Studies on Sublethal Toxicity of Carbosulfan on the Adults of Banana Pseudostem Weevil, *Odoiporus longicollis* Oliv. (Coleoptera: Curculionidae)

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DOCTOR OF PHILOSOPHY IN ZOOLOGY

Submitted by

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Under the guidance of

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CERTIFICATE

This is to certify that the thesis entitled **"Studies on sublethal toxicity of carbosulfan on the adults of Banana Pseudostem Weevil, *Odoiporus longicollis* Oliv. (Coleoptera: Curculionidae)"**submitted to University of Calicut, in partial fulfillment of the requirements for the award ofthe degree of Doctor of Philosophy in Zoology is a record of original and independent research work carried out by **Ms. Aiswarya, D**., Department of Zoology, University of Calicut, under my guidance and supervision. The thesis has not formed the basis for the award of any other Degree/ Diploma of this or any other University.

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The adjudicators of the thesis entitled "**Studies on Sublethal Toxicity of Carbosulfan on the adults of Banana pseudostem weevil, *Odoiporus longicollis* Oliv (Coleoptera : Curculionidae)"**. submitted by Ms. Aiswarya D. have not suggested for any corrections. Hence, as instructed by the Directorate of Research, two copies of thesis and one soft copy of the thesis in PDF format on CD is being sent to the office.

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DECLARATION

I do hereby declare that this thesis entitled **"Studies on sublethal toxicity of carbosulfan on the adults of Banana Pseudostem Weevil, *Odoiporus longicollis* Oliv. (Coleoptera: Curculionidae)"**, submitted to the University of Calicut in partial fulfillment for the Doctoral degree in Zoology is a bonafide research work done by me under the supervision and guidance of **Dr. E. Pushpalatha**, Head of the Department & Assosciate Professor, Department of Zoology, University of Calicut and no part of the thesis has been presented by me for the award of any other degree, dipoma or similar title.

Place: Calicut University **Aiswarya, D.**
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DEDICATED TO,

MY FAMILY

CONTENTS

ABBREVIATIONS

AChE	-	Acetylcholinesterase
ACTI	-	Acetylthiocholine iodide
BM	-	Basement membrane
C	-	Contaminated
CC	-	Columnar epithelium
CDNB	-	Chlorodinitrobenzene
CV	-	Canonical variate
CVA	-	Canonical Variate Analysis
DC	-	Digestive cells
DTNB	-	Dithiobis 2 nitro benzoic acid
EP	-	Epithelium
GL	-	Gut lumen
GST	-	Glutathione-S-transferase
HG	-	Hind Gut
HPL	-	High Performance Liquid Chromatograph
IN	-	Intima
LD_{50}	-	Lethal dosage for 50%
LD_{90}	-	Lethal dosage for 90%
MFO	-	Mixed function oxygenase
MG	-	Mid Gut
ML	-	Muscular layer
PCA	-	Principal Component Analysis

PCs	-	Principal components
PV	-	proventriculus
RC	-	regenerative cells
RG	-	regenerative crypts
SEM	-	scanning electron microscope
SR	-	sclerotized ridges
SV	-	stomodeal valve
TMB	-	tetramethylbenzidine
UC	-	Uncontaminated

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GENERAL INTRODUCTION

The history of agriculture dates back to thousands of years and about 10,000 years ago, nascent farmers began to gather wild grains and plant them to meet the requirement of food. Agriculture is the back bone of our economy, especially for developing countries like India. It is not only important for economic purpose but also has an influence on our social, political and cultural life. In the words of Jawaharlal Nehru, "Agriculture needed top most priority because the Government and the nation would each fail to succeed if agriculture couldn't be successful". Agriculture is the main occupation in India. Two-third of population relies on agriculture directly or indirectly. It is not merely a source of livelihood but a way of life. It is the most supply of food, fodder and fuel and is the basic foundation of economic development.

The modern agriculture is being practiced around the world on a far larger scale. In spite of the tremendous increase in the food production we constantly fall short of food supply owing to many reasons. However, the losses of crops caused due to insect pests are on the high. It is estimated that field and storage pests destroy approximately 43 per cent of potential production in developing Asian and African countries (Jacobson, 1982; Ogendo *et al*., 2004). Despite the use of three million metric tons of pesticides and also the varied biological and different non-chemical controls employed worldwide, international crop losses stay a matter of concern (Pimentel and Peshin, 2014).

Crops are attacked by pests or diseases in every stage of production and even after harvest. Pests include a large group of animals like insects, ticks, mites, slugs, snails, bacteria, weeds, fungi, nematodes, cestodes, viruses and other pathogens. Pest management is a way to reduce pest numbers up to an acceptable threshold level. Threshold level is the pest density at which some control should be exerted to prevent a pest population from increasing

further and causing economic loss. The goal of the economic threshold is to prevent a pest population from reaching the point where its damage causes losses that are equal to the cost of control. The economic threshold level is defined as "the pest density at which control measures should be applied to prevent an increasing pest population from reaching economic injury level". Subsequently, pest control needs to be regulated so that crops are kept free from pests and food production is maximized in every agricultural farm. Through time, several types of pest control strategies have emerged. Pest control mechanisms can be classified as chemical, biological, mechanical, cultural and genetic methods. Pest control is inevitable, as there has always been a need to keep crops free from pests.

Biological control involves the use of a pest's natural enemies (e.g., predators, pathogens, parasites and parasitoids), to control pest abundance. Cultural practices for pest control mechanisms include growing multiple crops in the same field which is called as intercropping, planting trap crops which attract pests away from harvest crops and which can later be treated with select application of pesticides, and delaying planting to escape from pest attack. Manual or mechanical removal or installation of physical barriers can be used to prevent pest species. Mechanical methods include hand picking of the insect, using sticky cards for insects, using insect traps and by removing the infected parts. Physical barriers such as fences, nets, and tree trunk guards can reduce pests and reduce the damage they inflict. Genetic control is another method for the successful control of the pest. Sterile males are introduced into the field and allowed to mate with females producing non-viable eggs. This method includes limitations such as how far the sterile insects will be able to mate by competing with naturally occurring males.

Integrated pest management or integrated pest control is broad spectrum approach for the successful eradication of the pest population. It

integrates all the other control measures and reduces the pest status below the economic injury level. The UN's Food and Agriculture Organization defines IPM as "the careful consideration of all available pest control techniques and subsequent integration of appropriate measures that discourage the development of pest populations and keep pesticides and other interventions to levels that are economically justified and reduce or minimize risks to human health and the environment". IPM emphasizes the growth of a healthy crop with the least possible disruption to agro-ecosystems and encourages natural pest control mechanisms (FAO, 2012).

Chemical pesticides date back 4,500 years. Currently, there are more than 1,055 active ingredients registered as pesticides, which are put together to produce over 16,000 pesticide products that are being marketed around the world.Any organism that causes an economic loss or damage to the physical wellbeing of human beings is a pest and chemical compounds that are used for the control of pests are called pesticides. According to food and agriculture organization (FAO, 2002), "pesticides are used as substances applied to crops either before or after harvest to protect the commodity from deterioration during storage and transport". Proper eradication of pests using pesticides enhance food production and also check the population of insects acting as vectors of many human diseases such as malaria, plague, filaria, dengue fever etc. The term pesticides include insecticides, fungicides, herbicide, nematicides etc. On the basis of chemical composition, there are different classes of pesticides like organo chlorine, organo phosphates, carbamates and inorganic insecticides. Chemical pest control is still the predominant type of pest control today, although a renewed interest in traditional and biological pest control developed towards the end of the 20th century and continues to this day (Emden *et al*., 2004).

Pesticides are very important as far as pest control is concerned. They help farmers to grow more food on less area and protect crops from pests, diseases and weeds as well as maximize productivity per hectare. All farmers use pesticides, both synthetic and natural in origin against pests. Organic farmers use organic fertilizers of natural origin. Without pesticides, more than half of our crops would be lost due to pests and diseases. Between 26 and 40 percent of the world's potential crop production is lost annually because of weeds, pests and diseases (FAO, 2012). Without crop protection, these losses could easily double. Pesticides provide farmers to make safe, quality foods at affordable prices. They also help farmers provide an abundance of nutritious, all-year-round foods, which are necessary for human health and also makes fruits and vegetables, which provide essential nutrients, more abundant and affordable.

Despite employing an array of pesticides, several factors affect the performance of pesticides. Insecticide resistance is one of the major obstacles to the successful control of insects. Inappropriate use of pesticides can result in target pest resurgence and secondary pest outbreaks by killing their natural enemies and become resistant to the pesticide. Interest in the problem of insect resistance to insecticides gained considerable momentum between the years 1950 and the early 1960s. Insecticide resistance has been shown to involve three mechanisms, i.e., enhanced detoxification, reduced penetration and target site insensitivity. Generally, these resistance factors do not occur alone and are known to interact with each other, especially penetration and metabolism to enhance the level of resistance. In the case of enhanced detoxification, a number of enzymes have been shown to be involved.

