenitrothion alone was calculated as 107.81µg/cm² for larvae (40 days old), and 2032.29µg/cm² for adults of exposure on treated filter paper. And on treated food media the LD₅₀ values were 170.39ppm and 385.77ppm for larvae and adults respectively after 72 hours' exposure to the treated food media.

The shares of Fenitrothion in the LD₅₀ value of mixture have been separated (table 44-47). The co-toxicity coefficient was determined as 534.68, 875.84, 928.41 and 1393.93 for larvae at the ratio 1:1, 1:3, 1:5 and 1:8 and 253.29, 450.95, 628.37 and 990.76 for adults at the ratios of 1:0.05, 1:0.1, 1:0.2 and 1:0.5 in RFM test respectively. And in TFM test the co-toxicity coefficients were 889.48, 1187.46, 1477.91 and 1747.57 for larvae at the ratios of 1:10, 1:15, 1:20 and 1:25 and 1130.41, 1599.41, 2042.23 and 2515.79 for adults at the ratios of 1:10, 1:15, 1:20 and 1:25 respectively.

The co-toxicity coefficients of mixtures of each ratio are greater than 100, following the principle of Sun and Johnson (1960). These values indicated that Fenitrothion + Azadirachtin offered synergistic action to the adults and larvae in the exposure period of 72 hours in both RFM and TFM tests. Larvae were found more susceptible than adults in both TFM and RFM tests.

The free hand curve fitting of isobolograms (Fig 69-72) has run below when the exposure period was 72 hours for both RFM and TFM tests.

RFM test

The LD₅₀ value of Fenitrothion for mature larvae was recorded 107.81µg/cm² after 72 hours exposure to the treated filter paper, but in the mixture, the shares of Fenitrothion were 20.17, 12.31, 11.62 and 7.74µg/cm² at the ratios of 1:1, 1:3, 1:5 and 1:8 when Azadirachtin caused reduction of the dose at the level of 81.21%, 88.59%, 89.23% and 92.83% respectively (Table-44). The shares of azadirachtin were 20.17, 36.93, 58.06 and 61.87µg/cm².

The LD₅₀ value for adults was recorded 2032.29µg/cm² after 72 hours exposure to the treated filter paper, but in the mixture, the shares of Fenitrothion were 802.37, 450.67, 323.43 and 205.13µg/cm² at the ratios of 1:0.05, 1:0.1, 1:0.2 and 1:0.5 when Azadirachtin caused the reduction of the dose at the levels of 60.52%, 77.83%,

84.09% and 89.91% respectively (Table- 45). The shares of azadirachtin were 40.12, 45.07, 64.69 and 102.57 $\mu\text{g}/\text{cm}^2$.

58.06 $\mu\text{g}/\text{cm}^2$ azadirachtin was needed for 89.23% dose reduction at the ratio 1:0.5 for larvae, but 102.57 $\mu\text{g}/\text{cm}^2$ azadirachtin was needed for 89.91% dose reduction at the ratio 1:5 which was the highest for adults. So, larvae were more susceptible than adults in the RFM test.

TFM test

LD₅₀ value of fenitrothion for mature larvae (40 days old larvae) after 72 hours exposure to the treated food media was 170.39ppm but in the mixture, the shares of fenitrothion were 19.16, 14.35, 11.53 and 9.75ppm at the ratios of 1:10, 1:15, 1:20 and 1:25 respectively when azadirachtin caused the reduction of the doses at the levels of 88.76%, 91.58%, 93.24% and 94.28% respectively (Table-46). The shares of azadirachtin were 191.57, 215.25, 230.58 and 243.87ppm.

LD₅₀ value of fenitrothion for adults after 72 hours exposure to the treated food media was 385.77ppm, but in the mixture, the shares of fenitrothion were 34.13, 24.12, 18.89 and 15.34ppm at the ratios of 1:10, 1:15, 1:20 and 1:25 respectively when azadirachtin caused the reduction of the doses at the levels of 91.16%, 93.75%, 95.11% and 96.03% respectively (Table-47). The shares of azadirachtin were 341.25, 361.71, 377.71 and 383.35ppm.

243.87ppm azadirachtin was needed for 94.278% dose reduction for larvae, on the contrary 361.71ppm azadirachtin was needed for 93.75% dose reduction which was the highest for adults. So, larvae were found more susceptible than adults in the TFM test.

Table 44: Effect of Fenitrothion with Azadirachtin ($\mu\text{g}/\text{cm}^2$) on 40 days old larvae of *A. diaperinus* exposed to treated filter paper for 72 h.

Insecticide	Life stage (Age)	Ratio of combination	LD ₅₀ of combination ($\mu\text{g}/\text{cm}^2$)	LD ₅₀ of insecticide ($\mu\text{g}/\text{cm}^2$)	LD ₅₀ of Azadirachtin ($\mu\text{g}/\text{cm}^2$)	Co-toxicity coefficient	Reduction of a. i. %
Fenitro (RFM)	40days old larvae	1:1	40.3245	20.16225	20.1622	534.674	81.291
		1:3	49.2392	12.309	36.929	875.834	88.583
		1:5	69.6691	11.612	58.058	928.405	89.229
		1:8	69.6011	7.734	61.868	1393.927	92.827

Table 45: Effect of Fenitrothion with Azadirachtin ($\mu\text{g}/\text{cm}^2$) on adults of *A. diaperinus* exposed to treated filter paper for 72 h.

Insecticide	Life stage (Age)	Ratio of combination	LD ₅₀ of combination ($\mu\text{g}/\text{cm}^2$)	LD ₅₀ of insecticide ($\mu\text{g}/\text{cm}^2$)	LD ₅₀ of Azadirachtin ($\mu\text{g}/\text{cm}^2$)	Co-toxicity coefficient	Reduction of a. i. %
Fenitro (RFM)	Adults	1:0.05	842.485	802.367	40.118	253.287	60.519
		1:0.1	495.736	450.669	45.067	450.949	77.825
		1:0.2	388.106	323.423	64.684	628.369	84.086
		1:0.5	307.688	205.125	102.562	990.757	89.907

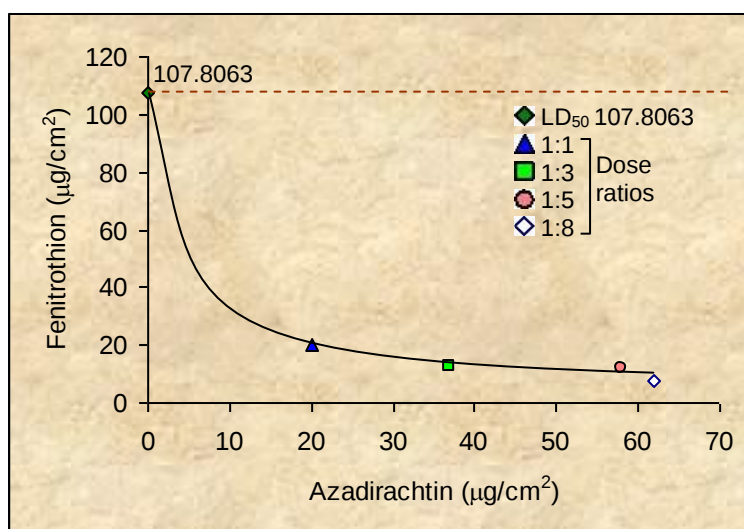


Fig 69: Isobologram of Fenitrothion with Azadirachtin against the mature larvae of *A. diaperinus* after 72 h exposure in RFM test.

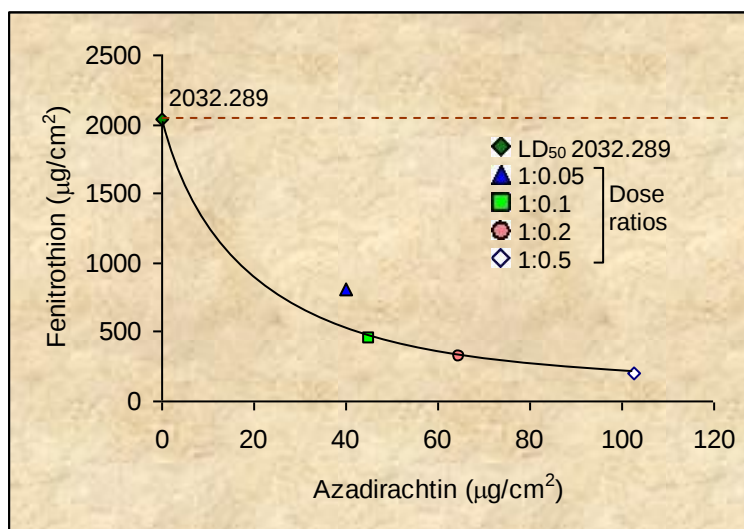


Fig 70: Isobologram of Fenitrothion with Azadirachtin against the adults of *A. diaperinus* after 72 h exposure in RFM test.

Table 46: Effect of Fenitrothion with Azadirachtin (ppm) on 40 days old larvae of *A. diaperinus* exposed to treated food media for 72 hours.

Insecticide	Life stage (Age)	Ratio of combination	LD ₅₀ of combination (ppm)	LD ₅₀ of insecticide (ppm)	LD ₅₀ of Azadirachtin (ppm)	Co-toxicity coefficient	Reduction of a. i. %
Fenitro (TFM)	40days old larvae	1:10	210.717	19.156	191.561	889.476	88.758
		1:15	229.597	14.341	215.246	1187.455	91.579
		1:20	242.105	11.529	230.576	1477.908	93.234
		1:25	253.621	9.75	243.867	1747.569	94.278

Table 47: Effect of Fenitrothion with the lethal dose of Azadirachtin (ppm) on adults of *A. diaperinus* exposed to treated food media for 72 hours.

Insecticide	Life stage (Age)	Ratio of combination	LD ₅₀ of combination (ppm)	LD ₅₀ of insecticide (ppm)	LD ₅₀ of Azadirachtin (ppm)	Co-toxicity coefficient	Reduction of a. i. %
Fenitro (TFM)	Adults	1:10	375.3657	34.124	341.242	1130.495	91.155
		1:15	385.9136	24.1196	361.794	1599.405	93.748
		1:20	396.6837	18.8897	377.794	2042.224	95.104
		1:25	398.6746	15.33364	383.341	2515.782	96.026

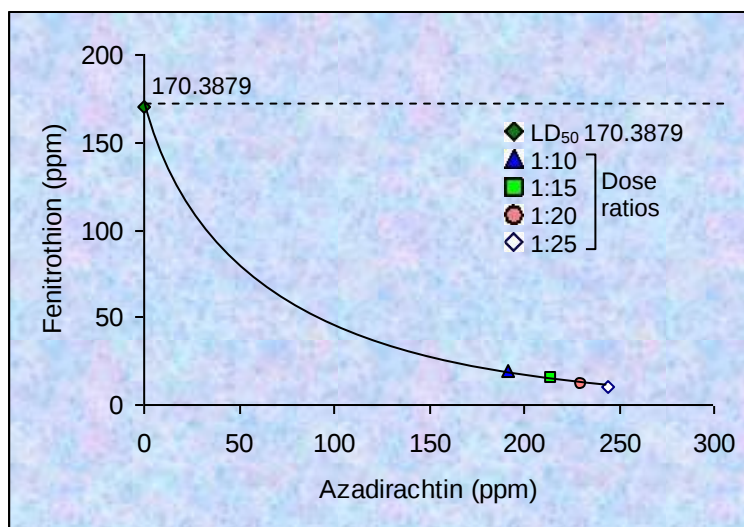


Fig 71: Isobologram of Fenitrothion with Azadirachtin against the mature larvae of *A. diaperinus* after 72 h exposure in TFM test.

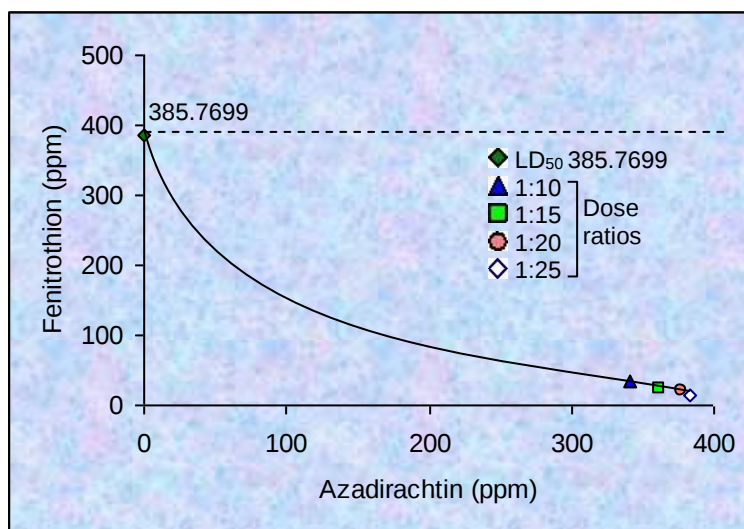


Fig 72: Isobologram of Fenitrothion with Azadirachtin against the adults of *A. diaperinus* after 72 h exposure in TFM test.

Chapter - 6

General Discussions

The present study has been made to investigate the effect of Deltamethrin, Imidacloprid, Thiodicarb, Fenitrothion and azadirachtin alone or in combination with azadirachtin on *Alphitobius diaperinus* in relation their potential use in the management of the *A. diaperinus* population. The longer exposure of both larvae and adults of *A. diaperinus* for ingestion of treated food have the toxic effect. At shorter exposure period the toxic effect was comparatively less than that of longer exposure. Moreover, contact action of all chemicals was found to be higher than the stomach action. The order of toxicity was as: Thiodicarb> Imidacloprid> Deltamethrin> Fenitrothion. Toxicity of these chemicals was very high when newly hatched larvae of *A. diaperinus* were exposed for the longer period which might be due to the cumulative effect which caused larval mortality (Mazid 2000). The present results indicated that the toxicity of these chemicals depends on larval age and exposure period (Mondal 1984a, c). Moreover, early larval instars were comparatively more susceptible than older ones. In case of adults, the mortality was also increased with the increase of doses and exposure period. Larvae of *A. diaperinus* of all ages were more susceptible than adults in both Residual Film Method and Treated Food Method.

At low doses both Azadirachtin> Thiodicarb> Deltamethrin were toxic to *A. diaperinus* adults (Fig. 105.A) in contact poisoning but in stomach poisoning this order was Thiodicarb> Imidacloprid> Azadirachtin (Fig 105.B). In contact poisoning Imidacloprid and Fenitrothion was less toxic for adults but in stomach poisoning it was high. Deltamethrin was less toxic for adults in treated food than other insecticides. Azadirachtin was more toxic for adults in RFM test than TFM test.

In the TFM experiments, the low LD₅₀ values for early larval instars (1 and 10 days old larvae) might be due to that although small amount of treated medium were being consumed by them, the amount of toxic substances consumed along with the food was sufficient enough in comparison to their body weight to kill them (Mondal 1984 a, b, c, Kamaruzzaman 2000). On the other hand, 30 days and 40 days old larvae showed more tolerance than early instars. This might be due to either increased tolerance resistance to these chemicals or to relatively small amounts of

chemicals treated medium being consumed in comparison with their body weight during the experimental period. The later effect may be due to the late instars particularly last instar being ready to pupate and in an inactive and non feeding stage (Mondal 1984a). In both experiments, the calculated LD₅₀ values for adults were higher than those of larvae indicating that, adults are more tolerant than larvae. The body cuticles of adults are very hard in comparison with that of larvae which do not allow the toxic substances to penetrate inside the adult body. Besides, adults consumed less amount of treated food in comparison with their body weight and accordingly, the amount of toxic substances consumed along with the flour medium were not sufficient enough to kill them. That is why the doses to kill the adults were comparatively higher than those of larvae (Fig: 4, 8, 12, 16, 20, 24, 28, 32, 36, and 40).

Deltamethrin was the most toxic for the early larval stages at contact poisoning than other insecticides but at stomach poisoning it was low. Thiodicarb was most toxic at stomach poisoning than other insecticides for newly hatched larvae. In both RFM and TFM tests, the larval instars were found to be more susceptible to Deltamethrin, Thiodicarb, Imidacloprid, Fenitrothion and Azadirachtin (Nimbecidine) than adults. Early larval instars were more susceptible than the older instars indicating that the tolerance to pesticides increases with the increase of larval age which is similar to the findings of Mondal (1984 a, c) and Hasnat (2003). Mondal (1984 a, c) reported that the LD₅₀ values of pirimphos-methyl against *T. castaneum* larvae increased with the age of larvae and larvae, up to fifth instar, showed an increased tolerance to the pirimphos-methyl. Similar results were also reported by Hasnat (2003) and Hasnat and Mondal (2003) working with technical grade of Deltamethrin against *T. castaneum* larvae.