Carbamate insecticides are competitive rather than irreversible inhibitors. Carbamate complexes with the enzymes without necessarily reacting it chemically (Casida *et al*., 1960; Kolbezen *et al*., 1954; Metcalf,

1962; Metcalf and Fukuto, 1965). Evidence is also available that, in some instances, a chemical reaction occurs yielding a carbamylated enzyme (Wilson *et al*., 1960, 1961; O'Brien *et al*., 1966). Carbamate insecticides are derivative of carbamic acids and carbazyl, the first carbamate insecticide, was introduced in 1956 (Thacker, 2002). They inhibit the acetyl cholinesterase enzyme (AChE) and cause over stimulation of nervous system.

Carbamates are esters of N-methyl carbamic acid. Aldicarb, carbaryl, propoxur, oxamyl, carbofuran, carbosulfan and terbucarb are carbamates. Although these pesticides differ chemically, they act similarly. When applied to crops or directly to the soil as systemic insecticides, organophosphates and carbamates generally persist from only a few hours to several months. The inhibition of AChE is the result of an actual chemical reaction between the enzyme and the organophosphate or carbamate ester (Aldridge and Davison, 1952; Wilson *et al.,* 1960; Cohen and Oosterbaan, 1963). The phosphorylated or carbamylated enzyme cannot hydrolyse AChE; this results in the accumulation of the neurotransmitter at a nerve synapse or neuromuscular junction.

Esterases confer resistance to carbamates and organo phosphates in many insect species (Lee, 1997; Hemingway *et al*., 2004; Liu *et al.,* 2006; Achaleke *et al*., 2009; Hotelier *et al*., 2010; Bass and Field*,* 2011) mainly due to the activity of carboxylesterases (Punta *et al*., 2012; Montella *et al*., 2012) and only in a few rare cases by arylesterases (aromatic esterases) (Lee, 1997). Among the metabolic based insecticide resistance mechanism, non-specific esterase, Glutathione S-transferase (GSTs) and, P450 mediated monooxygenase (MFOs) are known to be involved in the detoxification of organophosphate, pyrethroid, and carbamate insecticides (Hemingway and Ranson, 2000). In insects, AChE has been studied in relation to insecticide resistance because the enzyme is the target of organophosphate and

carbamates insecticides and its insensitivity to insecticides is one of the main factors accounting for resistance (Weill *et al.,* 2004); thereby these enzymes are used as reliable marker to assess the impact of toxic compounds on a range of test organisms (Smirle *et al*., 2010).

According to Winton *et al*. (1958) and Metcalf *et al*. (1960), the requirements for toxic action of a carbamate are "(a) structural complementarity to acetylcholine, the normal substrate for the enzyme cholinesterase, (b) sufficient stability to hydrolytic attack by cholinesterase to permit the compound to act as a competitive blocking agent for cholinesterase rather than as a substrate, and (c) proper lipoid solubility and absence of a permanent electrical charge to permit penetration into the lipoid sheath surrounding the insect nerve". In general, the stronger the ionization of the carbamate the lower is its insecticidal activity (O'Brien and Matthysse, 1961; O'Brien, 1967) even though the compound might be a potent *in vitro* cholinesterase inhibitor. In addition to a specific acetyl cholinesterase, nonspecific aliphatic esterase enzymes might be involved in the mode of action of, or resistance to the carbamates, as has been demonstrated for the organo phophorous compounds.

Major reason for the resistance mechanism is the increased detoxification of the xenobiotics by the insect. There are several enzymes involved in the insecticide degradation in insects. They degrade the insecticide and convert them to metabolites which can then be easily absorbed by insects through mineralization, finally achieving insecticide detoxification. These detoxification mechanisms help the insects to overcome insecticide and become resistant to it. Thus, knowledge about the detoxification mechanisms helps us to impart chemical resistance to crop plants and choose an effective insecticide against the pests. Carboxyl esterases, mixed function oxidases and glutathione-s-transferases are the major detoxification enzymes in insects.

Pyrethrins, pyrethroids, organophosphates (OP), carbamates, are degraded by hydrolysis. This forms the basic effect of insecticides and insects resistance mechanisms. Insecticide detoxification occurs by molecule hydrolysis and breaking chemical bonds such as ester, carboxylester and amide. Carboxylesterases are enzymes which break down carboxylesters into the corresponding alcohol and carboxylic acid. Esterases were first reported in 1906 (Loevenhart, 1906). They are involved in the detoxification of ester containing xenobiotics. So, they might be seen in tissues such as liver and gut epithelia which are exposed to xenobiotics containing ester chemo type. Carboxylesterases are involved in resistance to ester-containing insecticides such as organophosphorous, carbamate and pyrethroid insecticides.

GSTs are another group of detoxifying enzymes which catalyse the conjugation of the reduced form of glutathione (GSH) to electrophile xenobiotic substrates (Habig *et al*., 1974). GSTs are ubiquitously distributed in nature, being found in organisms such as microbes, insects, plants, fish, birds, and mammals. GSTs are group of enzymes involved in detoxification of xenobiotic and also associated with protection from oxidative stress. By degradation of insecticide, GSTs produce more water-soluble compounds which can be easily excreted out of the body. In addition, it eliminates toxic free radicals formed by the pesticide within the body of the insect. GSTs may also bind toxins and function as transport proteins, which gave rise to the early term for GSTs, ligandin (Litwack *et al.,* 1971; Leaver and George, 1998).

Mixed function oxidases are involved in degradation of lipophilic compounds. It primarily functions as an electron acceptor and terminal enzyme is Cytochrome P 450 and NADH provides reducing equivalents. In insect gut, fat body and malpighian tubules have high MFO activity. The MFO activity is generally higher in gut than the other two tissues in

herbivorous insects, e.g., in the southern armyworm, *Spodoptera eridania* (Gramer) (Krieger & Wilkinson, 1969); gypsy moth, *Lymantria dispar* L. (Ahmad & Forgash, 1973); saturniid moth, *Antherea pernyi* (Guerin) (Krieger *et al.*, 1976); black cutworm, *Agrotis ipsilon* (Hufnagel) (Thongsinthusak & Krieger, 1976); house cricket, *Acheta domesticus* L. (Benke & Wilkinson, 1971); and the roach, *Gromphadorhina portentosa* (Schaum) (Benke *et al.,* 1972). The high MFO activity in gut is for the effective removal of the dietary allelochemicals before their entry into haemolymph.

Pesticide resistance in a population is mainly caused by continuous and over dose usage of the same chemical pesticide. This enables the pest population to survive in the nature and become the dominant biotype. Pesticide resistance can be managed by several ways. The usage of the pesticide only when needed, use labeled doses, use alternatives for pesticides can make the growers to use these chemicals for the pest management in the fields.

India ranks second worldwide in farm outputs. As of 2018, Agriculture employed 50 per cent of the Indian work force and contributed 17-18 per cent to country's GDP (India economic survey, 2018). As per the FAO (2014) world agriculture statistics, India is the world's largest producer of fruits like banana, mango, guava, papaya, lemon and vegetables like chickpea, okra and milk, major spices like chili pepper, ginger, fibrous crops such as jute, staples such as millets and castor oil seed. India is the second largest producer of wheat and rice. India is the world's second or third largest producer of several dry fruits, agriculture-based textile raw materials, roots and tuber crops, pulses, farmed fish, eggs, coconut, sugarcane and numerous vegetables. India is ranked under the world's five largest producers of over 80% of agricultural produce items, including many cash crops such as coffee and cotton, in 2010 (FAOSTAT, 2014) .

Banana (*Musa* sp.) belonging to the family Musaceae. It is one of the major tropical fruits and India is world's largest producer and consumer of banana. In India, it is the second most important fruit crop next to mango. India has the most favourable tropical climatic condition throughout year, for the growth of banana plant. About 20 per cent of the crop area in India occupies banana. "Of the 40 million tons of fruits produced in India, banana occupies the top position with an annual output of 13.5 million tons from an area of 4,00,000 ha" (Justin *et al*., 2008; Padmanaban *et al*., 2001). It can grow in both alluvial and volcanic soil. Njalipoovan, Kathali, Ayrinka Poovan, Robusta, Karpooravalli, Nendran, Red Banana, Palayam Kodan, Monthan are some of the varieties of banana. Modern edible varieties are formed from *Musa acuminate* and *Musa balbisiana* species. There are several hybrid varieties of banana like BRS2, BRS1, FHIA-01, Udhayam etc.

For successful banana cultivation good, porous fertile soil is necessary. Soil should retain water and must have good drainage capacity. The pH of the soil must be neutral 6.5-7.5 for the cultivation.The temperature needed for the growth ranges between 15ºC-35ºC and relative humidity 75-85 per cent. Since the banana plant is succulent and shallow rooted, it requires large quantity of water for maximum production. But the banana plant root system is not capable of as much withdrawal of water. Banana cultivation must be supported by proper irrigation system. The water use efficiency has been reported improved by the application of drip irrigation, trench irrigation and mulching technology. Field capacity should be maintained properly by adequate quantity of water. Improper water management may cause deleterious effect to the plant establishment.

The planting materials for the banana used by 70 per cent of the farmers are suckers and rhizomes while the rest 30 per cent of farmers use tissue culture seedlings for the cultivation. Banana requires high amount of

nutrients. The nitrogen content of the soil must be high with proper quantity of potash and phosphorous. Traditional farmers use more urea than potash and phosphorous. The essential nutrient requirement for the banana farming is 10 kg FYM, 200 - 250gm N; 60-70gm P; 300gm K per plant. Banana plantation requires 7-8 Kg N, 0.7- 1.5 Kg P and 17-20 Kg K per metric ton yield. Application of liquid fertilizers through drip irrigation is encouraged because it prevents the loss of nutrients more effectively than conventional fertilizers.

For keeping the plantation weed free, spraying of Glyphosate before planting at the rate of 2 l/ha and Diuron (1Kg a.i./ha) is carried out. For improving the morphological, physiological and yield of banana, micronutrient foliar application of $ZnSO_4$ (0.5%), $CuSO_4$ (0.2%), H_3BO_3 (0.1%) can be adopted. Removal of male buds or denavelling helps to fruit development and bunch weight increase. Spraying of monocrotophos (0.2%) effectively removes thrips, which attack the fruit and discolor it. In the areas of high wind speed, pseudostems are propped with bamboo is necessary especially at the time of bunch emergence.

Bananas are staple starch food and are highly nutritious. Raw bananas contain 75% water (74.91 g/100g), 23% carbohydrates (22.84 g/100g), 1% protein (1.09 g/100g), and contain small amount of fat (0.33 g/100g). It contains vitamins and minerals. It provides an average energy 371kJ (89kcal) per 100g. Bananas are excellent source of potassium and fibre, which help in cardiovascular health. Each and every banana plant parts are important and beneficial. Banana peels contain plant chemicals like antioxidants, and have been used in traditional and folk medicine as an antiseptic and anti-inflammatory to promote wound healing such as for insect bites, minor burns, and sun burns (Pereira and Maraschin, 2015). From the banana shoot, fiber is extracted and is commercially used in textile industry. Banana flower is used as a vegetable and is used for preparing several dishes. The large water proof

leaves of the plant are used as eco-friendly plates and for food wrapping. The tender core of the plants trunk is also used for food. In addition to it there are several dishes prepared from banana fruit.

Banana plant is affected by several pests and diseases. The plant is usually attacked by bacteria, fungi, viruses, nematodes and insects. Bacterial wilt, gumming, rhizome rot, bugtok diseases, blood diseases are caused by bacteria like *Pseudomonas solanacearum, Ralstonia solanacearum, Erwinia carotovora, Erwinia chrysanthemi*etc. Crown rot, corm dry rot, eyespot, brown blotch, fruit rot, fungal root rot, leaf spot, panama disease, peduncle rot, sheath rot, stem end rot, verticillium tip rot etc. are some of the fungal diseases caused by *Fusarium pallidoroseum, Junghuhnia vincta, Pestalotiopsis leprogena, Drechslera gigantea, Botryosphaeriaribis, Fusarium solani, Nectria haematococca*, *Fusarium oxysporum*, *Rhizoctonia* sp., *Curvularia eragrostidis, Nectria foliicola, Mycosphaerella musicola, Verticillium theobromae etc.* Bract mosaic diseases, bunchy top diseases, and mosaic diseases are caused by viruses like Banana bract mosaic virus, Abaca bract mosaic virus, Banana bunchy top virus, Abaca bunchy top virus etc. *Radopholus similis, Meloidogyne arenaria, Meloidogyne incognita, Meloidogyne javanica, Pratylenchus reniformia, Helicotylenchus multicinctus, Helicotylenchus dihystera* are some of the nematodes causing root-knot, root lesion diseases.

Banana plant is infected by several insect pests. Banana pseudostem borer *Odoiporus longicollis* Oliv., rhizome weevil *Cosmopolites sordidus*, banana aphid *Pentalonia nigronervosa*, fruit and leaf scarring beetle *Colaspis hypochlora* are some of them. Among which the major key pest is *Odoiporus longicollis* Oliv. effecting badly the banana production (Visalakshi *et al*., 1989; Valmayor *et al*., 1994; Shukla & Kumar, 1970).

Cosmopolites sordidus attacks rhizome of the plant and make holes and tunnels in the corm. Tapering of the stem at crown region, poor bunch formation, yellowing and drying up of leaves are some of the symptoms of its attack. Morphologically it is similar to banana pseudo stem weevil, but the size of this pest is small compared with pseudostem weevil. Rhizome weevil can easily spread through infested suckers. Field sanitation is necessary to control the pest. Use of Chlorpyriphos 20 EC @ 2.5 ml/L in suckers before planting is necessary. Pheromonal traps like cosmolure are used to attract and kill pest. Thiamethoxam (1g/5 L) and fipronil (10g per plant) is also used for the effective control of the rhizome weevil.

Banana aphid *(Pentalonia nigronervosa)* is the vector of bunchy top diseases in banana. They are mostly seen at the leaf base. Dimethoate 30 EC@ 2ml/l or Oxy-demeton methyl 25 EC @ 2ml/l spray can control this pest. Flea beetles (Fruit / leaf scaring beetle) *Colaspis hypochlora* is another pest of banana. They infest the unfolded leaves and fruits. The pest is at the peak during rainy season.

Banana pseudostem weevil, *Odoiporus longicollis* Oliv. is an important pest of bananas and plantains. It starts attack in banana stem in 4.5 months of the banana plantations. About 90% of the banana yield loss is due to the attack of this pseudostem weevil. The origin of banana pseudostem weevil is in South and South East Asia. This insect is found in India, China, Malaysia, Indonesia and Thailand, causing major threat to banana production (Valmayor *et al*., 1994). Assam, West Bengal, Delhi, Bihar, Uttar Pradesh, Karnataka, Kerala, North East Hill States have been reported this pest of banana (Isahaque, 1978; Dutta and Maiti, 1972; Batra, 1952; Shukla and Kumar, 1970; Jayanthi and Varghese, 1999; Visalakshi *et al*., 1989; Prasad and Singh, 1987). In Kerala it is first noticed in 1987 in Ernakulum district.

Banana pseudostem weevil attack is most severe during flowering stage. The larval stage of this pest causes 80 per cent of the damage. The larvae are voracious feeders. They make tunnels inside the pseudostem. The tunneling may range from flower peduncle up to the rhizome region. Tunneling interferes with the water and nutrient transport of the plant and the chances of stem breakage by wind or by bunch weight are high. The larval stages damage the ascending flower bud in preflowering stage of the plant (Padmanaban *et al*., 2001). Attack of the pest causes delayed or non-flowering of the infested plants. If the attack of the pest is after flowering stage, it causes the improper development of fruit and reduces the bunch weight and reduces the overall plant vigour.

Grubs bore the stem by making holes in the pseudostem. Tunneling of the grubs causes decomposition and wilting of the pseudostem. Initial symptoms include holes made by the pest on the pseudostem, exudation of the gummy substance and blackened mass from the holes, decaying of the peduncle resulting in immature ripening of fruits, yellowing and wilting of leaves and finally reduce the yield.

For the prophylactic and cultural control of the pest, field sanitation is important factor. Measures to restrain the damage caused by weevil vary widely depending upon the type of banana production systems practiced. Large plantations resort to regular application of chemical insecticides to control the weevil. Resource limited marginal farmers cultivating banana as a subsistence crop are unable to undertake chemical pesticide interventions on a regular basis. In this situation, cultural control strategies assume greater significance due to their ease of application and their compatibility with other methods of control (Padmanaban & Sathiamoorthy, 2001). The volatiles from the cut stems attract the weevil and hence it is necessary to remove the parts of stem and rhizome after harvesting. Split-log traps are effectively used to

control the pest. The discon-stump with higher exudations of plant fluids and longitudinal split pseudostem traps are effective in pest number reduction. It is recommended to use any of the insecticide after removing the dry outer sheath of the infested and uninfected plants. If the attack is severe the plant should be uprooted and burned. Usually the holes made by the stem are plastered by mud. Hot-water treatment has been carried out tothe weevil control. By immersing the suckers in hot-water baths of 52-55ºC for 15-27 minutes is effective in killing the larvae (Gold *et al*., 1999). Clean and hygienic condition is necessary for the effective control of the pest.

Usually the farmers use the tissue culture plants for banana plantations to control disease and pest from crops. BRS-1, BRS-2 are resistant strains produced in banana research centers. FHIA-25, FHIA-23, FHIA-17, SH-3640, TMB-5925-1 are resistant strains developed outside India. Among the Nendran variety, Manjeri Nendran is high disease resistant with comparatively high yield, low height, early maturing variety. Pisang lilin, Yangambi Km5, Sanna Chen Kadli., Dudhsagar, Chinali etc. are also resistant varieties.