6.1 BIOASSAY OF INSECTICIDES ALONE

6.1.1 AZADIRACHTIN

Azadirachtin .03% (Nimbecidine) as a botanical was the highly toxic to kill both larvae and adults of *A. diaperinus* at contact poisoning. But at stomach poisoning it was less toxic, because insect feeds less, grow poorly and lay fewer eggs on foods treated with botanicals. Mortality of beetles is also attributed to starvation,

exhaustion or growth disruption, in addition to poisoning by direct contact or stomach toxicity (Saxena *et al.* 1989). The mortality of *A. diaperinus* due to nimbecidine (Azadirachtin .03%) might be due its growth inhibitor action. Chitin synthesis inhibitors act against the larval stages of insects which usually fail to survive due to incomplete moulting or disruption of cuticle formation (Fox 1990). If the larvae feed on these compounds and intake through cuticle, the lethal effect appears during any of the next moults (Hammann and Sirrenberg 1980)

The harmlessness of neem derivative products towards many predators and parasitoids has been reported extensively in the literature even though several negative delayed effects of these compounds were recorded mostly under laboratory conditions (Schmutterer 1997). The insecticidal properties of the neem derivatives (neem oil, neem seed powder, and neem extracts) was investigated against the different insect pests at different dose levels by Shapiro *et al* (1994).

Insects of different orders have different behavior to azadirachtin. Lepidoptera are extremely sensitive to azadirachtin, this sensitivity is expressed by a strong inhibition of food, with effective doses causing 50% inhibition of feeding (ED_{50}) of <1-50 ppm, depending on the species, whereas 100% inhibition of feeding in the Coleoptera, Hemiptera and Homoptera are achieved at doses of 100-600 ppm revealing so, their weak response to the azadirachtin (Mordue and Nisbet, 2000). Poland *et al.* (2006) reported that two species *Anoplophora glabripennis* and *Plectrodera scalator* (Coleoptera: Cerambycidae) show different sensitivity to azadirachtin with LC_{50} 23.55 and 1.58 ppm respectively.

The present study showed that regardless of dose, the azadirachtin causes mortality of *A. diaperinus*. This is similar to the findings of Mordue and Nisbet (2000). This result was also supported by Khan and Ahmed 2003. Our bioassay showed that the percentage of larvae killed by azadirachtin 72 h after treatment was significantly higher than that killed in 24 hours, reflecting a delayed toxicity of the tested product. The azadirachtin is toxic to the larvae of *A. diaperinus*. Martinez and van Emden (2001), reported that azadirachtin caused a significant increase in mortality of *Spodoptera littoralis*, which intensified during insect development. It induces a high response time (Chougourou *et al.*, 2012).

Rahman *et al.* 2001 found the toxicity of Azadirachtin (commercial product Nimbicidin®) to different larval instars and adults of *Cryptolestes pusillus* (Schon.) was recorded after 24 hours. The LC₅₀ values were 5.659, 13.962, 12.297, 10.203 µg/cm² for first to fourth instars larvae. This result is so close to the present findings.

In the present work it was noted that mortality rates are positively correlated to the different doses used, regardless of the duration of exposure of larvae to azadirachtin. Thus, the present results confirm the work of Rharrabe *et al.* (2008), who reported that the treatment of larval *Plodia interpunctella* Hübner by azadirachtin, showed a positive correlation between dose and observed mortality rate (7% to 2 ppm and 4 ppm to 10%) for a lethal time of 96h. The azadirachtin extracts had a significant increase in larval mortality when the dose increases (Chougourou *et al.*, 2012).

Khalequzzaman and Nahar (2008) also reported that the azadirachtin was more toxic than conventional insecticides used against different aphid species. Alouani *et al.* (2009) have observed high toxicity of azadirachtin on larvae and pupae of *Culex pipiens* with an LC₅₀ of 0.35 and 0.42 mg / L respectively after 24 h exposure.

As far as known, no work has been done on Azadirachtin on larval and adult mortality of *A. diaperinus* previously and hence, the comparison of the present data was not possible.

6.1.2 ORGANOPHOSPHORUS

The application of organophosphate (OP) 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 have received registration by ERA 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 showed clear stereo selectivity 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 having lower mammalian toxicity. After successful development of organophosphate as insecticides other carbamate compounds were also developed. They act as anticholinesterases. It has physiological properties of alkaloid, because it is a phenylester of methylcarbamic acid.

Lancaster and Simco (1969) tested several organophosphate insecticides in actual broiler house conditions. Dursban and ronnel at high doses were 100% effective for eight and three weeks exposure respectively. Dursban and ronnel were 100% effective for only one to two weeks. Unfortunately the exposure period was not sufficient enough to eliminate the beetle population of *A. diaperinus*.

In the present investigation commercial formulation of Fenitrothion (sumithion 50EC) was tested against adult and different ages of larvae of *A. diaperinus*. Toxicity was found to be increased with time and decreased with ages. It was more effective for adults as stomach poison than contact poison. It was more toxic for larvae than adults at contact poisoning. It was hypothesized by Johnson (1975) that OP compounds induced delayed neurotoxin ester (NTE) in nervous system, but not to AChE.

Kaufman *et al.* (2005) used tetrachlorvinfos as an organophosphate insecticide against susceptible strain of *A. diaperinus*. The 48h LD₅₀ values for tetrachlorvinfos were recorded as 0.080 µg/cm² for adult and 0.070 µg/cm² for larvae. If the temperature would be increased, the same mortality might be obtained at 24h of exposure. That means the toxicity was much related with temperature. It also happened in the present investigation.

The present results 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 both resistant and susceptible population of *A. diaperinus* to Chlorpyrifos by residual film method at 21°C ± 2. The LD₅₀ values for the susceptible laboratory population were found to be 0.097ug/gm for adult and 0.07ug/gm for larvae after 24h. The variation in the result could be attributed to the fact that technical grade of Chlorpyrifos was used in the previous experiment, but in the present investigation commercial formulation was used.

6.1.3 CARBAMATES

The toxicity of carbamate pesticides is due to the disruption of the nervous system of an invertebrate or a vertebrate through the inhibition of cholinesterase (ChE) enzymes. These enzymes are involved in transmitting normal nerve impulses throughout the nervous system. An acute pesticide dose reduces the activity of ChEs, and the nerve impulses cannot be transmitted normally. This can paralyze the nervous system, and it may lead to death, usually from respiratory failure (Hill, 1995).

In the environment, thiodicarb rapidly degrades to methomyl, which is also registered for use as an insecticide. Thiodicarb is a high efficient insecticide similar to methomyl, but it has lower toxicity compared with methomyl. It has mainly stomach toxicity, with little contact action. It can kill Lepidoptera, Coleoptera and Diptera pests effectively.

Larvin is primarily active as a stomach poison. In the present study Thiodicarb was examined against the lesser mealworm and LD₅₀ values were found to be as 78.468 and 376.394 µg/cm² for larvae and adults respectively at contact poisoning, but through the feeding bioassay the LD₅₀ values were 49.27849 and 194.2292 ppm after 24 hours for larvae and adults respectively and after 48 and 72 hours this values were decreased. Kerns and Tellez (1997) tested Thiodicarb against *Spodoptera exigua* (Hubner) through the feeding bioassay, and the LC₅₀ Value was found as 498.18 mg/kg, which is similar to the present investigation.

In the present investigation Larvin 75WP was most effective at TFM test which agrees with the findings of Bhoyar *et al.* (2004), Meena *et al.* (2006) and Muthukrishna *et al.* (2002) who reported the higher efficacy of this molecule.

So, Thiodicarb is the most effective against *A. diaperinus* adults and larvae. But among the two methods this insecticide was more effective in treated food method than residual film method. As Larvin is stomach poison so it was very effective with food contact.

6.1.4 DELTAMETHRIN

Deltamethrin is an insecticide in the synthetic pyrethroid family. Deltamethrin, like all synthetic pyrethroids, kills insects by disruption of the normal functioning of the nervous system. In insects as well as all other animals including humans, nerve

impulses travel along nerves when the nerves become momentarily permeable to sodium atoms, allowing sodium to flow into the nerve. Pyrethroids delay the closing of the "gate" that allows the sodium flow (Vijverberg and Bercken, 1990). This results in multiple nerve impulses instead of the usual single one. In turn, these impulses cause the nerve to release the neurotransmitter acetylcholine and stimulate other nerve (Ramadan, 1988).

Deltamethrin is widely used for post-harvest protection of grains. It is exceptionally potent against the whole spectrum of stored-product pests (Snelson 1987), whilst effective at very low dose level, it is stable on grains and shows no tendency to penetrate into grains (Jermannaud and Pochon 1994).

Longstaff and Desmarchelier (1983) reported that deltamethrin was significantly more toxic to *S. oryzae* than pirimiphos-methyl. Golob *et al.* (1985) tested deltamethrin by topical application to *P. truncatus* and found that it was from 5 to 50 times more effective than the most effective of 10 other insecticides. It was over 5000 times more potent than Malathion. Deltamethrin, when combined with the insect growth regulator (IGR) diflubenzuron, was found to produce greater adverse effect on the population of *T. castaneum* than by diflubenzuron or deltamethrin alone (Hasnat and Mondal 2003, Hasnat *et al.* 2003a, b).

Kljajic and Peric (1998) reported that the deltamethrin was significantly more toxic to adult *S. granarius*. In Europe, deltamethrin was found effective in controlling *O. surinamensis*, *R. dominica* and *S. oryzae* (Wilkin *et al.* 1998).

In the present study susceptibility of *A. diaperinus* to Deltamethrin was assessed. The LD₅₀ values were calculated as 3.05, 25.44, 142.94, 225.84, 361.78 and 1229.49 µg/cm² for 1 days (newly hatched larvae) 10, 20, 30, 40 days old larvae (mature larvae) and adult after 24 hours respectively and in treated food medium it was 168.90, 106.32, 298.57, 315.46, 1095.91 and 2044.41 ppm. The LD₅₀ values were calculated as 1.51, 11.96, 112.01, 180.75, 327.13 and 854.11 µg/cm² for 1 days (newly hatched larvae) 10, 20, 30, 40 days old larvae (mature larvae) and adult after 72 hours respectively and in treated food medium it was 48.73, 61.11, 690.93, 729.67, 778.62 and 1243.21 ppm. The findings of Khatun (2010) were that the LD₅₀ values of Deltamethrin were 55.28, 60.68, 281.61 µg/cm² for 1st inster larvae, 6th inster larvae, and adults of *Tribolium castaneum* respectively and in treated food

media the values were 69.17, 102.99, 353.89 ppm after 24 hours. The difference of the result is probably due to the difference of insect body weight and length (Mondal 1984 a, c, Kamaruzzaman 2000). The length of *A. diaperinus* is approximately 5.8 to 6.3mm (Kaufman *et al.* 2005) and of *Tribolium castaneum* is 2.3 to 4.4 mm (Dobie *et al.* 1991). On the otherhand, the difference may be due to the insect resistance. Resistance towards some commonly used synthetic pyrethroids, viz., permethrin, cypermethrin, deltamethrin and fenvalerate has already been reported (Sinha and Saxena 1999; Padhee *et al.*, 2002).

The present result is in general agreement with those of Steelman (2008) who investigated the toxicity of pyrethroid insecticides: cyfluthrin, permethrin and cypermethrin on adult and larvae of the beetle population collected from broiler chicken production firms in Arkansas that having different insecticide application - story. In the residual test Steelman (2008) found little difference in the susceptibility of adult and larvae to these insecticides. But in the present investigation a great difference has been found between the susceptibility of *A. diaperinus* adult, mature larva and newly hatched larvae to this insecticides tested.

6.1.5 IMIDACLOPRID

In the present investigation the toxicity of imidacloprid (Confidor) was examined against the lesser mealworm *A. diaperinus* and LD₅₀ values were calculated. The present finding with imidacloprid agrees with the result of Paul *et al.* (2006) who worked with imidacloprid against *Aedes aegypti*. Wu *et al.*, (2007) determined the toxicity of imidacloprid in susceptible F_{2i} progeny of *Diaeretiella rapae* (Hymenoptera: Aphidiidae) strains using the dry film method. LC₅₀ values were calculated as 0.17 (0.16-0.18) mg/l based on mortality at 24h.

Akter (2011) tasted imidacloprid against *A. diaperinus* and the LD₅₀ values were calculated as 0.69, 0.52 µg/cm² for adults and larvae respectively. 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). Akter (1011) used Imitaf 20 EC but in the present experiment Confidor 75 WP was used. Among several neonicotinoids, introduced into CPB management in '90s, imidacloprid effectively controlled pests resistant to conventional insecticides dominating the markets at that time (Elbert 2008).

Wang *et al* (2005) tested imidacloprid against Asian horn beetle *Anoplophora glabripennis* through the contact feeding bioassay and the calculated LD₅₀ value of imidacloprid was reported as 5.1 ppm after 24h exposure. It is apparent that the imidacloprid produces high toxicity for newly hatched larvae of *A. diaperinus* in the laboratory at residual film method. And 10 to 40 days old larvae showed similar activities in both TFM and RFM tests. In the present study treated food method was very effective than residual film method for adults.

In the present experiment it was found that when time duration was increased the toxicity also increased. Thorne and Breisch (2001) observed that the effect of imidacloprid was so negligible that the symptoms would disappear if termites were not exposed to imidacloprid for a longer period. They also noticed the delayed toxicity of imidacloprid and reported that death caused by imidacloprid took several days. The result of the present investigation is to some extent similar to the results of a previous study on *Culex quinquefasciatus* 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 against *Aedes aegypti*.

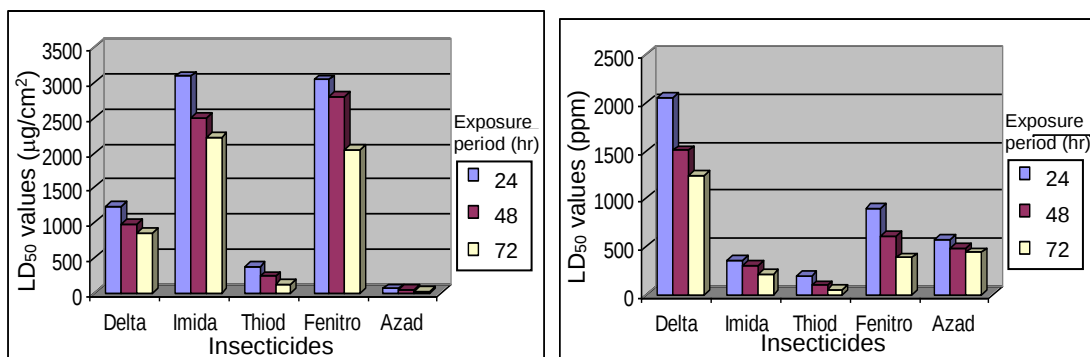


Fig105. LD₅₀ values of different insecticides on adult of *A. diaperinus* in RFM (A) and TFM (B) tests.

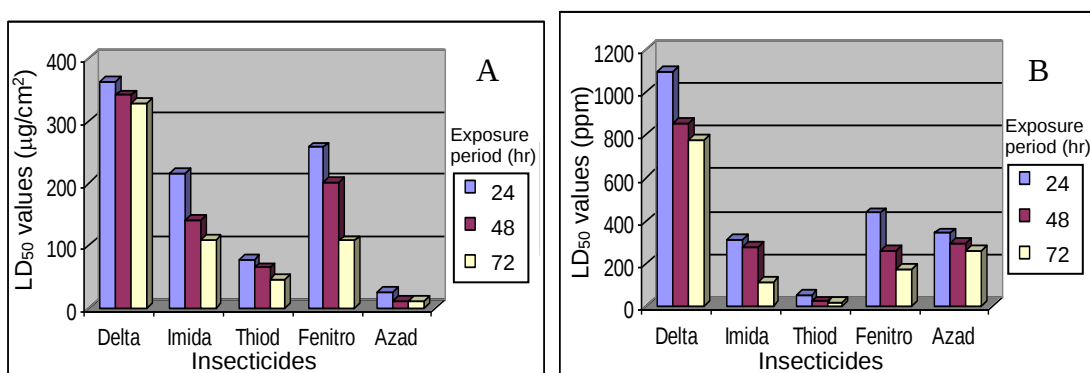


Fig106. LD₅₀ values of different insecticides on mature larvae of *A. diaperinus* in RFM (A) and TFM (B) tests.