Good husbandry practices, such as weeding, manuring and mulching produce pest resistant banana varieties (Feakin, 1971; Gowen, 2000).The cut surface of the longitudinal split traps can be swabbed with 20g of the formulation either entomopathogenic fungus, *Beauveria bassiana, Metarhizium anisopliae* or entomopathogenic nematode, *Heterorhabditis indica* (1x108 spores/ mg) and keep the split traps near the banana plant facing cut surface to soil is effective in control of the weevil. Injection of monocrotophos in the pseudostem at monthly interval from 5th to 8th month is effective in controlling the pest. Suckers can be dipped in monocrotophos for killing the pest. Two species of earwigs predate the pest by feeding the larvae and pupae of the pest. Acarid mites also parasitize the pest. They infest

the larval and adult stages. Fipronil 5% SC 3ml/1L in water is effectively used against the banana weevil. Carbosulfan 25EC 1.5ml/L in water is also used to protect the plant from this pest. At the heavy infested stage, chlorpyrifos 2.5 ml in 1L water is sprinkled on the plant for controlling the pest. Dersban (20%EC), chlorpyrifos (0.05%) at 2.5 ml/L application and psuedostem smearing with mud in Sevin 50WP (4 g/L) is used to protect banana from pseudostem weevil.

Carbosulfan is a carbamate pesticide widely used to control insects in a wide variety of field crops. Carbosulfan (2, 3-dihydro-2, 2-dimethyl-1-benzofuran-7-yl [(dibutylamino) sulfanyl] methycarbamate) is a broad spectrum systemic insecticide with contact and stomach action. By soil, foliar and seed treatment applications, carbosulfan is used in a variety of crops like banana, apple, sugar beets, rice, sorghum, potato and other vegetables.

The present study was aimed at investigating effect of sublethal toxicity of carbosulfan on adults of banana pseudostem weevil, *Odoiporus longicollis* Oliv. (Coleoptera: Curculionidae).

GENERAL REVIEW OF LITERATURE

The development of agriculture enabled the human population to grow many times larger than that could be sustained by hunting and gathering (Bocquet-Appel and Pierre, 2011). Major share of land is used for agriculture and over two thirds of water is used for agriculture around the world to raise about 85 major crops. Agriculture is not only for food, but also to obtain fibers, fuels, raw materials etc. These agricultural products support greater part of world economy. Modern agriculture along with the use of pesticides and fertilizers has increased the agriculture yields. But many human activities like deforestation, pollution, soil degradation etc. have slowed down the production. According to FAO's reports, out of traditionally cultivated 7000 species for food sources only 120 are being cultivated today. This has raised an issue that we may not be able to produce enough food that is required by the future populations.

Pests are important problem in successful cultivation as they reduce the quality of the production and the yield. 30-40 per cent of the world's potential production is lost annually due to pests, diseases, weeds etc. In India, crops are affected by over 200 major pests, 100 plant diseases, hundreds of weeds and other pests like nematodes, harmful birds, and rodents. "Food plants of the world are damaged by more than10,000 species of insects, 30,000 species of weeds, 100,000 diseases caused by fungi, viruses, bacteria and other microorganisms and 1000 species of nematodes" (Hall, 1995; Dhaliwal *et al*., 2007). However, major pests are only less than 10 per cent of generally identified pest species. For the increased output in food production, reduction in crop losses by pests is an obvious strategy. According to Boote *et al*., (1983) pests can be classified by their impacts, into the categories stand reducers (damping-off fungi), photosynthetic rate reducers (fungi, bacteria, viruses), leaf senescence accelerators (pathogens), light stealers (weeds, some

pathogens), assimilate sappers (nematodes, pathogens, sucking arthropods), and tissue consumers (necrotrophic pathogens).

Rodent damage is a serious impediment for agriculture. Its effects on agriculture are also complex because almost all crops are the target of rodent attack (Taylor, 1972; Fiedler, 1988; Singleton *et al*., 1999). They are responsible for substantial damage to food and cash crops and play an important role as reservoirs and carriers of zoonotic diseases. Survey reports have shown that on the average, rodents caused about 15 per cent loss of maize crop annually (Makundi *et al*., 1991). Although rodents have been identified as the most important mammalian agricultural pests at the global level (Cuong *et al*., 2002), birds also damage and/or destroy many crops prior to harvesting and the latter is a major pest for grain stored after harvesting. However, in recent years birds as pests in agriculture are attracting more attention in developed and developing countries alike. Dramatic rodent outbreaks have been reported in many countries where intensive and extensive cultivation of agricultural crops are major activities (Singleton and Redhead, 1990). Such outbreaks are common particularly in cereals such as maize, rice and guinea corn. The resultant effect of this damage is serious losses and spreading of the rodent populations as well as food shortages (Walker, 1990; Fayenuwo *et al*., 2000; Amusa *et al*., 2005). Damages ranging from negligible destruction to total crop loss have been experienced in major maize production of Africa.

Insects are the most diverse species of all animals on earth. An insect pest from an agriculturist's point of view is an insect causing sufficient damage to necessitate control measures (Kumar, 1984). On the other hand, from a human point of view, an insect pest may be defined as any insect in the wrong place – just as a rose bush is a weed when it is growing on a cabbage patch (Williams, 1947). Apart from the open ocean, insects can be found in all

habitats; swamps, jungles, deserts, even in highly harsh environments such as pools of crude petroleum (Imms, 1964). It is estimated that field and storage pests destroy approximately 43 per cent of potential production in developing Asian and African countries (Jackobson, 1982; Ahmed and Grainge, 1986; Ogendo *et al*., 2004).

Estimates of actual losses in crop production worldwide have been published by Cramer (1967) and Oerke *et al*. (1994). Insect pests are capable of evolving to biotypes that can adapt to new situations, for example, overcome the effect of toxic materials or bypass natural or artificial plant resistant, which further confounds the problem (Roush and McKenzie, 1987). Marlatt (1904) estimated pre-harvest losses caused by insect pests to be nearly 10 per cent. As per German authorities, in 1929 animal pests and fungal pathogens each caused a 10% loss of cereal yield, while, in potato, pathogens and animal pests reduced production by 25 and 5 per cent respectively, and in sugar-beet, production was reduced by 5 and 10 per cent due to pathogens and animal pests, respectively (Morstatt, 1929). Production losses in various field crops, fruits and vegetables in Great Britain were assessed by Ordish (1952). The first systematic attempt to estimate crop losses due to various pests globally was made by Cramer (1967), who estimated overall annual losses in major crops (including cereals, potato, vegetables, fruits, oil crops, fibre crops and natural rubber) to be about 34 per cent.

First, by changing or manipulating the environment, man has created conditions that permit certain species to increase their population densities (Ullyett, 1951). The rise of the Colorado potato beetle, *Leptinotarsa decemlineata* to pest status occurred in this manner. When the potato, as well as other solanaceous plants, were brought under wide spread cultivation in the United States, a change favorable to the beetle occurred in the environment which enabled this insect to become very quickly an important

pest. Similarly, when alfalfa, *Medicago sativa*, was introduced into California about 1850, the alfalfa butterfly, *Colias eurytheme*, which had previously occurred in low numbers on native legumes, found a wide spread and favorable new host plant in its environment, and it subsequently became an economic pest (Smith and Allen, 1954).

A second way in which arthropods have risen to pest status has been through their transportation across geographical barriers while leaving their specific predators, parasites and diseases behind (Smith and Allen, 1954). The increase in importance through such transportation is illustrated by the cottony cushion scale, *Icerya purchasi*. This scale insect was introduced into California from Australia on acacia in 1868. Within the following two decades, it increased in abundance to the point where it threatened economic disaster to the entire citrus industry in California. Fortunately, the timely importation and establishment of two of its natural enemies, *Rodolia cardinalis* and *Cryptochaetum iceryae*, resulted in the complete suppression of *I. purchasi* as a citrus pest (Doutt, 1958). The cottony cushion scale again achieved the status of a major pest when the wide spread use of DDT on citrus in the San Joaquin Valley eliminated the vedalia (Ewart and DeBach, 1947).

A third cause for the increasing number of pest arthropods has been the establishment of progressively lower economic thresholds. This can be illustrated by lygus bugs (*Lygus* sp.) on lima beans. Not too many years ago the blotches caused by lygus bugs feeding on an occasional lima bean were of little concern, and lygus bugs were considered a minor pest on this crop. However, with the emphasis on product appearance in the frozen-food industry, a demand was created for a near-perfect bean. For this reason, economic injury thresholds were established and lygus bugs are now considered serious pests of lima beans (stern *et al*., 1959).

A fourth way that insect can rise to pest status is by the elimination of natural enemies that hold a potential pest in check. For example, during the height of the emergency chemical campaign against the exotic spotted alfalfa aphid, *Therioaphis trifolii* in 1955 through 1957 in southern and central California, there were unprecedented numbers of a leaf miner, *Iriomyza* sp.; spider mites, *Tetranychus* sp.; pea aphid, *Acyrthosiphon pisum*; beet armyworm, *Spodoptera exigua*, and a leaf roller, *Platynota stultana*, causing damage in alfalfa. Circumstantially, at least, these pest upsurges seemed to have been correlated with the widespread and repeated use of the broadly toxic pesticides, parathion and malathion (Bosch and Stern, 1962). Certain of these pests caused considerable damage to alfalfa and *P. stultana*, spread to cotton where, for the first time, it caused serious damage to this crop in southern California (Atkins *et al*., 1957).

Fruits are known as protective foods because of their richness in vitamins, minerals and antioxidants, and their daily consumption protects human from various kinds of diseases. The current global fruit production is 599.3 million metric tonnes from an area of 55.08 million hectares. China, India and Brazil are the three leading fruit growing countries in terms of area and production (Anonymous, 2012). There are various insect pests of horticultural crops. They include stem and leaf eaters like the caterpillars of certain moths and butterflies, cabbage worm, tomato worm, certain beetles and their larvae such as the blister beetle, common bean beetle and the colorado potato beetle, the nymphs and adults of grasshoppers, stem borers, corn-borers, feeders on fleshy fruits, seed and storage organs like the bean weevil, tomato fruit worm etc. The feeding habit of the citrus thrips makes it a serious pest at low altitudes where an attempt to produce unblemished fruits is being made. It damages the fruit by producing a ring of scaly brown tissue around the stem end of the fruit and irregular areas of scarred tissue on the other parts of the fruit. The young leaves may also be damaged; aphids affect

citrus as well as a variety of other crops such as banana and maize (Hill, 1987).

More than 600 species of beetle pests, 70 species of moths, and about 355 species of mites, attack stored products of agricultural and animal origin causing quantitative and qualitative losses (Rajendran and Sriranjini, 2008) and insect contamination in food commodities is an important quality control problem of concern for food industries. In industrialized countries like Canada and Australia, there is zero tolerance for insects in food grains (White and Leesch, 1995). Control of stored-product insect populations is primarily dependent upon continued applications of insecticides (White and Leesch, 1995). In spite of its efficacy, their repeated use for several decades has disrupted biological control system by natural enemies and led to outbreaks of insect pests, wide spread development of resistance, undesirable effects on non-target organisms, and environmental and human health concerns (Subramanyam and Hagstrum, 1995).

These problems have highlighted the need for the development of new types of selective insect control alternatives. Plants may provide potential alternative to currently used insect control agents because they constitute a rich source of bioactive chemicals (Wink, 1993). Since these are often active against a limited number of species including specific target insect, they could lead to the development of new classes of insect control agents. Therefore, much effort has been focused on plant derived materials for potentially useful products as commercial insect control agents. Little work has been done to manage stored product insects by using aromatic medicinal plants despite their excellent pharmacological actions (Tang and Eisenbrand, 1992; Namba, 1993).

Secondary metabolites of plants are well known for their various properties such as larvicidal, bactericidal and fungicidal activities (Ikram and

Immanual, 1984; Sousa *et al.,* 1991). Several components having antimicrobial properties were also isolated and identified from plants (Li and Xu, 2008; Verma *et al.,* 2009) which have great significance in treatment of various microbial infections. Many plants and their crude extracts are used in traditional medicine for the treatment of various diseases such as fever, cough, diarrhea, skin diseases, gout, heart diseases, cuts and wounds and burns etc from time immemorial (Kirtikar and Basu, 1935).

Insect growth regulators (IGRs) are novel insecticides that interfere in the processes of molting and metamorphosis of insects. Two major insect specific target processes are the biosynthesis of chitin in cuticle and the activity of hormones such as juvenile hormone and the insect molting hormone, 20-hydroxyecdysone. Over the last decades several insect growth regulators have been developed such as chitin synthesis inhibitors (e.g., diflubenzuron and flufenoxuron), juvenile hormone analogues (e.g., pyriproxyfen), ecdysone agonists (e.g., RH-5849 and halofenozide) and azadirachtin-based products (e.g., Neem Azal T/S). Some IGRs are active against aphids as reported by Hatakoshi *et al*., (1991) and Kerns and Stewart, (2000).

Alternatives to traditional chemical insecticides, such as predators, parasitoids, microbes and natural products have been gaining interest among researchers concerned with developing integrated pest management (IPM) strategies for insect control (Copping and Menn, 2000). However, only a few of these alternative methods, such as pyrethrum and *Bacillus thuringiensis* (Bt)-based products, have been commercially successful in the pesticide market. For several years, we have been investigating a variety of alternatives to conventional chemical pesticides in stored product IPM. Controlling insect pests in stored grain and grain products can be very difficult because of the variety of species that can infest grain. Insect parasitoids have been shown to

be effective in suppressing a limited number of pest species both in bulk grain storages and in food processing facilities and warehouses (Scholler and Flinn, 2000). One of the more effective parasitoids is *Theocolax elegans* (Westwood), a small pteromalid wasp (1–2 mm) that attacks primary grain pests, whose immature stages develop inside the grain kernels, including the weevils, *Sitophilus* sp., lesser grain borer, *Rhyzopertha dominica*, drug store beetle, *Stegobium paniceum*, cowpea weevil, *Callosobruchus* sp., and Angoumois grain moth, *Sitotroga cerealella* (Burks, 1979; Flinn *et al*., 1996; Flinn, 1998; Flinn and Hagstrum, 2001). However, *T. elegans* does not parasitize species that are secondary grain pests, including the flour beetles, *Tribolium* sp., and the rusty grain beetle, *Cryptolestes ferrugineus*, whose immature stages develop outside of the grain kernel.

There are two main alternatives to pest control by the broad-spectrum eradicant pesticides currently in use. One method is the exploitation of semiochemicals, including pheromones, which are natural signals that affect changes in the behaviour or development of many organisms (Hardie and Minks, 1999; Matthes *et al*., 2003). The second comprises biological control agents, which range from pathogens of pests to other antagonistic organisms, including predators and parasitoids (Powell and Pickett, 2003). Plants bred conventionally for resistance, or genetically modified organisms (GMOs) expressing resistance traits, produce toxicants or agents which, by other modes of action, cause a lack of development or destruction of the pest and thus fall into the conventional approach, ie., incorporating toxic or direct physiological mechanisms. Semiochemicals, on the other hand, work by non-toxic modes of action and are often the same as or closely related to, food components or naturally derived or nature-identical food additives. These could, therefore, be exploited as non-toxic agents from GMOs, or as a result of more conventional breeding (Pickett and Poppy, 2001; Pickett *et al*., 1997). If the biological control agents act by antibiotic effects, then logically they

should fall into the first category, i.e., those to which we are seeking alternatives. However, those acting by pathogenicity would contribute to the new generation of alternatives. Already, synthetic but nature-identical insect pheromones are in wide use around the world for controlling pests of high-value horticultural crops (Trumble and Alvarado-Rodriguez, 1993; Boller and Hurter, 1998; Howse *et al*., 1998; Agelopoulos, 1999).

Since 2000 BC, humans have utilized pesticides to protect their crops. About 4500 years ago in ancient Mesopotamia the first known pesticide was elemental sulfur dusting was used. By the 15th century, Toxic chemicals like arsenic, mercury and lead were being applied to crops for pest control. In the 17th century, nicotine sulfate was extracted from tobacco leaves were used as an insecticide. The introduction of two more natural pesticides, pyrethrum, which is derived from chrysanthemums, and rotenone, which is derived from the roots of tropical vegetables was in the 19th century. In the 20th century the use of insecticides is believed to be one of the major factors behind the increase in agricultural productivity.

Almost 50 years ago, insecticide research led to the discoveries of the chlorinated hydrocarbons, organophosphates, methylcarbamates, and pyrethroids which are all neuroactive chemicals. Most of our current insecticides were discovered in this golden age. Flonicamid and pymetrozine are two novel insecticides with selective activity against Homoptera, acting as feeding inhibitors with high mortality due to starvation (Harrewijn and Kayser, 1997; Denholm *et al*., 1998; Morita *et al*., 2007). Imidacloprid is the most important neonicotinoid insecticide with good systemic activity that acts as an agonist of the insect nicotinyl acetylcholine receptors, causing the insect to reduce or stop feeding and mobility. It is particularly effective against aphids, whiteflies and plant hoppers (Boiteau and Osborn, 1997; Elbert *et al*., 1998; Nauen *et al*., 1998).

Chemical control has been practiced by farmers for maximum production. The chemical pesticides are applied in larger quantity than allowed limit caused pest resistance problems (Obeng-Ofori and Ankrah, 2002). Thus pest resistance, resurgence of pests, pesticide residues, destruction of beneficial fauna and environmental pollution may occur (AVRDC, 2003).

The effects of persistent organochlorine contaminants on raptor populations have been widely documented (Newton, 1979; Noble and Elliott, 1990). Organochlorines largely have been replaced by organophosphorous and carbamate insecticides that are considered less persistent, less bioaccumulative and therefore of lower risk for secondary poisoning of raptors. There are occasional published reports of anticholinesterase secondary poisoning of raptors by, for example, carbofuran (Balcomb, 1983). Recently, Porter (1993) concluded that secondary poisoning of raptors was a relatively common occurrence. Large numbers of waterfowl and raptors winter in agricultural fields of the Fraser Delta of British Columbia (Butler, 1992). Many fields are intensively farmed for vegetables and use of toxic, granular insecticides is common (Szcto and Price, 1991).

Development of resistance to insecticides always poses obstacles to the successful control of insects. The problem of insect resistance to insecticides started to attract our attention between the years 1950 and the early 1960s, but has subsided materially in later years. That is because, other than screening for new insecticides, no economical solution has been found to date, to remedy the resistance situation and no new, challenging, and thought provoking ideas have been forthcoming to give impetus to renewed interest in this scientific endeavor. Recently, however, such interest has experienced spontaneous rejuvenation due to knowledge gained from mammalian pharmacology, specifically from experimental evidence on the role of

microsomal enzymes in drug metabolism, the induction of this important enzyme system by drugs and insecticides, and its inhibition by so-called synergists.