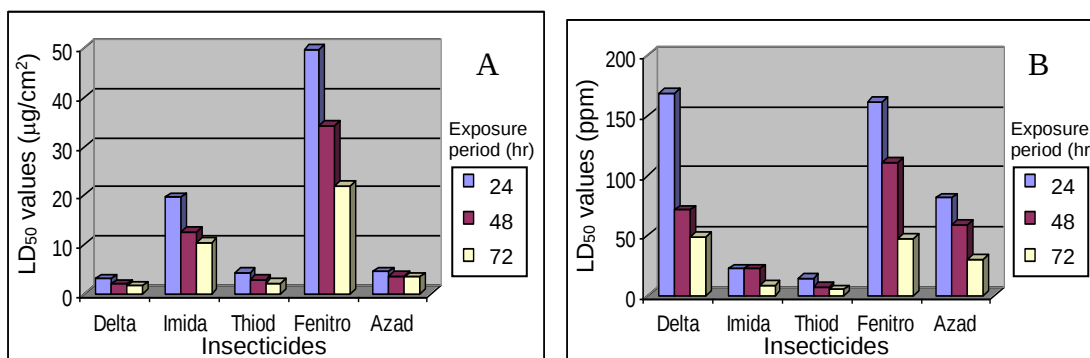


Fig107. LD₅₀ values of different insecticides on newly hatched larvae of *A. diaperinus* in RFM (A) and TFM (B) tests.

6.2 BIOASSAY WITH INSECTICIDES AND AZADIRACHTIN

Since, in recent years, there has been increased interest in natural plant-derived materials as alternative pesticides to conventional, broad-spectrum toxicants neem based biopesticides and neem extracts from the neem tree, *Azadirachta indica* Juss, have attracted considerable attention. Neem extracts have a wide range of effects against insect pests, including repellence, feeding, toxicity, sterility and growth regulator-activity (Schmutterer 1990) and low toxicity to vertebrates and natural enemy complex (Jacobson 1989, Mordue and Blackwell 1993) Moreover, allelochemicals (limonoids) present in the neem affect the biochemical and physiological process of insect system and nullify the insect detoxification mechanism thereby not allowing the pest to develop resistance (Thompson 1990, Murugan *et al.* 2000).

Moreover, as the neem possesses insect growth regulatory activity and low toxicity to birds, fish and mammals (Jacobson 1989); the neem-based biopesticides would be effective as a synergistic agent because of the fact that IGR (Nimbecidine) weakens the cuticle defense system of the larvae and easy penetration of pathogenic organism into the insect system (Murugan and Jeyabalan 1999).

Advantages of using botanical insecticides as mixtures with synthetics compounds may act synergistically (Berenbaum 1985), They may show greater overall bioactivity compared to the individual constituents (Berenbaum *et al.* 1991, Chan *et al.* 1995) and insect resistance is much less likely to develop in case of mixtures (Feng and Isman 1995). In toxicology, synergism is defined as the case where the toxicity of two compounds applied together is greater than would be expected from the sum of their individual effects. The use of synergists originally arose from the observation that sesame oil would potentiate the action of natural pyrethrins (Corbett *et al.* 1984). The first synergist was introduced in 1940. Since then many materials have appeared, but only a few are still marketed. Synergists are found in most household, livestock and pet aerosols to enhance the action of the fast knockdown insecticides pyrethrum, allethrin, and resmethrin, against flying insects. Current synergists, such as piperonyl butoxide, contain the methylenedioxyphenyl moiety; a molecule found in sesame oil and later named sesamin (Pap *et al.* 2001).

The mode of action of most synergists is that they inhibit cytochrome P450 dependent monooxygenases (P450s). This enzymes produced by

endoplasmic reticulum of cells especially in liver of mammals and in some insect tissues (e.g., fat bodies) (Becker *et al* 1984; Casida 1970). The earlier name for these enzymes was mixed-function oxidases (MFOs). These PSMOs bind the enzymes that degrade selected foreign substances, such as pyrethrum, allethrin, resmethrin or any other synergized compound. Synergists simply bind the oxidative enzymes and prevent them from degrading the toxicant (Bernard and Philogene 1993).

The toxicity of diazinon+ synergist (PBO) against cockroaches, *Pereplaneta americana* L. was found to be more than that observed in the toxicity of Diazinon alone (Rahman and Akter 2008). The toxicity of malathion+ PBO against *Rhizopertha dominica* also showed the similar activity (Rahman *et al* 2007). The mixture of chlorpyrifos and piperonyl butoxide demonstrated some synergistic effect to *A. diaperinus* (Rahman and Akter 2006).

Feeroza and Khaleqe (2005) carried out the laboratory bioassay of Coster WP (commercial preparation of *B. thuringiensis* Var. *Kurstaki*) in combination with Larvin (Thiodicarb) to determine the compatibility and interspecific relationship for synergism of two groups for management of *Helicoverpa armigera* (Hubn). In the present experiment it was also found that Larvin (Thiodicarb) in combination with azadirachtin caused the synergism for management of *A. diaperinus* in treated food method. This combination was more effective at treated food method than contact poisoning.

Celli *et al.* (1974) studied the effects of organophosphates and carbamate insecticides on *Bacillus thuringiensis* preparation against tobacco budworm larvae, *Heliothis veriscens* (F). They reported that among carbamate insecticide, the carbaryl was found to be the most synergistic among all others (Phosmet, methomyl and carbofuran). This is similar to the present result that the carbamate (Thiodicarb) in combination with azadirachtin was more synergistic than organophosphate insecticide fenitrothion.

Masood *et al.* (2001) reported that the insecticidal combination and the neem extracts at their recommended doses reduced 70.2- 95.3% pest infestation 2 days after the spray but gradually decreased to 6.5- 39.6% infestation 17 days after the spray against Jassid and Whitefly. This result is similar to the present results, where Azadirachtin in combination with Deltamethrin and Imidacloprid insecticide showed synergistic action after 72 hours on mature larvae and adults in both TFM and RFM tests.

Lower synergistic effect was found with imidacloprid in insectariums population of *Bemisia tabaci* whereas high level of synergism was observed in the field population in the same species (Kang *et al.*, 2006). The synergistic effect of PBO on active ingredients of imidacloprid varied depending on different species of insects. For example, no synergism of PBO with imidacloprid was found in the resistant strain of tobacco feeding *Myzus persicae* Sulzer (Nauen *et al.*, 1996).

In the present investigation it was observed that imidacloprid (Confidor) when used in conjunction with Azadirachtin offered significant synergism resulting low LD₅₀ values for imidacloprid in treated filter paper test against mature larvae of *A. diaperinus*. The combination of imidacloprid with azadirachtin was more effective for adults in RFM test, because imidacloprid and azadirachtin both gives a coefficient significantly greater than 100 at all ratios, which indicates a synergistic action. It was reported that imidacloprid was mainly taken up and transferred by body contact (Haagsma 2003, Saran and Rust 2007). So, larvae were more susceptible than adults at contact poisoning of imidacloprid when combined with azadirachtin.

Imidacloprid was more effective in treated food method than residual film method when combined with azadirachtin against adult *A. diaperinus*. Here co-toxicity coefficient of imidacloprid was the highest for adults in treated food method which indicated a synergistic action. But azadirachtin shows low co-toxicity coefficient value. This result indicated that imidacloprid reduced the efficacy of Azadirachtin and Azadirachtin enhanced the toxicity of imidacloprid. This is similar to the findings of Pan Luo (2010) who reported that imidacloprid reduced the efficacy of Fipronil whilst Fipronil enhanced the toxicity of imidacloprid. This is due to the fact that the feeding behavior depends upon both neural input from the insects' chemical senses (taste receptor on tarsi, mouthparts and oral cavity) and central nervous integration of this 'sensory code'. Azadirachtin stimulates specific 'deterrent' cells in chemoreceptors and also blocks the finding of 'sugar' receptor cells, which normally stimulate feeding (Blaney *et al.* 1990, Simmonds *et al.* 1990, Mordue (Luntz) *et al.* 1999). Adults were more susceptible than larvae in stomach poisoning of imidacloprid when combined with azadirachtin.

Sun and Johnson (1960) suggested that the synergistic effect of sesamex and related compounds was due to the inhibition of biological oxidation. The same mechanism of action may also occur in the combinations tested in the present investigation.

In the present study it was observed that deltamethrin was synergized by the azadirachtin at the highest levels in the ratio of 1:3 in treated food method. This was similar of the result of Bitran *et al.* 1983 who reported that, in Brazil, pyrethroid including deltamethrin was found to be highly effective efficacy when combined with piperonylbutoxide (1:5) in stored maize to control *S. zeamais* (Bitran *et al.* 1983a, b). It was also similar to the result of Coulon and Barres (1978) who reported the reproduction of *S. granarius* L could be prevented by treating wheat with 0.5 to 1.0 mg/kg deltamethrin combined with piperonylbutoxide in the ratio of 1:4 or by 0.25 to 0.5 mg/kg deltamethrin synergized in the ratio of 1:10 (Coulon and Barres 1978). It was effective against stored-product pests of maize and coffee in Brazil (Bitran and Campos 1978) including *S. granaries* and *T. castaneum* . Deltamethrin was also effective when combined with malathion to control *S. zeamais*, *R. dominica* and *Cryptolestes spp* (Xianjin *et al.* 1998). Deltamethrin plus piperonylbutoxide (2+8 mg/kg) controlled typical malathion-resistant strains of *S. oryzae*, *R. dominica*, *T. castaneum*, *Ephestia cautella* (Walk) (Bengston *et al.* 1984), *Callosobruchus maculatus* (F), *C. chinensis* (Duguet and Wu 1984). Deltamethrin (2mg/kg) plus piperonylbutoxide (10mg/kg) had proved satisfactory for the control of all major species of stored-product pests, including Malathion resistant insects, in Australian grain during 9 months storage (Bengston and Desmarchelier 1979). Fumigation followed by treatment with deltamethrin provided the best protection against *S. zeamais* and *S. cerealella*. Deltamethrin was found to be superior to malathion (Bitran *et al* 1981a,b).

Both cypermethrin and lambda- cyhalothrin are known to have a very high insecticidal activity either alone or in combination with synergist against various species of insects (Rahman 1996, Baki *et al* 2002, Rahman *et al* 2007). At high doses PBO increased the effectiveness of pyrethrin against the house flies by 300 fold (Testa and Jenner 1981, Rahman 1996). Correspondingly PBO brought down the dose level of lambda-cyhalothrin in the mixture at ratios 1:1 and greater (Rahman *et al* 2007). Kumar *et al* (1991) found the PBO as an effective synergist with natural pyrethrins and synthetic pyrethroids due to its ability to inhibit the detoxifying mechanism in the insects.

Khatun (2010) found the lowest adult emergence as recorded in the insecticide and nimbecidine combined treatment, which was probably due to higher larval and pupal

mortality because of the toxicity of cypermethrin and deltamethrin, and also in part, due to the synergistic action (Mondal 1984) of nimbidine.

In the present study it was found that deltamethrin was still the most toxic as larvaecide than adulticide. Besides, the mixture of azadirachtin and deltamethrin showed dramatic synergism at different ratios in both RFM and TFM tests after 72 hours for mature larvae. Most important of such alternatives are the pyrethroid derivatives, particularly synergized deltamethrin (Arthur 1994).

In the present study deltamethrin+ azadirachtin was the best combination for controlling of matured larvae in both RFM and TFM tests and imidacloprid+ azadirachtin was the best combination for controlling of adults in both RFM and TFM tests. Fenitrothion alone and combined with azadirachtin showed very low toxicity against both matured larvae and adults of *A. diaperinus*.

Application of low rates of pyrethroids and neonicotinoids mixed with azadirachtin will permit growers to attain the benefits of pest management strategy at reduced cost and insecticide input in the environment.

Nevertheless, avoiding the exceptions, the results of the present investigation clearly suggest the possibility of Azadirachtin as an effective synergist with both Deltamethrin and Imidacloprid against *A. diaperinus*.

Chapter-7

Summary

In the present experiments it was found that, Azadirachtin when combined with any insecticide enhanced the toxicity of insecticide. Nimbicidine 0.03% (Azadirachtin) used in the present experiment, contain a lot of oily substance, which easily affected the insects. But when combined with any insecticide these oily substances were reduced.

Single action

RFM

In RFM test with Azadirachtin 1 and 10 days old larvae of *A. diaperinus* were affected at similar doses. But in case of 20, 30 and 40 day's old larvae the LD₅₀ values were gradually increased. Adults were less affected, so the LD₅₀ value was the highest.

Deltamethrin was the best for controlling newly hatched larvae (1 day old) but Azadirachtin was the best for controlling 10, 20, 30, 40 days old larvae and adults in RFM test after 24, 48 and 72 hours exposure. The order of toxicity was Azadirachtin> Thiodicarb> Deltamethrin> Imidacloprid> Fenitrothion.

For 1 days old larvae the order of toxicity was Deltamethrin> Thiodicarb> Azadirachtin> Imidacloprid> Fenitrothion. For 10 days old larvae the order of toxicity was Azadirachtin> Deltamethrin> Imidacloprid>Thiodicarb> Fenitrothion. For 20 and 30days old larvae the order of toxicity was Azadirachtin> Thiodicarb> Fenitrothion>Imidacloprid >Deltamethrin. For 40 days old larvae the order of toxicity was Azadirachtin> Thiodicarb >Imidacloprid > Deltamethrin> Fenitrothion. For adults the order of toxicity was Azadirachtin> Thiodicarb > Deltamethrin > Fenitrothion> Imidacloprid.

TFM test

Thiodicarb was the best among all the insecticides for controlling all ages of larvae and adults of *A. diaperinus*. The order of toxicity was Thiodicarb> Imidacloprid> Fenitrothion >Azadirachtin>Deltamethrin.

For 1 days old larvae the order of toxicity was Thiodicarb> Imidacloprid> Deltamethrin> Azadirachtin>Fenitrothion. For 20, 30 and 40 days old larvae the order of toxicity was

Thiodicarb> Imidacloprid> Fenitrothion> Azadirachtin > Deltamethrin. For 10 days old larvae and adults, the order of toxicity was Thiodicarb> Imidacloprid> Azadirachtin> Fenitrothion> Deltamethrin.

The toxicity of all insecticides on all ages of larvae and adults was increased with the time in both RFM and TFM tests, so the amount of insecticides required was reduced with time

Combined action

RFM test

For mature larvae

17.844 $\mu\text{g}/\text{cm}^2$ azadirachtin was needed for 89.091% dose reduction of deltamethrin and 15.086 $\mu\text{g}/\text{cm}^2$ azadirachtin was needed for 93.089% dose reduction of imidacloprid. 41.708 $\mu\text{g}/\text{cm}^2$ azadirachtin was required for 93.908% dose reduction of thiodicarb and 58.058 $\mu\text{g}/\text{cm}^2$ azadirachtin for 89.229% dose reduction of fenitrothion for mature larvae. The co-toxicity coefficient of azadirachtin was 119.729-60.744 for deltamethrin at the ratio 1:0.1-1:0.5, 78.461-55.511 for imidacloprid at the ratio 1:1-1:5, 46.714-25.988 for thiodicarb at the ratio 1:3-1:15, 20.225-10.733 for fenitrothion at the ratio 1:1-1:8. The order of toxicity of the insecticides in combination with azadirachtin was Deltamethrin> Imidacloprid> Thiodicarb> Fenitrothion.

For adults

30.626 $\mu\text{g}/\text{cm}^2$ Azadirachtin was needed for 94.025% dose reduction of Deltamethrin and 31.23 $\mu\text{g}/\text{cm}^2$ for 92.949% dose reduction of Imidacloprid . 37.539 $\mu\text{g}/\text{cm}^2$ Azadirachtin was needed for 93.104% dose reduction of Thiodicarb and 102.562 $\mu\text{g}/\text{cm}^2$ for 89.907% dose reduction of Fenitrothion for adults. The co-toxicity coefficient of azadirachtin was 223.088-113.291 for Deltamethrin at the ratio 1:0.1-1:0.6, 312.717-111.108 for Imidacloprid at the ratio 1:0.02-1:0.2, 141.102-75.996 for Thiodicarb at the ratio 1:2-1:10, 86.493-33.833 for Fenitrothion at the ratio 1:0.05-1:0.5. The order of toxicity of the insecticides in combination with Azadirachtin was Imidacloprid> Deltamethrin> Thiodicarb> Fenitrothion.

TFM test

For mature larvae

122.024 ppm azadirachtin was needed for 93.732% dose reduction of deltamethrin and 200.492ppm of azadirachtin was needed for 94.733% dose reduction of imidacloprid. 235.457ppm azadirachtin was needed for 83.941% dose reduction of thiodicarb and 230.576ppm for 93.234% dose reduction of fenitrothion for mature larvae. The co-toxicity coefficient of azadirachtin was 24.713-20.701 for deltamethrin at the ratio 1:1-1:2.5, 17.222-12.605 for imidacloprid at the ratio 1:20-1:35, 20.225-10.733 for thiodicarb at the ratio 1:20-1:80, 13.193-10.363 for fenitrothion at the ratio 1:10-1:25. The order of toxicity of the insecticides in combination with azadirachtin was Deltamethrin> Thiodicarb> Imidacloprid> Fenitrothion.