By far the predominant factor in resistance is the ability of the resistant insect to detoxify the compound at a faster rate and in large quantities than its susceptible counterpart. It is now generally agreed that both susceptible and resistant insects basically contain the same detoxifying enzymes, so that differences between the strains are quantitative rather than qualitative.

Insecticide resistance poses a serious threat to increased agricultural production. By 1990 over 500 insect species were reported to be resistant to at least one insecticide class, and many were resistant to several. Melander (1914) is generally credited with the first publication on insect resistance and the now famous quotation, “Can insect become resistant to sprays?”, although observations on resistance had been made as early as 1887 (Babers and Pratt, 1951). It is widely accepted that the development of insecticide resistance by insects is due to the selection of variants in the population carrying preadaptive genes (Crow, 1957).

The rate at which resistance develops in a population may depend on the rate at which the species breeds (Kerr, 1963). In some instances of geographical isolation, confined populations may not contain the prerequisite resistant genes, and therefore do not develop resistance (Elliott, 1959), and highly inbred laboratory strains may fail to develop resistance if the gene pool contains no resistance factors (Crow, 1966; Harrison, 1952 ; Merrell and Underhill, 1956).

Synthetic organic insecticides provide effective insect control, but their wide spread use has resulted in toxicity to natural pest enemies, toxic residues in plants and the environment, and insect resistance. After insecticide

treatment, resurgence of pest on rice is becoming common. Such an abnormal increase in the pest population after insecticide application often far exceeds the economic injury level. Insecticide-induced pest outbreaks have been reported in walnut (Barlett and Ewart, 1951), hemlock (McClure, 1977), soybeans (Shepard *et al*., 1977), and cotton (Bottrell and Rummel, 1978).

Insects are faced with numerous xenobiotics in their life, some produced naturally by plants called allelochemicals and some produced by humans like insecticides. Insects have evolved detoxification mechanisms to survive the natural toxins. These same mechanisms also sometimes allow insects to overcome insecticides, and the level and type of mechanism differ greatly. Knowledge of detoxification helps us to incorporate chemical resistance mechanism in crop plants, and to choose the insecticides that will be effective when applied.

The three most important systems of detoxification in insects are the microsomal oxidases, the glutathione-s -transferases and the carboxyesterases, which degrade carbamate, organohosphate and pyrethroid insecticides. In order to survive, insects must deal with both naturally occurring plant toxins and synthetic insecticides in their diets. From the inception of synthetic insecticide usage in the 1940s, insects have been exposed to several major classes of insecticides including DDT, organochlorines (DDT, cyclodienes), organophosphates, carbamates, pyrethroids and insect growth regulators. The continual use of a limited number of these compounds has resulted in the evolution of insecticide resistances in more than 500 insect species mediated variously by esterases, glutathione-s-transferases (GSTs) and coupled cytochrome P450 monooxygenase (P450): NADPH-dependent P450 reductase systems (Feyereisen, 1999, 2005; Scott, 1999; Sheehan*et al*., 2001; Ranson *et al*., 2002; Li *et al*., 2007). Of these, detoxification by coupled

P450:P450 reductase systems represents one of the most prominent routes responsible for the inactivation of synthetic and natural toxins.

Since organophosphates, carbamates, and pyrethrins are all esters it might be expected that they would be readily degradable by esterases, but this appears not to be so. It should be pointed out that older reports of hydrolytic degradation based solely on the nature of the products found cannot be trusted, since it has been shown, mainly by the work of Nakatsugawa and Dahm (1968), that these products can also be derived by oxidation, in which case their formation is dependent on the presence of NADPH. Parathion thus is oxidized by microsomes and the products found are diethyl phosphorothioate and p-nitrophenol. Since these products are not really of a higher oxidation level, the author believes that one should assume the formation of an intermediate that is oxidized, this intermediate then giving rise to the products mentioned.

The report of Matsumura and Hogendijk (1964) of the hydrolytic detoxification of a P = S compound (parathion), could not be confirmed by Nakatsugawa *et al*. (1969). P = S compounds seem not to be attacked hydrolytically, except where additional ester groups are present, as in malathion. Hydrolytic degradation of phosphates does occur and is a resistance mechanism, but its role seems to be smaller than what was originally thought. Oppenoorth and van Asperen (1960) proposed the “mutant aliesterase" theory, which postulates the existence, in many organophosphate-resistant houseflies, of an organophosphate-hydrolysing enzyme replacing an esterase present in susceptible flies. This theory was severely criticized by O'Brien *et al*. (1966) on the grounds that the rate of detoxification found would be insufficient to explain the degree of resistance, and that enzymatic action had not been proved since products of hydrolysis had not been studied. This has recently been done by Welling (1971) for paraoxon, and this study

has proved beyond doubt that in parathion-resistant strains there is a phosphatase that slowly forms diethyl phosphate and p-nitrophenol. The enzyme is not dependent on NADPH, as has been suggested by Casida (1970), but is a true hydrolytic enzyme, which can still be irreversibly inhibited by some organophosphates (e.g., the n-propyl homologue of paraoxon). This enzyme may not be responsible for all of the resistance.

A systematic and complete overall picture of the importance of different detoxification routes is certainly not yet available. In addition to hydrolysis and microsomal oxidation there are such special features as a DDT dehydrochlorination enzyme, which is glutathione-dependent, and dealkylation enzymes present in the soluble cell fraction, which transfer alkyl (mainly methyl) groups from organophosphates to glutathione (Lewis, 1969; O'Brien and Yamamoto, 1970). Whereas the former is the earliest and classic example of a detoxifying enzyme causing resistance, the role of the latter is only just becoming recognized and will require much further study. Owing to the considerable improvement in techniques in recent years, much progress in elucidating the relative importance of the different detoxification routes is to be expected in the near future.

It is quite apparent from toxicological observations that there exists a dynamic variation in tolerance to a poison among individuals of a given population. However, truly sublethal doses of insecticides do not induce resistance in susceptible populations of the house fly (Beard, 1952, 1965; Brown, 1964), or increase the levels of DDT dehydrochlorinase (Moorefield, 1958). Rather, the cumulative effect of daily sublethal amounts of DDT, γ-BHC, dieldrin or diazinon renders the housefly more susceptible (Hadaway, 1956).

Continuous use of insecticides has resulted in Brown plant hopper (BPH) resistance to insecticides in Taiwan (Lin *et al.,* 1979), Japan (Nagata,

1979), and the Philippines (Heinrichs, 1979). After application of insecticides, BPH resurgence was reported in Bangladesh (Alam and Karim, 1977), India (Varadharajan *et al.,* 1977; Chandy, 1979), Indonesia (Oka, 1978; Soekarna, 1979), the Philippines (IRRI, 1979), and the Solomon lslands (Stapley *et al.,* 1979). Most of the hopper burned fields reported or observed in India, Indonesia, Philippines, and Sri Lanka received insecticides before the outbreak. Detailed investigations have been made in the past few years on the insecticide-induced BPH resurgence in rice (Chelliah, 1979; Chelliah and Heinrichs, 1980; Chelliah *et al*., 1980; Raman, 1981; Heinrichs *et al.,* 1982; Reissig *et al.,* 1982). Degree of resurgence is dependent on the method, timing, and number of insecticide applications and the level of varietal resistance to BPH.

Suppression of natural enemies following intensive broad-spectrum insecticide application was suggested as an important factor for BPH resurgence in rice (Kiritani, 1972, 1975; Kiritani *et al.,* 1971; Kobayashi, 1961; Miyashita, 1963). Dyck and Orlido (1977) reported that reduction in the population of the mirid predator *Cyrtorhinus lividipennis* after regular spraying with methyl parathion caused BPH resurgence. However, extensive field studies conducted later did not show adequate evidence that reduction in the *C. lividipennis* population caused the resurgence (IRRI, 1978; Chelliah, 1979). Reissig *et al*. (1982) indicated that when BPH resurgence occurred in the field, the population of the most important predators such as spider's *C. lividipennis* and *Microvelia atrolineata* could not increase to a sufficient level to suppress the increasing BPH population. Their investigations further indicated that when resurgence-inducing insecticides were applied in the field, they stimulated BPH population growth regardless of their relative toxicity to natural enemies. Natural enemy destruction was a minor factor (Chelliah, 1979; Heinrichs *et al*., 1982).

It is also well established that insecticides are not mutagenic. Genetic variability for resistance already exists in natural populations which are not previously exposed to insecticides. This is also evident from the fact that resistance develops slowly in highly inbred lines. If new mutations were to arise, inbred lines under selection pressure would develop resistance as rapidly as heterogenous populations (Crow, 1966). Genetic variability in DDT dehydrochlorinase content is also evident from analysis of individuals in a random population of houseflies selected with DDT, showing a clear genetic relationship between DDT-dehydrochlorinase and resistance (Lovell and Kearns, 1959).

Pesticides are widely used to combat diseases and pests. Residues of those compounds can sometimes find their way to the human consumers or to the environmental compartments (Ecobichon, 2001). The organophosphorus insecticides, malathion, fenitrothion etc., are regularly sprayed on stored grain to protect against grain weevils on the assumption that residues in subsequent products are negligible. However, studies have shown that whole meal bread prepared from treated grain can contain up to 50 per cent of the original dose (Wilkin and Fishwick, 1981). Pesticide residues may also occur in the air we breathe. Every year in Britain over 1 billion gallons of pesticide sprays are dispersed over crops and more than 20 per cent of this spray is released as droplets too small to settle; they can thus drift widely in the wind (Soil Association, 1984). Anyone living close to areas where spraying regularly occurs is almost certain to breath some contaminated air.

Heinrichs *et al*. (1979) reported that, in rice, application of granular formulations of carbofuran, isazophos, ethoprop, and acephate significantly increases plant height. Spraying rice plants with methyl parathion induced tillering (Chelliah, 1979). Recently, Raman (1981) reported that foliar application of deltamethrin and methyl parathion resulted in increased number

of tillers and leaves and increased plant height. The phytotonic effect (healthy, green plants) of certain insecticides may attract more macropterous hoppers immigrating into rice fields. The alighting followed by increased feeding, reproduction, and longevity would increase BPH resurgence.

In spite of official assurances that all is safe, and often after many years of heavy usage, regularly in recent years individual pesticides have been shown to be dangerous and have been banned or heavily restricted. Compounds such as dieldrin, endrin, aldrin, DDT, Kelthane, methidathion, parathion, trifluralin, captan and DBCP have been suspected or demonstrated to be carcinogenic (Ames, 1979; Davies, 1977; Jackson *et al*., 1982). The true state of affairs is that pesticides are subjected to rigorous safety testing before they are used commercially and that significant pesticide residues in our food are rare.

The use of pesticides in agriculture led to an improvement in the crop yield. Studies have established a possible correlation relationship between the quantity of pesticides used per hectare and the amount of crop yields per hectare (Hellar, 2002).

CHAPTER 1

EFFECT OF CARBOSULFAN ON THE ENZYMES OF GST, MFO, AChE OF ADULT BANANA PSEUDOSTEM WEEVIL

1.1 INTRODUCTION

Banana is one of the major fruit crops in Kerala. Among the different varieties of banana grown, Nendran production is about 57% compared to others. Robusta, Palayankodan and Poovan are also cultivated in Kerala on a limited scale. In spite of the predominance of the fruit crops of Kerala, the productivity in the state is only 14.2 tonnes/ha as against the national average of 26.40 tonnes/ha. Major problem that limit banana cultivation is the banana pseudostem weevil, *Odoiporus longicollis.* In India, nineteen species have been reported to infest banana (Padmanaban and Sathiamoorthy, 2002) and of these, the banana stem weevil (BSW), known as pseudostem weevil/pseudo stem borer *Odoiporus longicollis* Oliver (Coleoptera: Curculionidae) is a serious pest causing heavy loss to the grower which affects both production and productivity of bananas and plantains (Ostmark, 1974)

Banana weevil is an important pest of banana, plantain (*Musa* sp.), and ensete (*Ensete* sp.). It is one of the major constraints for banana production in most of the countries. The pest control is difficult due to the fact that the pest life cycle is completed within the pseudostem. Chemical control measures are the best method for managing the pest. To control the pest the stem injection of monocrotophos is used (Sathiamoorthy *et al.*, 1998). Swabbing with surfactants and mud slurry containing the insecticide may also use for the control of the pest (Mathew *et al*., 1997). The region or site of egg laying on pseudostem needs to be covered by insecticidal spray to prevent egg laying (Dutt and Maiti, 1972). Dutt and Maiti (1972) observed maximum mortality of adults when exposed to contact insecticides or when adults come in contact with insecticide treated soil. Carbaryl 50 WP at 0.1 per cent spray also gave good results (Isahaque, 1978). Application of phorate or carbofuran @ 25 g / plant at the basal region upto six months from planting is effective in

controlling the pest (Visalakshi *et al*., 1989). Chemical control is regarded by farmers as easy to manage, fast acting and effective (Gold *et al*., 1994). In India infested pseudostems have been fumigated after the initiation of flowering, otherwise there was a phytotoxic effect. Three aluminium phosphide tablets per plant were inserted 15 cm above ground level, at a depth of a quarter of the pseudostem diameter. After insertion, the entry hole was sealed (Anon, 1977).

Banana pest management is an important activity in commercial banana production programme. Insecticide resistance is one of the major obstacles to the successful cultivation. When naturally occurring genetic variation allows a small proportion of the population to resist and survive the effects of the insecticide to develop resistance. Host plant resistance is one of the long term control strategy for the banana weevil within the framework of integrated pest management (Seshu-Reddy & Lubega, 1993). The banana weevils can decrease root growth, nutrient uptake and plant vigour. It leads to small fruit bunches and yields, and weaken the overall stability of the plant. Infestations in newly planted fields results in crop failure. Highland bananas and plantains are more susceptible varieties. The objective of the study is to evaluate the effect of carbosulfan on the adult banana weevil and on its detoxifying enzymes.

1.2 REVIEW OF LITERATURE

The pseudostem weevil was first reported by Fletcher (1914) at Pusa, Bihar. The pest was also reported from Sri Lanka, Java (Frogatt, 1928), Hong Kong and Hawai Islands (Hoffman, 1932), Formosa (Kung, 1955), and China (Luo *et al*., 1985). The occurrence of this insect pest in Kerala in alarming proportion was experienced during the late eighties only (Sheriff and Thomas, 1988). In Kerala all the varieties of banana such as Nendran, Palyankodan

and Poovan varieties are vulnerably attacked by this pest causing heavy production loss for the commercial growers.

Chemical control of the beetle has been employed since the early 20th century. Pesticides consisted mainly of Paris Green, followed by the use of organochlorines like BHC and DDT (Froggatt, 1925; Cuillé, 1950; Simmonds, 1966; Treverrow *et al*., 1992). The chemicals were usually applied with flour or other substances as baits (Froggatt, 1925; Cuillé, 1950; Simmonds, 1966; Treverrow *et al*., 1992). The method was not very effective (Simmonds, 1966) and the persistent cyclodienes, dieldrin and aldrin, showed high efficacy as a soil treatment against the banana weevil (Braithwaite, 1958). Cyclodienes was used extensively around the world from the mid 1950's (Edge, 1974) and was found to be effective for up to 2 years after application (Braithwaite, 1967). Before 1970, however, resistance to cyclodienes was widely diagnosed (Vilardebó, 1967; Shanahan & Goodyer, 1974). Aldicarb, terbufos, carbofuran, carbosulfan, oxamyl, fenamiphos (Román *et al*., 1983; Cárdenas, 1984; De Jager *et al*., 1991; Vittayaruk *et al*., 1994; Chavarria-Carvajal & Irizarry, 1997; Fogain *et al*., 2002), isofenphos, isazofos (Bujulu *et al*., 1983), phoxim (Nuno & Ribeiro, 2002) tebupirimiphos and cadusafos (Quilici, 1993), fosthiazate (Chabrier *et al*., 2002), phorate, disulfoton, quinalphos (Viswanath, 1977), acephate, diethyl, pada, monocrotophos, deltamethrin (pyrethroid) (Maolin,1994), fipronil (phenyl pyrazole) (Price, 1995; Fogain *et al*., 2002) and bifenthrin (pyrethroid) (Smith, 1995) were also found to be effective.

On susceptible banana varieties, infestation might be controlled by a drench with endosulfan (35% EC) or Carbaryl (50% WP) each at 0.1% strength within the leaf whorls or on the leaf sheath at monthly interval during March-September (Ishaque, 1978). According to Luo *et al*. (1985) Decis (Deltamethrin) is effective against adult weevils. Mathew *et al.* (1996) studied

the effectiveness of the two chemical insecticdes, carbaryl and chlorpyriphos against the stem borer. Results indicate that swabbing chlorpyriphos (0.05%) was the best treatment giving complete protection of the treated plants.

Aldicarb, carbofuran, chlorpyrifos, cyclodiene, dusband, organophosphates and pirimiphos-ethyl are the insecticides for chemical control of pest (Aba *et al*., 2011; Marilene *et al*., 2013; Bwogi *et al*., 2014; Carval *et al*., 2016). Chemical control may be used for its high mortality of pest and for fast acting, manageable and effective method (Aby, 2015; Tinzaara *et al.*, 2015). In Tanzania, chemical application in weevil control has met little success by complex undescribed banana distribution patterns in different farming systems and also high cost (Bujulu *et al*., 1983; Rannestad *et al*., 2013). However, chemical control is a fast solution for the banana weevil control while its long-time application resulted in weevil resistance (Gokool *et al*., 2010; Bortoluzzi *et al*., 2013; Bwogi *et al*., 2014; Aby *et al*., 2015).