For adults

200.159ppm azadirachtin was needed for 93.559% dose reduction of deltamethrin and 180.056ppm azadirachtin was needed for 93.241% dose reduction of imidacloprid. 254.472ppm azadirachtin was required for 84.463% dose reduction of thiodicarb and 361.794ppm for 93.748% dose reduction of fenitrothion for adults. The co-toxicity coefficient of azadirachtin was 32.253-30.609 for deltamethrin at the ratio 1:1.5-1:3, 35.361-30.351 for imidacloprid at the ratio 1:10-1:20, 35.115-24.305 for thiodicarb at the ratio 1:10-1:30, 18.138-16.135 for fenitrothion at the ratio 1:10-1:25. The order of toxicity of the insecticides in combination with azadirachtin was Imidacloprid> Thiodicarb>Deltamethrin> Fenitrothion.

The combination of deltamethrin with azadirachtin was more effective for mature larvae in RFM test, because deltamethrin and azadirachtin both gave a coefficient which was significantly greater than 100 only at the first ratio indicating a synergistic action.

The co-toxicity coefficient of azadirachtin indicates synergistic action when combined with deltamethrin and imidacloprid on adults in RFM test. So, the combination of deltamethrin and imidacloprid with azadirachtin was the most effective for controlling of adults in RFM test after 72 hours.

Azadirachtin and thiodicarb were most effective for all ages of larvae and adults in RFM and TFM tests respectively. Fenitrothion and deltamethrin were less effective in RFM and TFM tests respectively. But at combined action the deltamethrin was most effective.

Deltamethrin+ azadirachtin was the best combination for controlling of matured larvae in both RFM and TFM tests. Imidacloprid+ azadirachtin was the best combination for controlling of adults in both RFM and TFM tests. Fenitrothion alone

and combined with azadirachtin showed very low toxicity against *A. diaperinus* matured larvae and adults.

Summarizing the results with neem products under field conditions, simple aqueous and alcoholic, as well as enriched, formulated products have a high potential for pest control especially in developing countries where the raw material is present in abundance. However, to succeed, certain strategies must be followed, because the application of neem products differs from most of the synthetic compounds.

They are as follows:

1. As neem products are ultra-violet sensitive stomach insecticides, the target insects must take them up as soon as possible during feeding; the more active material they consume the better. The application of neem products should therefore coincide with the most active feeding phases of the target insects.
2. Neem products must be applied against the most sensitive larval instars of the target insects, as there are also remarkable differences in sensitivity during metamorphosis.
3. Because of their delayed effect neem products may be unsuitable if no further damage to treated plants is tolerable and if no insects should be present on plants during marketing. It can be said that perhaps unique mode of action of azadirachtin, which means its controlling effect on insects hormones, especially ecdysone, and the favorable toxicological and selective properties of neem products provide a basis for a new promising way of environmentally sound pest control with bio-rational pesticides within the framework of integrated pest management. Due to the longer residual and systemic effects pesticides based on neem are more suitable than most juvenoids

Azadirachtin from neem affects insects in a variety of different ways: as an antifeedent, insect growth regulator and sterilant. As antifeedant sensitivity varies greatly between insects the overriding efficacy of neem insecticide use lies in its physiological toxic effects. An understanding of the physiological effects of azadirachtin in neem has been reached and biochemical approaches have begun to define its mode of action at the cellular level. Further work is however required to fully understand its mode of action. It is now accepted that neem based insecticides have a wide margin of safety for both user and consumer. Increasing knowledge of how to use neem insecticides in the field is proving a solid base from which successful market penetration may be achieved.

(<http://www.ukessays.com/essays/biology/effect-of-azadirachtin-on-insects-biology-essay>.)

Chapter-8

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Chapter-9

Appendices

Appendix Table 1

Calculation of log/ Probit regression line for the dose mortality experiment in which *A. diaperinus* larvae (1 day old) were exposed to the filter paper treated with different concentrations of Azadirachtin (Exposure period= 24h).

[illegible]

Appendix Table 2

Calculation of log/ Probit regression line for the dose mortality experiment in which *A. diaperinus* larvae (10 days old) were exposed to the filter paper treated with different concentrations of Azadirachtin (Exposure period= 24h).

[illegible]

Appendix Table 3

Calculation of log/ Probit regression line for the dose mortality experiment in which *A. diaperinus* larvae (20 days old) were exposed to the filter paper treated with different concentrations of Azadirachtin (Exposure period= 24h).

[illegible]

Appendix Table 4

Calculation of log/ Probit regression line for the dose mortality experiment in which *A. diaperinus* larvae (30 days old) were exposed to the filter paper treated with different concentrations of Azadirachtin (Exposure period= 24h).

[illegible]

Appendix Table 5

Calculation of log/ Probit regression line for the dose mortality experiment in which *A. diaperinus* larvae (40 days old) were exposed to the filter paper treated with different concentrations of Azadirachtin (Exposure period= 24h).

[illegible]

Appendix Table 6

Calculation of log/ Probit regression line for the dose mortality experiment in which *A. diaperinus* adults were exposed to the filter paper treated with different concentrations of Azadirachtin (Exposure period= 24h).

[illegible]

Appendix Table 7

Calculation of log/ Probit regression line for the dose mortality experiment in which *A. diaperinus* larvae (1 day old) were exposed to the filter paper treated with different concentrations of Azadirachtin (Exposure period= 48h).

[illegible]

Appendix Table 8

Calculation of log/ Probit regression line for the dose mortality experiment in which *A. diaperinus* larvae (10 days old) were exposed to the filter paper treated with different concentrations of Azadirachtin (Exposure period= 48h).

[illegible]

Appendix Table 13

Calculation of log/ Probit regression line for the dose mortality experiment in which *A. diaperinus* larvae (1 day old) were exposed to the filter paper treated with different concentrations of Azadirachtin (Exposure period= 72h).

[illegible]

Appendix Table 14

Calculation of log/ Probit regression line for the dose mortality experiment in which *A. diaperinus* larvae (10 days old) were exposed to the filter paper treated with different concentrations of Azadirachtin (Exposure period= 72h).

[illegible]

Appendix Table 15

Calculation of log/ Probit regression line for the dose mortality experiment in which *A. diaperinus* larvae (20 days old) were exposed to the filter paper treated with different concentrations of Azadirachtin (Exposure period=72h).

[illegible]

Appendix Table 16

Calculation of log/ Probit regression line for the dose mortality experiment in which *A. diaperinus* larvae (30 days old) were exposed to the filter paper treated with different concentrations of Azadirachtin (Exposure period= 72h).

[illegible]

Appendix Table 37

Calculation of log/ Probit regression line for the dose mortality experiment in which *A. diaperinus* 1 days old larvae were exposed to the filter paper treated with different concentrations of Deltamethrin (Exposure period= 24h).

Dose µg/cm ²	Log dose	# used	# Kill	% Kill	Corr. %	Emp. probit	Expt probit	Work probit	Weight	Final probit
0.90	0.954	50	6	12	12	3.82	3.825	3.822	18.50	3.799
1.80	1.255	50	17	34	34	4.59	4.504	4.572	29.05	4.482
3.60	1.556	50	27	54	54	5.10	4.183	5.09	31.70	5.166
7.20	1.857	50	38	76	76	5.71	5.863	5.664	25.15	5.849
14.40	2.158	50	49	98	98	7.05	6.542	6.836	13.45	6.534
Y = 1.631496 + 2.271258 X LOG LD ₅₀ IS 1.4831 LD ₅₀ IS 3.041587					CHI-SQUARED IS 2.525597 WITH 3 DEGREES OF FREEDOM NO SIG HETEROGENEITY 95% CONF LIMITS ARE 2.530506 TO 3.655889					

Appendix Table 38

Calculation of log/ Probit regression line for the dose mortality experiment in which *A. diaperinus* 10 days old larvae were exposed to the filter paper treated with different concentrations of Deltamethrin (Exposure period= 24h).

Dose µg/cm ²	Log dose	# used	# Kill	% Kill	Corr. %	Emp. probit	Expt probit	Work probit	Weight	Final probit
7.20	0.857	50	5	10	10	3.72	3.782	3.720	16.8	3.772
14.40	1.158	50	17	34	34	4.59	4.445	4.600	27.90	4.447
28.80	1.459	50	26	52	52	5.05	5.108	5.040	31.70	5.121
57.60	1.760	50	37	74	74	5.64	5.771	5.638	26.60	5.795
115.20	2.061	50	48	96	96	6.75	6.435	6.692	15.10	6.470
Y = 1.851606 + 2.240287 X LOG LD ₅₀ IS 1.405353 LD ₅₀ IS 25.43039					CHI-SQUARED IS 2.314827 WITH 3 DEGREES OF FREEDOM NO SIG HETEROGENEITY 95% CONF LIMITS ARE 21.10275 TO 30.64552					

Appendix Table 39

Calculation of log/ Probit regression line for the dose mortality experiment in which *A. diaperinus* 20 days old larvae were exposed to the filter paper treated with different concentrations of Deltamethrin (Exposure period= 24h).

Dose µg/cm ²	Log dose	# used	# Kill	% Kill	Corr. %	Emp. probit	Expt probit	Work probit	Weight	Final probit
88.40	1.946	50	8	16	16	4.01	3.813	4.026	18.50	3.800
123.76	2.092	50	17	34	34	4.59	4.634	4.578	30.05	4.640
159.12	2.201	50	26	52	52	5.05	5.247	5.072	31.55	5.267
194.48	2.289	50	37	74	74	5.64	5.737	5.638	26.60	5.768
229.80	2.361	50	48	96	96	6.75	6.145	6.546	20.25	6.186
Y = -7.388528 + 5.748341 X LOG LD ₅₀ IS 2.155149 LD ₅₀ IS 142.9383					CHI-SQUARED IS 5.346268 WITH 3 DEGREES OF FREEDOM NO SIG HETEROGENEITY 95% CONF LIMITS ARE 133.1168 TO 153.4844					

Appendix Table 40

Calculation of log/ Probit regression line for the dose mortality experiment in which *A. diaperinus* 30 days old larvae were exposed to the filter paper treated with different concentrations of Deltamethrin (Exposure period= 24h).

Dose µg/cm ²	Log dose	# used	# Kill	% Kill	Corr. %	Emp. probit	Expt probit	Work probit	Weight	Final probit
159.12	2.201	50	6	12	12	3.82	3.695	3.864	15.10	3.696
194.48	2.289	50	14	28	28	4.42	4.466	4.420	27.90	4.443
229.84	2.361	50	25	50	50	5.00	5.107	4.990	31.70	5.065
265.20	2.424	50	33	66	66	5.41	5.656	5.400	27.90	5.598
300.56	2.478	50	46	92	92	6.41	6.136	6.362	20.25	6.064
Y = -15.18247 + 8.574482 X LOG LD ₅₀ IS 2.353783 LD ₅₀ IS 225.8308					CHI-SQUARED IS 3.516327 WITH 3 DEGREES OF FREEDOM NO SIG HETEROGENEITY 95% CONF LIMITS ARE 215.3414 TO 236.8311					

Appendix Table 41

Calculation of log/ Probit regression line for the dose mortality experiment in which *A. diaperinus* 40 days old larvae were exposed to the filter paper treated with different concentrations of Deltamethrin (Exposure period= 24h).

Dose $\mu\text{g}/\text{cm}^2$	Log dose	# used	# Kill	% Kill	Corr. %	Emp. probit	Expt probit	Work probit	Weight	Final probit
300.56	2.478	50	7	14	14	3.92	3.810	3.924	18.50	3.804
335.92	2.526	50	16	32	32	4.53	4.530	4.516	29.05	4.521
371.28	2.570	50	27	54	54	5.10	5.178	5.09	31.70	5.167
406.64	2.609	50	36	72	72	5.58	5.768	5.574	26.60	5.754
442	2.645	50	48	96	96	6.75	6.308	6.598	16.80	6.292
Y = -33.00723 + 14.85563 X LOG LD ₅₀ IS 2.55844 LD ₅₀ IS 361.776					CHI-SQUARED IS 2.932686 WITH 3 DEGREES OF FREEDOM NO SIG HETEROGENEITY 95% CONF LIMITS ARE 351.9246 TO 371.9031					

Appendix Table 42

37. Calculation of log/ Probit regression line for the dose mortality experiment in which *A. diaperinus* adults were exposed to the filter paper treated with different concentrations of Deltamethrin (Exposure period= 24h).

Dose $\mu\text{g}/\text{cm}^2$	Log dose	# used	# Kill	% Kill	Corr. %	Emp. probit	Expt probit	Work probit	Weight	Final probit
707.21	2.850	50	4	8	8	3.59	3.421	3.63	11.90	3.440
972.41	2.988	50	13	26	26	4.36	4.336	4.362	26.60	4.338
1237.62	3.093	50	23	46	46	4.90	5.029	4.900	31.85	5.018
1502.82	3.177	50	32	64	64	5.36	5.587	5.332	29.05	5.566
1768.03	3.247	50	47	94	94	6.55	6.053	6.374	21.95	6.024
Y = -15.06803 + 6.495089 X LOG LD ₅₀ IS 3.089724 LD ₅₀ IS 1229.488					CHI-SQUARED IS 5.15667 WITH 3 DEGREES OF FREEDOM NO SIG HETEROGENEITY 95% CONF LIMITS ARE 1154.285 TO 1309.589					

Appendix Table 43

Calculation of log/ Probit regression line for the dose mortality experiment in which *A. diaperinus* 1 days old larvae were exposed to the filter paper treated with different concentrations of Deltamethrin (Exposure period= 48h).

Dose $\mu\text{g}/\text{cm}^2$	Log dose	# used	# Kill	% Kill	Corr. %	Emp. probit	Expt probit	Work probit	Weight	Final probit
0.45	0.651	50	4	8	8	3.59	3.632	3.596	15.10	3.659
0.90	0.952	50	13	26	26	4.36	4.286	4.354	25.15	4.292
1.80	1.253	50	24	48	48	4.95	4.940	4.940	31.70	4.926
3.60	1.554	50	35	70	70	5.52	5.594	5.500	29.05	5.559
7.20	1.855	50	45	90	90	6.28	6.248	6.230	18.50	6.193
Y = 2.288426 + 2.104413 X LOG LD ₅₀ IS 1.288518 LD ₅₀ IS 1.943203					CHI-SQUARED IS .2896042 WITH 3 DEGREES OF FREEDOM NO SIG HETEROGENEITY 95% CONF LIMITS ARE 1.596973 TO 2.364497					

Appendix Table 44

Calculation of log/ Probit regression line for the dose mortality experiment in which *A. diaperinus* 10 days old larvae were exposed to the filter paper treated with different concentrations of Deltamethrin (Exposure period= 48h).

Dose $\mu\text{g}/\text{cm}^2$	Log dose	# used	# Kill	% Kill	Corr. %	Emp. probit	Expt probit	Work probit	Weight	Final probit
3.854	0.554	50	5	10	10	3.72	3.742	3.72	16.80	3.785
7.168	0.855	50	16	32	32	4.53	4.379	4.554	26.60	4.392
14.336	1.156	50	23	46	46	4.90	4.016	4.900	31.85	4.998
28.672	1.457	50	35	70	70	5.52	5.653	5.52	27.90	5.604
57.344	1.758	50	46	92	92	6.41	6.290	6.332	18.50	6.211
Y = 2.668379 + 2.014452 X LOG LD ₅₀ IS 1.157447 LD ₅₀ IS 14.36968					CHI-SQUARED IS 1.5494 WITH 3 DEGREES OF FREEDOM NO SIG HETEROGENEITY 95% CONF LIMITS ARE 11.7273 TO 17.60745					

Appendix Table 53

Calculation of log/ Probit regression line for the dose mortality experiment in which *A. diaperinus* 40 days old larvae were exposed to the filter paper treated with different concentrations of Deltamethrin (Exposure period= 72h).

Dose µg/cm²	Log dose	# used	# Kill	% Kill	Corr. %	Emp. probit	Expt probit	Work probit	Weight	Final probit
265.20	2.424	50	5	10	10	3.72	3.750	3.720	16.80	3.753
300.56	2.478	50	18	36	36	4.64	4.494	4.660	27.90	4.497
335.92	2.526	50	25	50	50	5.00	5.155	4.990	31.70	5.157
371.28	2.570	50	38	76	76	5.71	5.749	5.702	26.60	5.752
406.64	2.609	50	47	94	94	6.55	6.290	6.434	18.50	6.292
Y = -29.38725 + 13.67445 X LOG LD ₅₀ IS 2.514709 LD ₅₀ IS 327.1215					CHI-SQUARED IS 2.068199 WITH 3 DEGREES OF FREEDOM NO SIG HETEROGENEITY 95% CONF LIMITS ARE 317.376 TO 337.166					

Appendix Table54

Calculation of log/ Probit regression line for the dose mortality experiment in which *A. diaperinus* adults were exposed to the filter paper treated with different concentrations of Deltamethrin (Exposure period= 72h).

Dose µg/cm²	Log dose	# used	# Kill	% Kill	Corr. %	Emp. probit	Expt probit	Work probit	Weight	Final probit
442	2.645	50	7	14	14	3.92	3.760	3.952	16.80	3.743
707.21	2.850	50	16	32	32	4.53	4.669	4.524	30.05	4.640
972.41	2.988	50	28	56	56	5.15	5.284	5.176	31.35	5.247
1237.62	3.093	50	36	72	72	5.58	5.751	5.574	26.60	5.707
1502.82	3.177	50	46	92	92	6.41	6.126	6.362	20.25	6.078
Y = -7.874621 + 4.391804 X LOG LD ₅₀ IS 2.931511 LD ₅₀ IS 854.1034					CHI-SQUARED IS 3.401383 WITH 3 DEGREES OF FREEDOM NO SIG HETEROGENEITY 95% CONF LIMITS ARE 777.9675 TO 937.6903					

Appendix Table 55

Calculation of log/ Probit regression line for the dose mortality experiment in which *A. diaperinus* 1 day old larvae were exposed to the flour medium treted with different concentrations of Deltamethrin (Exposure period= 24h).

Dose ppm	Log dose	# used	# Kill	% Kill	Corr. %	Emp. probit	Expt probit	Work probit	Weight	Final probit
40	1.602	50	4	8	8	3.59	3.423	3.630	11.90	3.355
80	1.903	50	11	22	22	4.23	4.474	4.24	27.90	4.424
160	2.204	50	24	48	48	4.95	4.999	4.940	31.70	4.958
320	2.505	50	35	70	70	5.52	5.524	5.500	29.05	5.492
640	2.806	50	44	88	88	6.18	6.050	6.128	21.95	6.027
Y = 1.046537 + 1.774727 X LOG LD ₅₀ IS 2.227646 LD ₅₀ IS 168.9062					CHI-SQUARED IS 2.07827 WITH 3 DEGREES OF FREEDOM NO SIG HETEROGENEITY 95% CONF LIMITS ARE 134.234 TO 212.5343					

Appendix Table 56

Calculation of log/ Probit regression line for the dose mortality experiment in which *A. diaperinus* 10 days old larvae were exposed to the flour medium treated with different concentrations of Deltamethrin (Exposure period= 24h).

Dose ppm	Log dose	# used	# Kill	% Kill	Corr. %	Emp. probit	Expt probit	Work probit	Weight	Final probit
40	1.602	50	2	4	4	3.25	3.011	3.320	6.55	3.023
80	1.903	50	8	16	16	4.01	4.146	4.018	23.55	4.152
160	2.204	50	19	38	38	4.69	4.713	4.688	30.80	4.717
320	2.505	50	31	62	62	5.31	5.281	5.332	31.35	5.282
640	2.806	50	41	82	82	5.92	5.848	5.868	25.15	5.846
Y = .5829625 + 1.875665 X LOG LD ₅₀ IS 2.354918 LD ₅₀ IS 226.4216					CHI-SQUARED IS 1.119812 WITH 3 DEGREES OF FREEDOM NO SIG HETEROGENEITY 95% CONF LIMITS ARE 180.9819 TO 283.2699					

Appendix Table 57

Calculation of log/ Probit regression line for the dose mortality experiment in which *A. diaperinus* 20 days old larvae were exposed to the flour medium treated with different concentrations of Deltamethrin (Exposure period= 24h).

<i>Dose ppm</i>	<i>Log dose</i>	<i># used</i>	<i># Kill</i>	<i>% Kill</i>	<i>Corr. %</i>	<i>Emp. probit</i>	<i>Expt probit</i>	<i>Work probit</i>	<i>Weight</i>	<i>Final probit</i>
600	2.778	50	7	14	14	3.92	3.660	3.998	15.10	3.665
800	2.903	50	13	26	26	4.36	4.488	4.360	27.90	4.488
1000	2.999	50	24	48	48	4.95	5.130	4.94	31.70	5.126
1200	3.079	50	36	72	72	5.58	5.655	5.580	27.90	5.647
1400	3.146	50	47	94	94	6.55	6.098	6.374	21.95	6.088
Y = -14.62587 + 6.583902 X LOG LD ₅₀ IS 2.980888 LD ₅₀ IS 956.9463					CHI-SQUARED IS 5.144303 WITH 3 DEGREES OF FREEDOM NO SIG HETEROGENEITY 95% CONF LIMITS ARE 899.5316 TO 1018.025					

Appendix Table 58

Calculation of log/ Probit regression line for the dose mortality experiment in which *A. diaperinus* 30 days old larvae were exposed to the flour medium treated with different concentrations of Deltamethrin (Exposure period= 24h).

<i>Dose ppm</i>	<i>Log dose</i>	<i># used</i>	<i># Kill</i>	<i>% Kill</i>	<i>Corr. %</i>	<i>Emp. probit</i>	<i>Expt probit</i>	<i>Work probit</i>	<i>Weight</i>	<i>Final probit</i>
600	2.778	50	4	8	8	3.59	3.426	3.630	11.90	3.345
800	2.903	50	10	20	20	4.16	4.288	4.150	25.15	4.230
1000	2.999	50	21	42	42	4.80	4.957	4.790	31.70	4.917
1200	3.079	50	33	66	66	5.41	5.503	5.388	29.05	5.478
1400	3.146	50	44	88	88	6.18	5.965	6.174	23.55	5.952
Y = -16.33951 + 7.085431 X LOG LD ₅₀ IS 3.011745 LD ₅₀ IS 1027.412					CHI-SQUARED IS 3.029312 WITH 3 DEGREES OF FREEDOM NO SIG HETEROGENEITY 95% CONF LIMITS ARE 969.6004 TO 1088.669					

Appendix Table 59

Calculation of log/ Probit regression line for the dose mortality experiment in which *A. diaperinus* 40 day old larvae were exposed to the flour medium treated with different concentrations of Deltamethrin (Exposure period= 24h).

<i>Dose ppm</i>	<i>Log dose</i>	<i># used</i>	<i># Kill</i>	<i>% Kill</i>	<i>Corr. %</i>	<i>Emp. probit</i>	<i>Expt probit</i>	<i>Work probit</i>	<i>Weight</i>	<i>Final probit</i>
600	2.778	50	2	4	4	3.25	3.179	3.268	7.70	3.111
800	2.903	50	8	16	16	4.01	4.053	3.996	21.95	4.013
1000	2.999	50	18	36	36	4.64	4.731	4.636	30.80	4.713
1200	3.079	50	30	60	60	5.25	5.284	5.280	31.35	5.284
1400	3.146	50	40	80	80	5.85	5.753	5.830	26.60	5.768
Y = -16.94525 + 7.219374 X LOG LD ₅₀ IS 3.039772 LD ₅₀ IS 1095.903					CHI-SQUARED IS .4825821 WITH 3 DEGREES OF FREEDOM NO SIG HETEROGENEITY 95% CONF LIMITS ARE 1033.803 TO 1161.731					

Appendix Table 60

Calculation of log/ Probit regression line for the dose mortality experiment in which *A. diaperinus* adults were exposed to the flour medium which treated with different concentrations of Deltamethrin (Exposure period= 24h).

<i>Dose ppm</i>	<i>Log dose</i>	<i># used</i>	<i># Kill</i>	<i>% Kill</i>	<i>Corr. %</i>	<i>Emp. probit</i>	<i>Expt probit</i>	<i>Work probit</i>	<i>Weight</i>	<i>Final probit</i>
1000	2.999	50	4	8	8	3.59	3.398	3.678	10.40	3.305
1500	3.176	50	10	20	20	4.16	4.329	4.170	26.60	4.266
2000	3.301	50	21	42	42	4.80	4.989	4.790	31.70	4.948
2500	3.398	50	33	66	66	5.41	5.502	5.388	29.05	5.477
3000	3.477	50	44	88	88	6.18	5.921	6.174	23.55	5.909
Y = -13.06815 + 5.457717 X LOG LD ₅₀ IS 3.310569 LD ₅₀ IS 2044.416					CHI-SQUARED IS 4.369385 WITH 3 DEGREES OF FREEDOM NO SIG HETEROGENEITY 95% CONF LIMITS ARE 1896.433 TO 2203.944					

Appendix Table 61

Calculation of log/ Probit regression line for the dose mortality experiment in which *A. diaperinus* 1 day old larvae were exposed to the flour medium treated with different concentrations of Deltamethrin (Exposure period= 48h).

[illegible]

Appendix Table 62

Calculation of log/ Probit regression line for the dose mortality experiment in which *A. diaperinus* 10 days old larvae were exposed to the flour medium treted with different concentrations of Deltamethrin (Exposure period= 48h).

[illegible]

Appendix Table 63

Calculation of log/ Probit regression line for the dose mortality experiment in which *A. diaperinus* 20 days old larvae were exposed to the flour medium treated with different concentrations of Deltamethrin (Exposure period= 48h).

[illegible]

Appendix Table 64

Calculation of log/ Probit regression line for the dose mortality experiment in which *A. diaperinus* 30 days old larvae were exposed to the flour medium treted with different concentrations of Deltamethrin (Exposure period= 48h).

[illegible]

Appendix Table 65

Calculation of log/ Probit regression line for the dose mortality experiment in which *A. diaperinus* 40 days old larvae were exposed to the flour medium treated with different concentrations of Deltamethrin (Exposure period= 48h).

Dose ppm	Log dose	# used	# Kill	% Kill	Corr. %	Emp. probit	Expt probit	Work probit	Weight	Final probit
400	2.602	50	3	6	6	3.45	3.335	3.466	10.40	3.257
600	2.778	50	10	20	20	4.16	4.234	4.15	25.15	4.185
800	2.903	50	19	38	38	4.69	4.872	4.708	31.35	4.843
1000	2.999	50	32	64	64	5.36	5.367	5.344	30.80	5.354
1200	3.079	50	41	82	82	5.92	5.772	5.894	26.60	5.771
Y = -10.45696 + 5.27042 X LOG LD ₅₀ IS 2.932776 LD ₅₀ IS 856.5946					CHI-SQUARED IS 1.456596 WITH 3 DEGREES OF FREEDOM NO SIG HETEROGENEITY 95% CONF LIMITS ARE 792.7374 TO 925.5958					

Appendix Table 66

Calculation of log/ Probit regression line for the dose mortality experiment in which *A. diaperinus* adults were exposed to the flour medium which treated with different concentrations of Deltamethrin (Exposure period= 48h).

Dose ppm	Log dose	# used	# Kill	% Kill	Corr. %	Emp. probit	Expt probit	Work probit	Weight	Final probit
500	2.699	50	4	8	8	3.59	3.421	3.63	11.90	3.352
1000	2.999	50	13	26	26	4.36	4.454	4.36	27.90	4.390
1500	3.176	50	20	40	40	4.75	5.058	4.75	31.85	4.997
2000	3.301	50	32	64	64	5.36	5.487	5.348	30.05	4.428
2500	3.398	50	44	88	88	6.18	5.820	6.072	25.15	5.762
Y = -5.953027 + 3.447704 X LOG LD ₅₀ IS 3.176905 LD ₅₀ IS 1502.814					CHI-SQUARED IS 5.497494 WITH 3 DEGREES OF FREEDOM NO SIG HETEROGENEITY 95% CONF LIMITS ARE 1337.702 TO 1688.304					

Appendix Table 67

Calculation of log/ Probit regression line for the dose mortality experiment in which *A. diaperinus* 1 day old larvae were exposed to the flour medium treated with different concentrations of Deltamethrin (Exposure period= 72h).

Dose ppm	Log dose	# used	# Kill	% Kill	Corr. %	Emp. probit	Expt probit	Work probit	Weight	Final probit
20	1.301	50	8	16	16	4.01	3.958	4.016	20.25	3.974
40	1.602	50	16	32	32	4.53	4.423	4.54	27.90	4.423
80	1.903	50	27	54	54	5.10	5.353	5.084	30.80	5.321
160	2.204	50	38	76	76	5.71	5.818	5.664	25.15	5.770
320	2.505	50	48	96	96	6.75	6.282	6.536	18.50	6.219
Y = 2.482739 + 1.491464 X LOG LD ₅₀ IS 1.687779 LD ₅₀ IS 48.72798					CHI-SQUARED IS 4.289055 WITH 3 DEGREES OF FREEDOM NO SIG HETEROGENEITY 95% CONF LIMITS ARE 36.95286 TO 64.2553					

Appendix Table 68

Calculation of log/ Probit regression line for the dose mortality experiment in which *A. diaperinus* 10 day old larvae were exposed to the flour medium treated with different concentrations of Deltamethrin (Exposure period= 72h).

Dose ppm	Log dose	# used	# Kill	% Kill	Corr. %	Emp. probit	Expt probit	Work probit	Weight	Final probit
20	1.301	50	6	12	12	3.82	3.801	3.822	18.50	3.814
40	1.602	50	14	28	28	4.42	4.272	4.422	25.15	4.268
80	1.903	50	24	48	48	4.95	5.212	4.968	31.35	5.176
160	2.204	50	35	70	70	5.52	5.682	5.52	27.90	5.631
320	2.505	50	46	92	92	6.41	6.153	6.362	20.25	6.085
Y = 2.306036 + 1.508338 X LOG LD ₅₀ IS 1.786048 LD ₅₀ IS 61.10099					CHI-SQUARED IS 3.856369 WITH 3 DEGREES OF FREEDOM NO SIG HETEROGENEITY 95% CONF LIMITS ARE 46.6455 TO 80.03626					

Appendix Table 69

Calculation of log/ Probit regression line for the dose mortality experiment in which *A. diaperinus* 20 day old larvae were exposed to the flour medium treated with different concentrations of Deltamethrin (Exposure period= 72h).

Dose ppm	Log dose	# used	# Kill	% Kill	Corr. %	Emp. probit	Expt probit	Work probit	Weight	Final probit
400	2.602	50	8	16	16	4.01	3.796	4.068	16.80	3.813
600	2.778	50	19	38	38	4.69	4.695	4.686	30.05	4.694
800	2.903	50	25	50	50	5.00	5.333	4.98	30.80	5.318
1000	2.999	50	39	78	78	5.77	5.828	5.732	25.15	5.803
1200	3.079	50	49	98	98	7.05	6.231	6.638	18.50	6.198
Y = -9.194282 + 4.998991 X LOG LD ₅₀ IS 2.83943 LD ₅₀ IS 690.9224					CHI-SQUARED IS 8.313538 WITH 3 DEGREES OF FREEDOM VARIANCE HAS BEEN ADJUSTED FOR HETEROGENEITY 95% CONF LIMITS ARE 600.2 TO 795.3577					

Appendix Table 70

Calculation of log/ Probit regression line for the dose mortality experiment in which *A. diaperinus* 30 days old larvae were exposed to the flour medium treated with different concentrations of Deltamethrin (Exposure period= 72h).

Dose ppm	Log dose	# used	# Kill	% Kill	Corr. %	Emp. probit	Expt probit	Work probit	Weight	Final probit
400	2.602	50	6	12	12	3.82	3.631	3.864	15.10	3.616
600	2.778	50	17	34	34	4.59	2.547	4.572	29.05	4.550
800	2.903	50	23	46	46	4.90	5.198	4.890	31.70	5.211
1000	2.999	50	37	74	74	5.64	5.702	5.638	26.60	5.725
1200	3.079	50	48	96	96	6.75	6.113	6.546	20.25	6.145
Y = -10.17187 + 5.299066 X LOG LD ₅₀ IS 2.863122 LD ₅₀ IS 729.6625					CHI-SQUARED IS 7.680023 WITH 3 DEGREES OF FREEDOM NO SIG HETEROGENEITY 95% CONF LIMITS ARE 675.0147 TO 788.7345					

Appendix Table 71

Calculation of log/ Probit regression line for the dose mortality experiment in which *A. diaperinus* 40 days old larvae were exposed to the flour medium treated with different concentrations of Deltamethrin (Exposure period= 72h).

Dose ppm	Log dose	# used	# Kill	% Kill	Corr. %	Emp. probit	Expt probit	Work probit	Weight	Final probit
400	2.602	50	5	10	10	3.72	3.608	3.73	15.10	3.565
600	2.778	50	15	30	30	4.48	4.475	4.48	27.90	4.438
800	2.903	50	21	42	42	4.80	5.090	4.80	31.85	5.058
1000	2.999	50	35	70	70	5.52	5.568	5.500	29.05	5.539
1200	3.079	50	44	88	88	6.18	5.958	6.174	23.55	5.932
Y = -9.342322 + 4.960473 X LOG LD ₅₀ IS 2.891322 LD ₅₀ IS 778.612					CHI-SQUARED IS 4.011131 WITH 3 DEGREES OF FREEDOM NO SIG HETEROGENEITY 95% CONF LIMITS ARE 718.3122 TO 843.9758					

Appendix Table 72

Calculation of log/ Probit regression line for the dose mortality experiment in which *A. diaperinus* adults were exposed to the flour medium which treated with different concentrations of Deltamethrin (Exposure period= 72h)

Dose ppm	Log dose	# used	# Kill	% Kill	Corr. %	Emp. probit	Expt probit	Work probit	Weight	Final probit
500	2.699	50	7	14	14	3.92	3.710	3.952	16.80	3.682
1000	2.999	50	17	34	34	4.59	4.744	4.584	30.80	4.685
1500	3.176	50	26	52	52	5.05	5.348	5.032	30.80	5.272
2000	3.301	50	36	72	72	5.58	5.777	5.574	26.60	5.688
2500	3.398	50	47	94	94	6.55	6.110	6.454	20.25	6.010
Y = -5.308184 + 3.331086 X LOG LD ₅₀ IS 3.094542 LD ₅₀ IS 1243.201					CHI-SQUARED IS 7.628441 WITH 3 DEGREES OF FREEDOM NO SIG HETEROGENEITY 95% CONF LIMITS ARE 1099.583 TO 1405.579					

[illegible][illegible][illegible]

Appendix Table 89

Calculation of log/ Probit regression line for the dose mortality experiment in which *A. diaperinus* 1 day old larvae were exposed to the treted filter paper with different concentrations of Imidacloprid (Exposure period= 24h).

Dose µg/cm²	Log dose	# used	# Kill	% Kill	Corr. %	Emp. probit	Expt probit	Work probit	Weight	Final probit
5.81	0.764	50	6	12	12	3.82	3.825	3.822	18.50	3.799
11.61	1.064	50	17	34	34	4.59	4.504	4.572	29.05	4.482
23.21	1.365	50	27	54	54	5.10	5.183	5.09	31.70	5.166
46.42	1.667	50	38	76	76	5.71	5.863	5.664	25.15	5.850
92.83	1.968	50	49	98	98	7.05	6.542	6.836	13.45	6.534
Y = 2.062727 + 2.272356 X LOG LD ₅₀ IS 1.292612 LD ₅₀ IS 19.61605					CHI-SQUARED IS 2.523705 WITH 3 DEGREES OF FREEDOM NO SIG HETEROGENEITY 95% CONF LIMITS ARE 16.32139 TO 23.57577					

Appendix Table 90

Calculation of log/ Probit regression line for the dose mortality experiment in which *A. diaperinus* 10 day old larvae were exposed to the treted filter paper with different concentrations of Imidacloprid (Exposure period= 24h).

Dose µg/cm²	Log dose	# used	# Kill	% Kill	Corr. %	Emp. probit	Expt probit	Work probit	Weight	Final probit
9.67	0.985	50	5	10	10	3.72	3.782	3.72	16.80	3.772
19.34	1.286	50	17	34	34	4.59	4.445	4.60	27.90	4.446
38.68	1.587	50	26	52	52	5.05	5.108	5.04	31.70	5.121
77.36	1.888	50	37	74	74	5.64	5.771	5.638	26.60	5.795
154.71	2.189	50	48	96	96	6.75	6.434	6.692	15.10	6.469
Y = 1.564603 + 2.240317 X LOG LD ₅₀ IS 1.533443 LD ₅₀ IS 34.15409					CHI-SQUARED IS 2.315109 WITH 3 DEGREES OF FREEDOM NO SIG HETEROGENEITY 95% CONF LIMITS ARE 28.34195 TO 41.15814					

Appendix Table 91

Calculation of log/ Probit regression line for the dose mortality experiment in which *A. diaperinus* 20 day old larvae were exposed to the treted filter paper with different concentrations of Imidacloprid (Exposure period= 24h).

Dose µg/cm²	Log dose	# used	# Kill	% Kill	Corr. %	Emp. probit	Expt probit	Work probit	Weight	Final probit
23.21	1.365	50	8	16	16	4.01	3.933	4.016	20.25	3.928
46.42	1.667	50	17	34	34	4.59	4.542	4.572	29.05	4.538
92.83	1.967	50	26	52	52	5.05	5.152	5.04	31.70	5.148
185.65	2.268	50	37	74	74	5.64	5.761	5.638	26.60	5.758
371.29	2.569	50	48	96	96	6.75	6.371	6.598	16.80	6.368
Y = 1.160968 + 2.026337 X LOG LD ₅₀ IS 1.894568 LD ₅₀ IS 78.44539					CHI-SQUARED IS 1.831223 WITH 3 DEGREES OF FREEDOM NO SIG HETEROGENEITY 95% CONF LIMITS ARE 64.13582 TO 95.94765					

Appendix Table 92

Calculation of log/ Probit regression line for the dose mortality experiment in which *A. diaperinus* 30 day old larvae were exposed to the treted filter paper with different concentrations of Imidacloprid (Exposure period= 24h).

Dose µg/cm²	Log dose	# used	# Kill	% Kill	Corr. %	Emp. probit	Expt probit	Work probit	Weight	Final probit
46.42	1.667	50	6	12	12	3.82	3.778	3.836	16.80	3.811
92.83	1.967	50	14	28	28	4.42	4.394	4.426	26.60	4.398
185.65	2.268	50	25	50	50	5.00	5.011	5.00	31.85	4.985
371.29	2.569	50	33	66	66	5.41	5.629	5.40	27.90	5.572
742.58	2.870	50	46	92	92	6.41	6.246	6.332	18.50	6.159
Y = .5605683 + 1.95038 X LOG LD ₅₀ IS 2.276188 LD ₅₀ IS 188.881					CHI-SQUARED IS 1.41732 WITH 3 DEGREES OF FREEDOM NO SIG HETEROGENEITY 95% CONF LIMITS ARE 153.1334 TO 232.9736					

Appendix Table 93

Calculation of log/ Probit regression line for the dose mortality experiment in which *A. diaperinus* 40 days old larvae were exposed to the treated filter paper with different concentrations of Imidacloprid (Exposure period= 24h).

[illegible]

Appendix Table 94

Calculation of log/ Probit regression line for the dose mortality experiment in which *A. diaperinus* adults were exposed to the treated filter paper with different concentrations of Imidacloprid (Exposure period= 24h).

[illegible]

Appendix Table 95

Calculation of log/ Probit regression line for the dose mortality experiment in which *A. diaperinus* 1 day old larvae were exposed to the treated filter paper with different concentrations of Imidacloprid (Exposure period= 48h).

[illegible]

Appendix Table 96

Calculation of log/ Probit regression line for the dose mortality experiment in which *A. diaperinus* 10 day old larvae were exposed to the treated filter paper with different concentrations of Imidacloprid (Exposure period= 48h).

[illegible]

Appendix Table 97

Calculation of log/ Probit regression line for the dose mortality experiment in which *A. diaperinus* 20 days old larvae were exposed to the treted filter paper with different concentrations of Imidacloprid (Exposure period= 48h).

Dose µg/cm²	Log dose	# used	# Kill	% Kill	Corr. %	Emp. probit	Expt probit	Work probit	Weight	Final probit
11.61	1.064	50	3	6	6	3.45	3.448	3.45	11.90	3.451
23.21	1.365	50	10	20	20	4.16	4.113	4.17	23.55	4.110
46.42	1.667	50	20	40	40	4.75	4.779	4.74	30.80	4.768
92.83	1.967	50	32	64	64	5.36	5.445	5.348	30.05	5.427
185.65	2.268	50	44	88	88	6.18	6.112	6.178	20.25	6.085
Y = 1.122405 + 2.187714 X LOG LD ₅₀ IS 1.772442 LD ₅₀ IS 59.21636					CHI-SQUARED IS .4705124 WITH 3 DEGREES OF FREEDOM NO SIG HETEROGENEITY 95% CONF LIMITS ARE 48.84561 TO 71.789					

Appendix Table 98

Calculation of log/ Probit regression line for the dose mortality experiment in which *A. diaperinus* 30 days old larvae were exposed to the treted filter paper with different concentrations of Imidacloprid (Exposure period= 48h).

Dose µg/cm²	Log dose	# used	# Kill	% Kill	Corr. %	Emp. probit	Expt probit	Work probit	Weight	Final probit
23.21	1.365	50	4	8	8	3.59	3.671	3.596	15.10	3.718
46.42	1.667	50	13	26	26	4.36	4.270	4.354	25.15	4.295
92.83	1.967	50	24	48	48	4.95	4.867	4.968	31.35	4.872
185.65	2.268	50	32	64	64	5.36	5.465	5.348	30.05	5.449
371.29	2.569	50	43	86	86	6.08	6.064	6.046	21.95	6.026
Y = 1.099379 + 1.917513 X LOG LD ₅₀ IS 2.034209 LD ₅₀ IS 108.1954					CHI-SQUARED IS .916008 WITH 3 DEGREES OF FREEDOM NO SIG HETEROGENEITY 95% CONF LIMITS ARE 87.52403 TO 133.7491					

Appendix Table 99

Calculation of log/ Probit regression line for the dose mortality experiment in which *A. diaperinus* 40 days old larvae were exposed to the treted filter paper with different concentrations of Imidacloprid (Exposure period= 48h).

Dose µg/cm²	Log dose	# used	# Kill	% Kill	Corr. %	Emp. probit	Expt probit	Work probit	Weight	Final probit
30.94	1.490	50	2	4	4	3.25	3.525	3.288	13.45	3.508
61.88	1.791	50	13	26	26	4.36	4.193	4.398	23.55	4.187
123.76	2.092	50	22	44	44	4.85	4.861	4.864	31.35	4.865
247.52	2.393	50	34	68	68	5.47	5.529	5.444	29.05	5.544
495.04	2.694	50	45	90	90	6.28	6.197	6.27	20.25	6.222
Y = .1498766 + 2.253583 X LOG LD ₅₀ IS 2.152184 LD ₅₀ IS 141.9658					CHI-SQUARED IS 2.038635 WITH 3 DEGREES OF FREEDOM NO SIG HETEROGENEITY 95% CONF LIMITS ARE 118.0232 TO 170.7655					

Appendix Table 100

Calculation of log/ Probit regression line for the dose mortality experiment in which *A. diaperinus* adults were exposed to the treted filter paper with different concentrations of Imidacloprid (Exposure period= 48h).

Dose µg/cm²	Log dose	# used	# Kill	% Kill	Corr. %	Emp. probit	Expt probit	Work probit	Weight	Final probit
1237.62	3.092	50	4	8	8	3.59	3.547	3.596	13.45	3.543
1856.43	3.268	50	14	28	28	4.42	4.403	4.42	27.90	4.387
2475.24	3.393	50	23	46	46	4.9	5.010	4.90	31.85	4.986
3094.05	3.490	50	33	66	66	5.41	5.481	5.402	30.05	5.451
3712.86	3.569	50	42	84	84	5.99	5.866	5.936	25.15	5.830
Y = -11.27969 + 4.793294 X LOG LD ₅₀ IS 3.396346 LD ₅₀ IS 2490.843					CHI-SQUARED IS .6521454 WITH 3 DEGREES OF FREEDOM NO SIG HETEROGENEITY 95% CONF LIMITS ARE 2292.158 TO 2706.748					

Appendix Table 101

Calculation of log/ Probit regression line for the dose mortality experiment in which *A. diaperinus* 1 day old larvae were exposed to the treted filter paper with different concentrations of Imidacloprid (Exposure period= 72h).

<i>Dose µg/cm²</i>	<i>Log dose</i>	<i># used</i>	<i># Kill</i>	<i>% Kill</i>	<i>Corr. %</i>	<i>Emp. probit</i>	<i>Expt probit</i>	<i>Work probit</i>	<i>Weight</i>	<i>Final probit</i>
2.91	0.463	50	6	12	12	3.82	3.794	3.836	16.80	3.805
5.81	0.764	50	15	30	30	4.48	4.462	4.48	27.90	4.457
11.61	1.064	50	27	54	54	5.10	5.131	5.09	31.70	5.111
23.21	1.365	50	38	76	76	5.71	5.800	5.664	25.15	5.765
46.42	1.667	50	47	94	94	6.55	6.470	6.558	15.10	6.419
Y = 2.797208 + 2.173288 X LOG LD ₅₀ IS 1.013576 LD ₅₀ IS 10.31753					CHI-SQUARED IS .5913468 WITH 3 DEGREES OF FREEDOM NO SIG HETEROGENEITY 95% CONF LIMITS ARE 8.505444 TO 12.51568					

Appendix Table 102

Calculation of log/ Probit regression line for the dose mortality experiment in which *A. diaperinus* 10 day old larvae were exposed to the treted filter paper with different concentrations of Imidacloprid (Exposure period= 72h).

<i>Dose µg/cm²</i>	<i>Log dose</i>	<i># used</i>	<i># Kill</i>	<i>% Kill</i>	<i>Corr. %</i>	<i>Emp. probit</i>	<i>Expt probit</i>	<i>Work probit</i>	<i>Weight</i>	<i>Final probit</i>
4.83	0.683	50	7	14	14	3.92	3.904	3.924	20.25	3.888
9.67	0.985	50	18	36	36	4.64	4.536	4.628	29.05	4.531
19.34	1.286	50	26	52	52	5.05	5.168	5.04	31.70	5.172
38.68	1.587	50	38	76	76	5.71	5.799	5.702	26.60	5.812
77.36	1.888	50	48	96	96	6.75	6.430	6.692	15.10	6.453
Y = 2.432563 + 2.129355 X LOG LD ₅₀ IS 1.205735 LD ₅₀ IS 16.0596					CHI-SQUARED IS 2.033623 WITH 3 DEGREES OF FREEDOM NO SIG HETEROGENEITY 95% CONF LIMITS ARE 13.24161 TO 19.47729					

Appendix Table 103

Calculation of log/ Probit regression line for the dose mortality experiment in which *A. diaperinus* 20 day old larvae were exposed to the treted filter paper with different concentrations of Imidacloprid (Exposure period= 72h).

<i>Dose µg/cm²</i>	<i>Log dose</i>	<i># used</i>	<i># Kill</i>	<i>% Kill</i>	<i>Corr. %</i>	<i>Emp. probit</i>	<i>Expt probit</i>	<i>Work probit</i>	<i>Weight</i>	<i>Final probit</i>
11.61	1.064	50	6	12	12	3.82	3.740	3.836	16.80	3.748
23.21	1.365	50	15	30	30	4.48	4.397	4.490	26.60	4.405
46.42	1.667	50	23	46	46	4.90	5.054	4.90	31.85	5.063
92.83	1.967	50	37	74	74	5.64	5.711	5.638	26.60	5.720
185.65	2.268	50	48	96	96	6.75	6.368	6.598	16.80	6.378
Y = 1.422481 + 2.184458 X LOG LD ₅₀ IS 1.637715 LD ₅₀ IS 43.42249					CHI-SQUARED IS 2.159706 WITH 3 DEGREES OF FREEDOM NO SIG HETEROGENEITY 95% CONF LIMITS ARE 35.90061 TO 52.52035					

Appendix Table 104

Calculation of log/ Probit regression line for the dose mortality experiment in which *A. diaperinus* 30 days old larvae were exposed to the treted filter paper with different concentrations of Imidacloprid (Exposure period= 72h).

<i>Dose µg/cm²</i>	<i>Log dose</i>	<i># used</i>	<i># Kill</i>	<i>% Kill</i>	<i>Corr. %</i>	<i>Emp. probit</i>	<i>Expt probit</i>	<i>Work probit</i>	<i>Weight</i>	<i>Final probit</i>
23.21	1.365	50	7	14	14	3.92	3.907	3.924	20.25	3.918
46.42	1.667	50	18	36	36	4.64	4.534	4.628	29.05	4.528
92.83	1.967	50	26	52	52	5.05	5.159	5.04	31.70	5.138
185.65	2.268	50	37	74	74	5.64	5.785	5.638	26.60	5.748
371.29	2.569	50	47	94	94	6.55	6.412	6.558	15.10	6.358
Y = 1.151554 + 2.026017 X LOG LD ₅₀ IS 1.899513 LD ₅₀ IS 79.34386					CHI-SQUARED IS 1.520592 WITH 3 DEGREES OF FREEDOM NO SIG HETEROGENEITY 95% CONF LIMITS ARE 64.82878 TO 97.10886					

Appendix Table 109

Calculation of log/ Probit regression line for the dose mortality experiment in which *A. diaperinus* 20 days old larvae were exposed to the treated flour medium with different concentrations of Imidacloprid (Exposure period= 24h).

[illegible]

Appendix Table 110

Calculation of log/ Probit regression line for the dose mortality experiment in which *A. diaperinus* 30 days old larvae were exposed to the treated flour medium with different concentrations of Imidacloprid (Exposure period= 24h).

[illegible]

Appendix Table 111

Calculation of log/ Probit regression line for the dose mortality experiment in which *A. diaperinus* 40 days old larvae were exposed to the treated flour medium with different concentrations of Imidacloprid (Exposure period= 24h).

[illegible]

Appendix Table 112

Calculation of log/ Probit regression line for the dose mortality experiment in which *A. diaperinus* adults were exposed to the treated flour medium with different concentrations of Imidacloprid (Exposure period= 24h).

[illegible]

Appendix Table 113

Calculation of log/ Probit regression line for the dose mortality experiment in which *A. diaperinus* 1 day old larvae were exposed to the treated flour medium with different concentrations of Imidacloprid (Exposure period= 48h).

[illegible]

Appendix Table 114

Calculation of log/ Probit regression line for the dose mortality experiment in which *A. diaperinus* 10 days old larvae were exposed to the treated flour medium with different concentrations of Imidacloprid (Exposure period= 48h).

[illegible]

Appendix Table 115

Calculation of log/ Probit regression line for the dose mortality experiment in which *A. diaperinus* 20 days old larvae were exposed to the treated flour medium with different concentrations of Imidacloprid (Exposure period= 48h).

[illegible]

Appendix Table 116

Calculation of log/ Probit regression line for the dose mortality experiment in which *A. diaperinus* 30 days old larvae were exposed to the treated flour medium with different concentrations of Imidacloprid (Exposure period= 48h).

[illegible]

Appendix Table 117

Calculation of log/ Probit regression line for the dose mortality experiment in which *A. diaperinus* 40 days old larvae were exposed to the treated flour medium with different concentrations of Imidacloprid (Exposure period= 48h).

[illegible]

Appendix Table 118

Calculation of log/ Probit regression line for the dose mortality experiment in which *A. diaperinus* adults were exposed to the treated flour medium with different concentrations of Imidacloprid (Exposure period= 48h).

[illegible]

Appendix Table 119

Calculation of log/ Probit regression line for the dose mortality experiment in which *A. diaperinus* 1 day old larvae were exposed to the treated flour medium with different concentrations of Imidacloprid (Exposure period= 72h).

[illegible]

Appendix Table 120

Calculation of log/ Probit regression line for the dose mortality experiment in which *A. diaperinus* 10 days old larvae were exposed to the treated flour medium with different concentrations of Imidacloprid (Exposure period= 72h).

[illegible]

[illegible]

Appendix Table 126

Effect of Imidacloprid with Azadirachtin 1:2 on *A. diaperinus* 40 days old larvae after 72 h exposure to treated filter paper.

[illegible]**Appendix Table 127**

Effect of Imidacloprid with Azadirachtin 1:3.5 on *A. diaperinus* 40 days old larvae after 72 h exposure to treated filter paper.

[illegible]

Appendix Table 128

Effect of Imidacloprid with Azadirachtin 1:5 on *A. diaperinus* 40 days old larvae after 72 h exposure to treated filter paper.

[illegible]

Appendix Table 133

Effect of Imidacloprid with Azadirachtin 1:20 on *A. diaperinus* 40 days old larvae after 72 h exposure to treated food media.

[illegible]

Appendix Table 134

Effect of Imidacloprid with Azadirachtin 1: 25 on *A. diaperinus* 40 days old larvae after 72 h exposure to treated food media.

[illegible]

Appendix Table 135

Effect of Imidacloprid with Azadirachtin 1:30 on *A. diaperinus* 40 days old larvae after 72 h exposure to treated food media.

[illegible]

Appendix Table 136

Effect of Imidacloprid with Azadirachtin 1:35 on *A. diaperinus* 40 days old larvae after 72 h exposure to treated food media.

[illegible]

Appendix Table 137

Effect of Imidacloprid with Azadirachtin 1:10 on *A. diaperinus* adults after 72 h exposure to treated food media.

[illegible]

Appendix Table 138

Effect of Imidacloprid with Azadirachtin 1:12 on *A. diaperinus* adults after 72 h exposure to treated food media.

[illegible]

Appendix Table 139

Effect of Imidacloprid with Azadirachtin 1:15 on *A. diaperinus* adults after 72 h exposure to treated food media.

[illegible]

Appendix Table 140

Effect of Imidacloprid with Azadirachtin 1:20 on *A. diaperinus* adults after 72 h exposure to treated food media.

[illegible]

Appendix Table 141

Calculation of log/ Probit regression line for the dose mortality experiment in which *A. diaperinus* 1 day old larvae were exposed to the filter paper treated with different concentrations of Thiodicarb (Exposure period= 24h).

<i>Dose µg/cm²</i>	<i>Log dose</i>	<i># used</i>	<i># Kill</i>	<i>% Kill</i>	<i>Corr. %</i>	<i>Emp. probit</i>	<i>Expt probit</i>	<i>Work probit</i>	<i>Weight</i>	<i>Final probit</i>
1.04	1.017	50	2	4	4	3.25	3.620	3.328	15.10	3.630
2.08	1.318	50	17	34	34	4.59	4.287	4.626	25.15	4.286
4.16	1.619	50	24	48	48	4.95	4.953	4.94	31.70	4.942
8.32	1.92	50	32	64	64	5.36	5.62	5.34	27.90	5.598
16.64	2.221	50	47	94	94	6.55	6.290	6.434	18.50	6.254
Y = 3.59308 + 2.179677 X LOG LD ₅₀ IS .6454721 LD ₅₀ IS 4.420507					CHI-SQUARED IS 6.741387 WITH 3 DEGREES OF FREEDOM NO SIG HETEROGENEITY 95% CONF LIMITS ARE 3.65438 TO 5.347248					

Appendix Table 142

Calculation of log/ Probit regression line for the dose mortality experiment in which *A. diaperinus* 10 days old larvae were exposed to the filter paper, treated with different concentrations of Thiodicarb (Exposure period= 24h).

<i>Dose µg/cm²</i>	<i>Log dose</i>	<i># used</i>	<i># Kill</i>	<i>% Kill</i>	<i>Corr. %</i>	<i>Emp. probit</i>	<i>Expt probit</i>	<i>Work probit</i>	<i>Weight</i>	<i>Final probit</i>
8.29	0.918	50	4	8	8	3.59	3.70	3.604	16.80	3.746
16.58	1.219	50	15	30	30	4.48	4.334	4.49	26.6	4.360
33.16	1.520	50	24	48	48	4.95	4.968	4.94	31.70	4.975
66.32	1.822	50	37	74	74	5.64	5.602	5.640	27.90	5.588
132.64	2.123	50	44	88	88	6.18	6.236	6.128	18.50	6.203
Y = 1.872489 + 2.040075 X LOG LD ₅₀ IS 1.533037 LD ₅₀ IS 34.12223					CHI-SQUARED IS 1.001457 WITH 3 DEGREES OF FREEDOM NO SIG HETEROGENEITY 95% CONF LIMITS ARE 27.91747 TO 41.70599					

Appendix Table 143

Calculation of log/ Probit regression line for the dose mortality experiment in which *A. diaperinus* 20 day old larvae were exposed to the filter paper treated with different concentrations of Thiodicarb (Exposure period= 24h).

<i>Dose µg/cm²</i>	<i>Log dose</i>	<i># used</i>	<i># Kill</i>	<i>% Kill</i>	<i>Corr. %</i>	<i>Emp. probit</i>	<i>Expt probit</i>	<i>Work probit</i>	<i>Weight</i>	<i>Final probit</i>
13.26	1.122	50	5	10	10	3.72	3.789	3.72	16.80	3.820
26.52	1.423	50	17	34	34	4.59	4.366	4.618	26.60	4.378
53.04	1.725	50	19	38	38	4.69	4.944	4.69	31.70	4.938
106.08	2.026	50	37	74	74	5.64	5.521	5.612	29.05	5.496
212.16	2.326	50	43	86	86	6.08	6.098	6.046	21.95	6.054
Y = 1.737474 + 1.855559 X LOG LD ₅₀ IS 1.758245 LD ₅₀ IS 57.31185					CHI-SQUARED IS 4.023743 WITH 3 DEGREES OF FREEDOM NO SIG HETEROGENEITY 95% CONF LIMITS ARE 46.1506 TO 71.17247					

Appendix Table 144

Calculation of log/ Probit regression line for the dose mortality experiment in which *A. diaperinus* 30 days old larvae were exposed to the filter paper treated with different concentrations of Thiodicarb (Exposure period= 24h).

<i>Dose µg/cm²</i>	<i>Log dose</i>	<i># used</i>	<i># Kill</i>	<i>% Kill</i>	<i>Corr. %</i>	<i>Emp. probit</i>	<i>Expt probit</i>	<i>Work probit</i>	<i>Weight</i>	<i>Final probit</i>
16.58	1.219	50	3	6	6	3.45	3.564	3.442	13.45	3.616
33.16	1.520	50	10	20	20	4.16	4.169	4.17	23.55	4.209
66.32	1.822	50	25	50	50	5.00	4.774	5.00	30.80	4.802
132.64	2.122	50	33	66	66	5.41	5.379	5.396	30.80	5.396
265.28	2.423	50	40	80	80	5.85	5.984	5.87	23.55	5.989
Y = 1.212657 + 1.970786 X LOG LD ₅₀ IS 1.921743 LD ₅₀ IS 83.51076					CHI-SQUARED IS 1.978851 WITH 3 DEGREES OF FREEDOM NO SIG HETEROGENEITY 95% CONF LIMITS ARE 67.83163 TO 102.8141					

Appendix Table 149

Calculation of log/ Probit regression line for the dose mortality experiment in which *A. diaperinus* 20 days old larvae were exposed to the filter paper treated with different concentrations of Thiodicarb (Exposure period= 48h).

Dose µg/cm²	Log dose	# used	# Kill	% Kill	Corr. %	Emp. probit	Expt probit	Work probit	Weight	Final probit
6.63	0.821	50	2	4	4	3.25	3.235	3.248	9.00	3.211
13.26	1.122	50	7	14	14	3.92	3.847	3.924	18.50	3.832
26.52	1.423	50	11	22	22	4.23	4.458	4.240	27.90	4.454
53.04	1.724	50	30	60	60	5.25	5.069	5.25	31.85	5.075
106.08	2.025	50	37	74	74	5.64	5.680	5.640	27.90	5.697
Y = 1.51488 + 2.064753 X LOG LD ₅₀ IS 1.687912 LD ₅₀ IS 48.74291					CHI-SQUARED IS 2.50544 WITH 3 DEGREES OF FREEDOM NO SIG HETEROGENEITY 95% CONF LIMITS ARE 39.27673 TO 60.49057					

Appendix Table 150

Calculation of log/ Probit regression line for the dose mortality experiment in which *A. diaperinus* 30 days old larvae were exposed to the filter paper treated with different concentrations of Thiodicarb (Exposure period= 48h).

Dose µg/cm²	Log dose	# used	# Kill	% Kill	Corr. %	Emp. probit	Expt probit	Work probit	Weight	Final probit
8.29	0.918	50	4	8	8	3.59	3.734	3.604	16.80	3.796
16.58	1.219	50	13	26	26	4.36	4.270	4.354	25.15	4.309
33.16	1.520	50	24	48	48	4.95	4.806	4.968	31.35	4.821
66.32	1.821	50	32	64	64	5.36	5.342	5.344	30.80	5.332
132.64	2.123	50	39	78	78	5.77	5.878	5.732	25.15	5.845
Y = 2.234448 + 1.700978 X LOG LD ₅₀ IS 1.625861 LD ₅₀ IS 42.2533					CHI-SQUARED IS 1.679077 WITH 3 DEGREES OF FREEDOM NO SIG HETEROGENEITY 95% CONF LIMITS ARE 33.38719 TO 53.47384					

Appendix Table 151

Calculation of log/ Probit regression line for the dose mortality experiment in which *A. diaperinus* 40 days old larvae were exposed to the filter paper treated with different concentrations of Thiodicarb (Exposure period= 48h).

Dose µg/cm²	Log dose	# used	# Kill	% Kill	Corr. %	Emp. probit	Expt probit	Work probit	Weight	Final probit
9.95	0.998	50	2	4	4	3.25	3.363	3.254	10.40	3.436
19.90	1.298	50	8	16	16	4.01	3.965	4.016	20.25	4.014
39.80	1.600	50	19	38	38	4.69	4.568	4.684	29.05	4.592
79.60	1.901	50	30	60	60	5.25	5.170	5.24	31.70	5.171
159.2	2.202	50	37	74	74	5.64	5.772	5.638	26.60	5.750
Y = 1.518883 + 1.921369 X LOG LD ₅₀ IS 1.81179 LD ₅₀ IS 64.83203					CHI-SQUARED IS 1.067146 WITH 3 DEGREES OF FREEDOM NO SIG HETEROGENEITY 95% CONF LIMITS ARE 51.83288 TO 81.09123					

Appendix Table 152

Calculation of log/ Probit regression line for the dose mortality experiment in which *A. diaperinus* adults were exposed to the filter paper treated with different concentrations of Thiodicarb (Exposure period= 48h).

Dose µg/cm²	Log dose	# used	# Kill	% Kill	Corr. %	Emp. probit	Expt probit	Work probit	Weight	Final probit
53.04	1.724	50	4	8	8	3.59	3.694	3.596	15.10	3.736
106.08	2.025	50	13	26	26	4.36	4.288	4.354	25.15	4.315
212.16	2.326	50	23	46	46	4.90	4.882	4.916	31.15	4.895
424.32	2.627	50	37	74	74	5.64	5.476	5.618	30.05	5.475
848.64	2.928	50	41	82	82	5.93	6.069	5.882	21.95	6.054
Y = .4159141 + 1.925299 X LOG LD ₅₀ IS 2.380974 LD ₅₀ IS 240.4216					CHI-SQUARED IS 1.614868 WITH 3 DEGREES OF FREEDOM NO SIG HETEROGENEITY 95% CONF LIMITS ARE 194.7059 TO 296.8713					

Appendix Table 153

Calculation of log/ Probit regression line for the dose mortality experiment in which *A. diaperinus* 1 day old larvae were exposed to the filter paper treted with different concentrations of Thiodicarb (Exposure period= 72h).

<i>Dose µg/cm²</i>	<i>Log dose</i>	<i># used</i>	<i># Kill</i>	<i>% Kill</i>	<i>Corr. %</i>	<i>Emp. probit</i>	<i>Expt probit</i>	<i>Work probit</i>	<i>Weight</i>	<i>Final probit</i>
0.52	0.716	50	6	12	12	3.82	3.889	3.822	18.50	3.907
1.04	1.017	50	15	30	30	4.48	4.444	4.48	27.90	4.457
2.08	1.318	50	27	54	54	5.10	5.00	5.10	31.85	5.006
4.16	1.619	50	35	70	70	5.52	5.556	5.50	29.05	5.556
8.32	1.920	50	43	86	86	6.08	6.112	6.086	20.25	6.107
Y = 2.598434 + 1.82706 X LOG LD ₅₀ IS 1.314443 LD ₅₀ IS 2.062731					CHI-SQUARED IS .5270462 WITH 3 DEGREES OF FREEDOM NO SIG HETEROGENEITY 95% CONF LIMITS ARE 1.657253 TO 2.567418					

Appendix Table 154

Calculation of log/ Probit regression line for the dose mortality experiment in which *A. diaperinus* 10 days old larvae were exposed to the filter paper treted with different concentrations of Thiodicarb (Exposure period= 72h).

<i>Dose µg/cm²</i>	<i>Log dose</i>	<i># used</i>	<i># Kill</i>	<i>% Kill</i>	<i>Corr. %</i>	<i>Emp. probit</i>	<i>Expt probit</i>	<i>Work probit</i>	<i>Weight</i>	<i>Final probit</i>
4.14	0.617	50	8	16	16	4.01	3.847	4.026	18.50	3.835
8.28	0.918	50	14	28	28	4.42	4.418	4.42	27.90	4.413
16.56	1.219	50	22	44	44	4.85	4.990	4.84	31.70	4.990
33.12	1.520	50	33	66	66	5.41	5.560	5.388	29.05	5.568
66.24	1.821	50	47	94	94	6.55	6.132	6.454	20.25	6.145
Y = 2.651138 + 1.918791 X LOG LD ₅₀ IS 1.224137 LD ₅₀ IS 16.75469					CHI-SQUARED IS 4.259308 WITH 3 DEGREES OF FREEDOM NO SIG HETEROGENEITY 95% CONF LIMITS ARE 13.60276 TO 20.63697					

Appendix Table 155

Calculation of log/ Probit regression line for the dose mortality experiment in which *A. diaperinus* 20 days old larvae were exposed to the filter paper treted with different concentrations of Thiodicarb (Exposure period= 72h).

<i>Dose µg/cm²</i>	<i>Log dose</i>	<i># used</i>	<i># Kill</i>	<i>% Kill</i>	<i>Corr. %</i>	<i>Emp. probit</i>	<i>Expt probit</i>	<i>Work probit</i>	<i>Weight</i>	<i>Final probit</i>
6.63	0.821	50	4	8	8	3.59	3.456	3.63	11.90	3.409
13.26	1.122	50	9	18	18	4.08	4.118	4.094	23.55	4.082
26.52	1.423	50	16	32	32	4.53	4.78	4.532	30.80	4.756
53.04	1.725	50	35	70	70	5.52	5.542	5.51	30.05	5.429
106.08	2.026	50	44	88	88	6.18	6.104	6.178	20.25	6.102
Y = 1.572203 + 2.236188 X LOG LD ₅₀ IS 1.532875 LD ₅₀ IS 34.10949					CHI-SQUARED IS 2.438378 WITH 3 DEGREES OF FREEDOM NO SIG HETEROGENEITY 95% CONF LIMITS ARE 28.24704 TO 41.18863					

Appendix Table 156

Calculation of log/ Probit regression line for the dose mortality experiment in which *A. diaperinus* 30 days old larvae were exposed to the filter paper treted with different concentrations of Thiodicarb (Exposure period= 72h).

<i>Dose µg/cm²</i>	<i>Log dose</i>	<i># used</i>	<i># Kill</i>	<i>% Kill</i>	<i>Corr. %</i>	<i>Emp. probit</i>	<i>Expt probit</i>	<i>Work probit</i>	<i>Weight</i>	<i>Final probit</i>
8.29	0.918	50	7	14	14	3.92	3.962	3.924	20.25	3.978
16.58	1.220	50	18	36	36	4.64	4.534	4.628	29.05	4.536
33.16	1.521	50	26	52	52	5.05	5.106	5.04	31.70	5.093
66.32	1.822	50	37	74	74	5.64	5.678	5.640	27.90	5.651
132.64	2.123	50	45	90	90	6.28	6.250	6.23	18.50	6.208
Y = 2.27656 + 1.852337 X LOG LD ₅₀ IS 1.470273 LD ₅₀ IS 29.53064					CHI-SQUARED IS .4083328 WITH 3 DEGREES OF FREEDOM NO SIG HETEROGENEITY 95% CONF LIMITS ARE 23.77096 TO 36.68587					

Appendix Table 157

Calculation of log/ Probit regression line for the dose mortality experiment in which *A. diaperinus* 40 days old larvae were exposed to the filter paper treted with different concentrations of Thiodicarb (Exposure period= 72h).

[illegible]

Appendix Table 158

Calculation of log/ Probit regression line for the dose mortality experiment in which *A. diaperinus* adults were exposed to the filter paper treated with different concentrations of Thiodicarb (Exposure period= 72h).

[illegible]

Appendix Table 159

Calculation of log/ Probit regression line for the dose mortality experiment in which *A. diaperinus* 1 day old larvae were exposed to the treated flour medium with different concentrations of Thiodicarb (Exposure period= 24h).

[illegible]

Appendix Table 160

Calculation of log/ Probit regression line for the dose mortality experiment in which *A. diaperinus* 10 days old larvae were exposed to the treated flour medium with different concentrations of Thiodicarb (Exposure period= 24h).

[illegible]

Appendix Table 161

Calculation of log/ Probit regression line for the dose mortality experiment in which *A. diaperinus* 20 days old larvae were exposed to the treated flour medium with different concentrations of Thiodicarb (Exposure period= 24h).

[illegible]

Appendix Table 162

Calculation of log/ Probit regression line for the dose mortality experiment in which *A. diaperinus* 30 days old larvae were exposed to the treated flour medium with different concentrations of Thiodicarb (Exposure period= 24h).

[illegible]

Appendix Table 163

Calculation of log/ Probit regression line for the dose mortality experiment in which *A. diaperinus* 40 days old larvae were exposed to the treated flour medium with different concentrations of Thiodicarb (Exposure period= 24h).

[illegible]

Appendix Table 164

Calculation of log/ Probit regression line for the dose mortality experiment in which *A. diaperinus* adults were exposed to the treted flour medium with different concentrations of Thiodicarb (Exposure period= 24h).

[illegible]

Appendix Table 165

Calculation of log/ Probit regression line for the dose mortality experiment in which *A. diaperinus* 1 day old larvae were exposed to the treted flour medium with different concentrations of Thiodicarb (Exposure period= 48h).

<i>Dose ppm</i>	<i>Log dose</i>	<i># used</i>	<i># Kill</i>	<i>% Kill</i>	<i>Corr. %</i>	<i>Emp. probit</i>	<i>Expt probit</i>	<i>Work probit</i>	<i>Weight</i>	<i>Final probit</i>
1.5	0.176	50	4	8	8	3.59	3.642	3.596	15.10	3.684
3	0.477	50	13	26	26	4.36	4.249	4.354	25.15	4.266
6	0.778	50	23	46	46	4.90	4.856	4.916	31.35	4.847
12	1.079	50	30	60	60	5.25	5.463	5.24	30.05	5.428
24	1.380	50	44	88	88	6.18	6.07	6.128	21.95	6.009
Y = 3.344418 + 1.930885 X LOG LD ₅₀ IS .8574213 LD ₅₀ IS 7.201473					CHI-SQUARED IS 1.836662 WITH 3 DEGREES OF FREEDOM NO SIG HETEROGENEITY 95% CONF LIMITS ARE 5.831221 TO 8.893713					

Appendix Table 166

Calculation of log/ Probit regression line for the dose mortality experiment in which *A. diaperinus* 10 days old larvae were exposed to the treted flour medium with different concentrations of Thiodicarb (Exposure period= 48h).

<i>Dose ppm</i>	<i>Log dose</i>	<i># used</i>	<i># Kill</i>	<i>% Kill</i>	<i>Corr. %</i>	<i>Emp. probit</i>	<i>Expt probit</i>	<i>Work probit</i>	<i>Weight</i>	<i>Final probit</i>
2	0.301	50	2	4	4	3.25	3.353	3.254	10.40	3.410
4	0.602	50	10	20	20	4.16	4.016	4.16	21.95	4.048
8	0.903	50	19	38	38	4.69	4.68	4.686	30.05	4.685
16	1.204	50	31	62	62	5.31	5.343	5.292	30.80	5.323
32	1.505	50	42	84	84	5.99	6.006	5.964	21.95	5.960
Y = 2.772636 + 2.117838 X LOG LD ₅₀ IS 1.051716 LD ₅₀ IS 11.26461					CHI-SQUARED IS .559639 WITH 3 DEGREES OF FREEDOM NO SIG HETEROGENEITY 95% CONF LIMITS ARE 9.207493 TO 13.78133					

Appendix Table 167

Calculation of log/ Probit regression line for the dose mortality experiment in which *A. diaperinus* 20 days old larvae were exposed to the treted flour medium with different concentrations of Thiodicarb (Exposure period= 48h).

<i>Dose ppm</i>	<i>Log dose</i>	<i># used</i>	<i># Kill</i>	<i>% Kill</i>	<i>Corr. %</i>	<i>Emp. probit</i>	<i>Expt probit</i>	<i>Work probit</i>	<i>Weight</i>	<i>Final probit</i>
3	0.477	50	7	14	14	3.92	3.878	3.924	18.50	3.877
6	0.778	50	15	30	30	4.48	4.454	4.48	27.90	4.447
12	1.079	50	24	48	48	4.95	5.03	4.95	31.85	5.017
24	1.380	50	35	70	70	5.52	5.606	5.52	27.90	5.588
48	1.681	50	45	90	90	6.28	6.182	6.27	20.25	6.158
Y = 2.973384 + 1.894238 X LOG LD ₅₀ IS 1.069885 LD ₅₀ IS 11.74585					CHI-SQUARED IS .5977096 WITH 3 DEGREES OF FREEDOM NO SIG HETEROGENEITY 95% CONF LIMITS ARE 9.500711 TO 14.52155					

Appendix Table 168

Calculation of log/ Probit regression line for the dose mortality experiment in which *A. diaperinus* 30 days old larvae were exposed to the treted flour medium with different concentrations of Thiodicarb (Exposure period= 48h).

<i>Dose ppm</i>	<i>Log dose</i>	<i># used</i>	<i># Kill</i>	<i>% Kill</i>	<i>Corr. %</i>	<i>Emp. probit</i>	<i>Expt probit</i>	<i>Work probit</i>	<i>Weight</i>	<i>Final probit</i>
3	0.477	50	5	10	10	3.72	3.730	3.72	16.80	3.746
6	0.778	50	13	26	26	4.36	4.313	4.362	26.60	4.318
12	1.079	50	22	44	44	4.85	4.896	4.864	31.35	4.889
24	1.380	50	34	68	68	5.47	5.479	5.456	30.05	5.462
48	1.681	50	43	86	86	6.08	6.061	6.046	21.95	6.034
Y = 2.838545 + 1.900944 X LOG LD ₅₀ IS 1.137043 LD ₅₀ IS 13.71017					CHI-SQUARED IS 8.792114 WITH 3 DEGREES OF FREEDOM NO SIG HETEROGENEITY 95% CONF LIMITS ARE 11.09856 TO 16.93631					

Appendix Table 169

Calculation of log/ Probit regression line for the dose mortality experiment in which *A. diaperinus* 40 days old larvae were exposed to the treated flour medium with different concentrations of Thiodicarb (Exposure period= 48h).

[illegible]

Appendix Table 170

Calculation of log/ Probit regression line for the dose mortality experiment in which *A. diaperinus* adults were exposed to the treted flour medium with different concentrations of Thiodicarb (Exposure period= 48h).

[illegible]

Appendix Table 171

Calculation of log/ Probit regression line for the dose mortality experiment in which *A. diaperinus* 1 day old larvae were exposed to the treated flour medium with different concentrations of Thiodicarb (Exposure period= 72h).

[illegible]

Appendix Table 172

Calculation of log/ Probit regression line for the dose mortality experiment in which *A. diaperinus* 10 days old larvae were exposed to the treated flour medium with different concentrations of Thiodicarb (Exposure period= 72h).

[illegible]

Appendix Table 177

Effect of Thiodicarb with Azadirachtin 1:3 on *A. diaperinus* 40 days old larvae after 72 h exposure to treated filter paper.

[illegible]

Appendix Table 178

Effect of Thiodicarb with Azadirachtin 1:7 on *A. diaperinus* 40 days old larvae after 72 h exposure to treated filter paper.

[illegible]

Appendix Table 179

Effect of Thiodicarb with Azadirachtin 1:10 on *A. diaperinus* 40 days old larvae after 72 h exposure to treated filter paper.

[illegible]

Appendix Table 180

Effect of Thiodicarb with Azadirachtin 1:15 on *A. diaperinus* 40 days old larvae after 72 h exposure to treated filter paper.

[illegible]

Appendix Table 181

Effect of Thiodicarb with Azadirachtin on *A. diaperinus* adults after 72 h exposure to treated filter paper.

[illegible]

Appendix Table 182

Effect of Thiodicarb with Azadirachtin 1:4 on *A. diaperinus* adults after 72 h exposure to treated filter paper.

[illegible]**Appendix Table 183**

Effect of Thiodicarb with Azadirachtin 1:6 on *A. diaperinus* adults after 72 h exposure to treated filter paper.

[illegible]

Appendix Table 184

Effect of Thiodicarb with Azadirachtin 1:10 on *A. diaperinus* adults after 72 h exposure to treated filter paper.

[illegible]

Appendix Table 193

Calculation of log/ Probit regression line for the dose mortality experiment in which *A. diaperinus* 1 day old larvae were exposed to the treated filter paper with different concentrations of Fenitrothion (Exposure period= 24h).

[illegible]

Appendix Table 194

Calculation of log/ Probit regression line for the dose mortality experiment in which *A. diaperinus* 10 days old larvae were exposed to the treated filter paper with different concentrations of Fenitrothion (Exposure period= 24h).

[illegible]

Appendix Table 195

Calculation of log/ Probit regression line for the dose mortality experiment in which *A. diaperinus* 20 days old larvae were exposed to the treated filter paper with different concentrations of Fenitrothion (Exposure period= 24h).

[illegible]

Appendix Table 196

Calculation of log/ Probit regression line for the dose mortality experiment in which *A. diaperinus* 30 days old larvae were exposed to the treated filter paper with different concentrations of Fenitrothion (Exposure period= 24h).

[illegible]

Appendix Table 201

Calculation of log/ Probit regression line for the dose mortality experiment in which *A. diaperinus* 20 days old larvae were exposed to the treated filter paper with different concentrations of Fenitrothion (Exposure period= 48h).

[illegible]

Appendix Table 202

Calculation of log/ Probit regression line for the dose mortality experiment in which *A. diaperinus* 30 days old larvae were exposed to the treated filter paper with different concentrations of Fenitrothion (Exposure period= 48h).

[illegible]

Appendix Table 203

Calculation of log/ Probit regression line for the dose mortality experiment in which *A. diaperinus* 40 days old larvae were exposed to the treated filter paper with different concentrations of Fenitrothion (Exposure period= 48h).

[illegible]

Appendix Table 204

Calculation of log/ Probit regression line for the dose mortality experiment in which *A. diaperinus* adults were exposed to the treated filter paper with different concentrations of Fenitrothion (Exposure period= 48h).

[illegible]

Appendix Table 209

Calculation of log/ Probit regression line for the dose mortality experiment in which *A. diaperinus* 40 days old larvae were exposed to the treated filter paper with different concentrations of Fenitrothion (Exposure period= 72h).

[illegible]

Appendix Table 210

Calculation of log/ Probit regression line for the dose mortality experiment in which *A. diaperinus* adults were exposed to the treated filter paper with different concentrations of Fenitrothion (Exposure period= 72h).

[illegible]

Appendix Table 211

Calculation of log/ Probit regression line for the dose mortality experiment in which *A. diaperinus* 1 day old larvae were exposed to the treated flour medium with different concentrations of Fenitrothion (Exposure period= 24h).

[illegible]

Appendix Table 212

Calculation of log/ Probit regression line for the dose mortality experiment in which *A. diaperinus* 10 days old larvae were exposed to the treated flour medium with different concentrations of Fenitrothion (Exposure period= 24h).

[illegible]

Appendix Table 217

Calculation of log/ Probit regression line for the dose mortality experiment in which *A. diaperinus* 1day old larvae were exposed to the treated flour medium with different concentrations of Fenitrothion (Exposure period= 48h).

[illegible]

Appendix Table 218

Calculation of log/ Probit regression line for the dose mortality experiment in which *A. diaperinus* 10 days old larvae were exposed to the treated flour medium with different concentrations of Fenitrothion (Exposure period= 48h).

[illegible]

Appendix Table 219

Calculation of log/ Probit regression line for the dose mortality experiment in which *A. diaperinus* 20 days old larvae were exposed to the treated flour medium with different concentrations of Fenitrothion (Exposure period= 48h).

[illegible]

Appendix Table 220

Calculation of log/ Probit regression line for the dose mortality experiment in which *A. diaperinus* 30 days old larvae were exposed to the treated flour medium with different concentrations of Fenitrothion (Exposure period= 48h).

[illegible]

Appendix Table 237

Effect of Fenitrothion with Azadirachtin 1:10 on *A. diaperinus* 40 days old larvae after 72 h exposure to treated food media.

[illegible]

Appendix Table 238

Effect of Fenitrothion with Azadirachtin 1:15 on *A. diaperinus* 40 days old larvae after 72 h exposure to treated food media.

[illegible]

Appendix Table 239

Effect of Fenitrothion with Azadirachtin 1:20 on *A. diaperinus* 40 days old larvae after 72 h exposure to treated food media.

[illegible]

Appendix Table 240

Effect of Fenitrothion with Azadirachtin 1:25 on *A. diaperinus* 40 days old larvae after 72 h exposure to treated food media.

[illegible]

Appendix Table 241

Effect of Fenitrothion with Azadirachtin 1:10 on *A. diaperinus* aults after 72 h exposure to treated food media.

[illegible]

Appendix Table 242

Effect of Fenitrothion with Azadirachtin 1:15 on *A. diaperinus* aults after 72 h exposure to treated food media.

[illegible]

Appendix Table 243

Effect of Fenitrothion with Azadirachtin 1:20 on *A. diaperinus* aults after 72 h exposure to treated food media.

[illegible]

Appendix Table 244

Effect of Fenitrothion with Azadirachtin 1:25 on *A. diaperinus* aults after 72 h exposure to treated food media.

[illegible]