Weevil resistance towards these chemicals has recently been reported in some countries (Collins *et al.*, 1991; Gold *et al.,* 1999). In recent years, there has been increased interest in the development of host plant resistance to banana weevil (INIBAP, 2001). However, very little work has been done on screening for banana weevil resistance. Available reports (Pavis & Lemaire, 1997; Kiggundu *et al*., 1999; Gold *et al*., 2002) were inconclusive defining resistant clones. Much of the information on weevil damage levels on different cultivars had been collected in surveys (e.g. Gold *et al*., 1994) and therefore, confounded by site differences. Fogain & Price (1994), Ortiz *et al*. (1995), Rajamony *et al*. (1993, 1994, and 1995) and Anitha *et al*. (1996) conducted screening trials to identify existing clones displaying resistance to weevil. Banana farmers have limited knowledge on weevil biology. Some farmers believe that larvae are more destructive than adult and others believe

the opposite (Ssennyonga *et al.,* 1998; Okech *et al.,* 2006). Chemical insecticides cause unwanted long-term effect, including insecticide resistance (Gold and Messiaen, 2000), pest resurgence, pest outbreak, ground water contamination and radical effects on beneficial insects (David and Vasantharaj, 2008).

The present study aims at investigating the effect of carbosulfan on the enzymes like GST, MFO and AChE in the different tissues of the insect banana pseudostem weevil, *Odoiporus longicollis* Oliv. The study also aims at investigating the presence of enzymes that breakdown the insecticide, carbosulfan, in the different tissues of the insect, banana pseudostem weevil, *Odoiporus longicollis* Oliv.

1.3. MATERIALS AND METHODS

1.3.1. Target Insect

Insects selected for the present study, the Banana pseudostem weevil, *Odoiporus longicollis* Oliv. (Coleoptera: Curculionidae) is an important pest of banana and plantain. The adult weevil is black and measures 23-39 mm (Plate 1.1). In India, red-coloured morphs of the BSW are also present (Padmanaban and Sathiamoorthy, 2001). According to Dutt and Maiti (1972) colour difference is due to non-sex-limited variation and not due to sexual dimorphism.

Banana stem weevil is mostly seen in between the leaf sheaths, inside the pseudostem, in the soil at the base of the plant, and also in the the crop residues. It is free living insect. The weevil is active during night and easily susceptible to desiccation. Since the adults rarely fly, it may remain at the same mat for long time. The adult weevils rarely fly. Dissemination is primarily through infested planting material. The pest banana pseudostem weevil enjoys a wide distribution throughout the tropics, and advancement of

infestation by the weevil during the late pre-flowering stage culminates in the failure of ascending flower bud and peduncle (Padmanabhan *et al*., 2001). Incidence of BPW has been reported from different parts of India and it is becoming very serious in Southern India, particularly in Tamil Nadu and Kerala (Reghunath *et al.,* 1992; Justin *et al.,* 2008). The oviposition punctures inflicted by female weevils and the tunnels made by the grubs turn the plant fragile and weak, causing premature falling (Ravi and Palaniswami, 2002; Anitha, 2004). Infestation by weevil perpetrates hefty crop loss (Padmanaban *et al*., 1999; Gold *et al.,* 2001) to a tune of 10-90%, depending on the intensity of ravage and management efficiency (Prasuna *et al.,* 2008).

A. Female **B. Male**

Plate 1.1. A& B Adult banana pseudostem weevil *Odoiporus longicollis* Oliv.

The life span of most of the adult banana pseudostem weevil is about one year but some may live for four years. Since they are mostly seen associated with moist substrates, they can survive for several months without feeding. Mostly the insect lay about 1egg/day and 1:1 is the sex ratio. The pre-oviposition period is 15-30 days. The female adult insect make slits in the pseudostem using its rostrum and gravid female lay yellowish white elliptical eggs in it. Inside the leaf sheath the oviposition take place. 'The rate of egg laying decreases as the number of adults increases due to the existence of a spacing pheromone, epideictic compounds which act as a deterrent to conspecific females' (Ranjith and Lalitha, 2001).

The creamy eggs are cylindrical in shape. After 3-8 days, eggs hatch into yellowish white, apodous larvae (Plate 1.2). The emerging larvae preferentially feed on the pseudostem. They make tunnels inside the stem (Plate 1.3). There are about 5-8 larval instars present. "The larva causes damages in the advanced pre flowering stage of the plant by feeding on the flower bud and the peduncle inside the pseudostem resulting in the non-emergence and decay of the flower bud" (Padmanaban and Sathiamoorthy, 2001). After the 5^{th} larval instar stage, it enters into the pupal stage for about a week. Pupation takes place inside the cocoon formed by the fibrous leaf sheaths. On the surface of the pseudo stem pupation take place. Temperature affects the rate of development due to which the duration of the life stages is longer in winter than in summer. The whole life cycle completes in about 35-40 days.

Plate 1.2. Larva of banana pseudostem weevil *Odoiporus longicollis* Oliv.

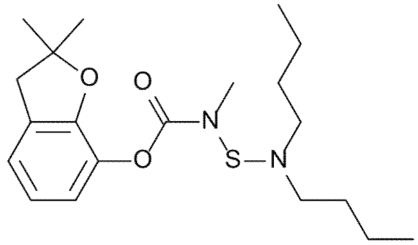
1.3.2. Collection of the insects and culture maintenance

Insects were collected from Annassery, Vellalassery, Feroke, Chathamangalam, Nadakkavu, Kuttiady and Thottilpalam areas in Kozhikode district (Plate 1.4). From the damaged banana stems adults and larvae were collected and maintained in the lab. Insects required for the experiment were drawn from the culture just before the experiment.

1.3.3. Carbosulfan

Bioassays were carried out with Carbosulfan standard. Carbosulfan is a broad-spectrum insecticide, nematicide and miticide effective against pests and mites. Carbosulfan is safe to crops and effective to both pests and larvae by good systemic properties, low residue and long-term effect. The oral LD_{50} for rats is 90 to 250 mg/kg bw, inhalation LC_{50} is 0.61 mg/L. Carbosulfan is only slightly absorbed through skin. The mechanism of toxicity is based on reversible inhibition of acetyl cholinesterase. Carbosulfan is used for the protection of the fruit and vegetable crops like apple, citrus, corn, potato, rice,

sorghum, soybean, sugar beets, and sugarcane by soil, foliar and seed treatment applications.



Carbosulfan

Carbosulfan standard, PENSTANAL analytical grade with molecular formula $C_{20}H_{32}N_2O_3S$ was purchased from Sigma-Aldrich and is used for the assay.

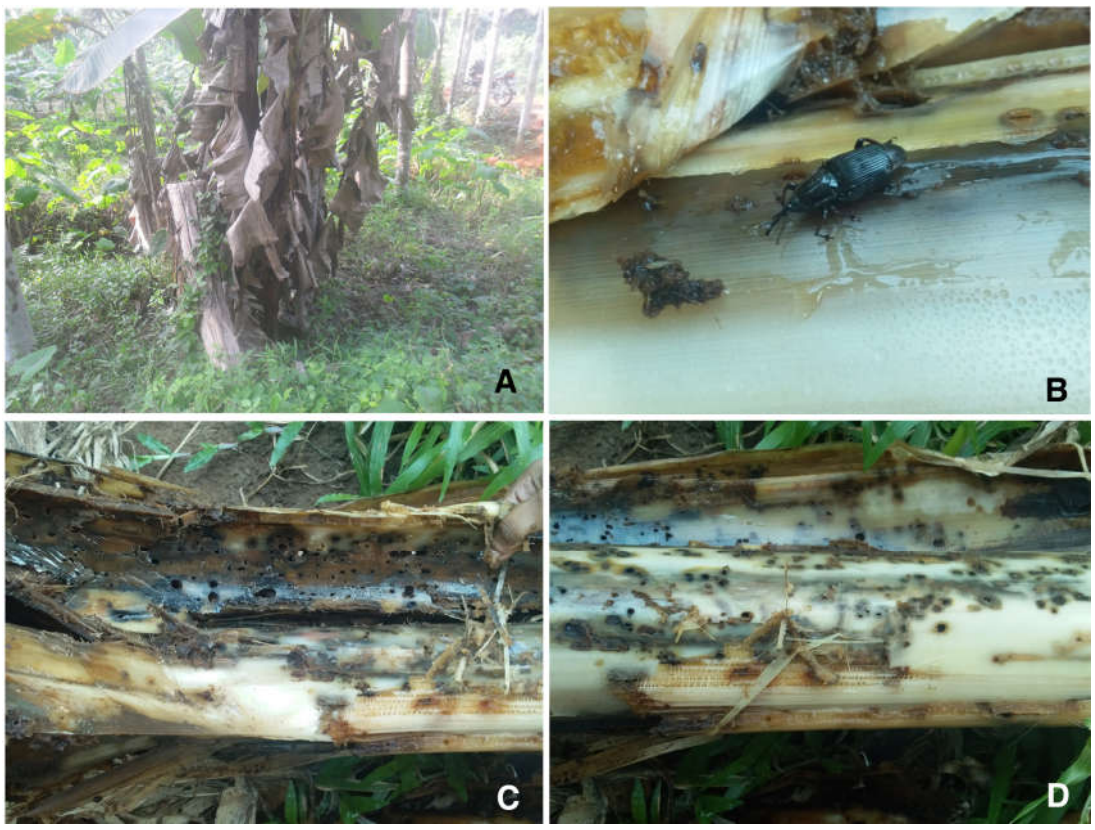
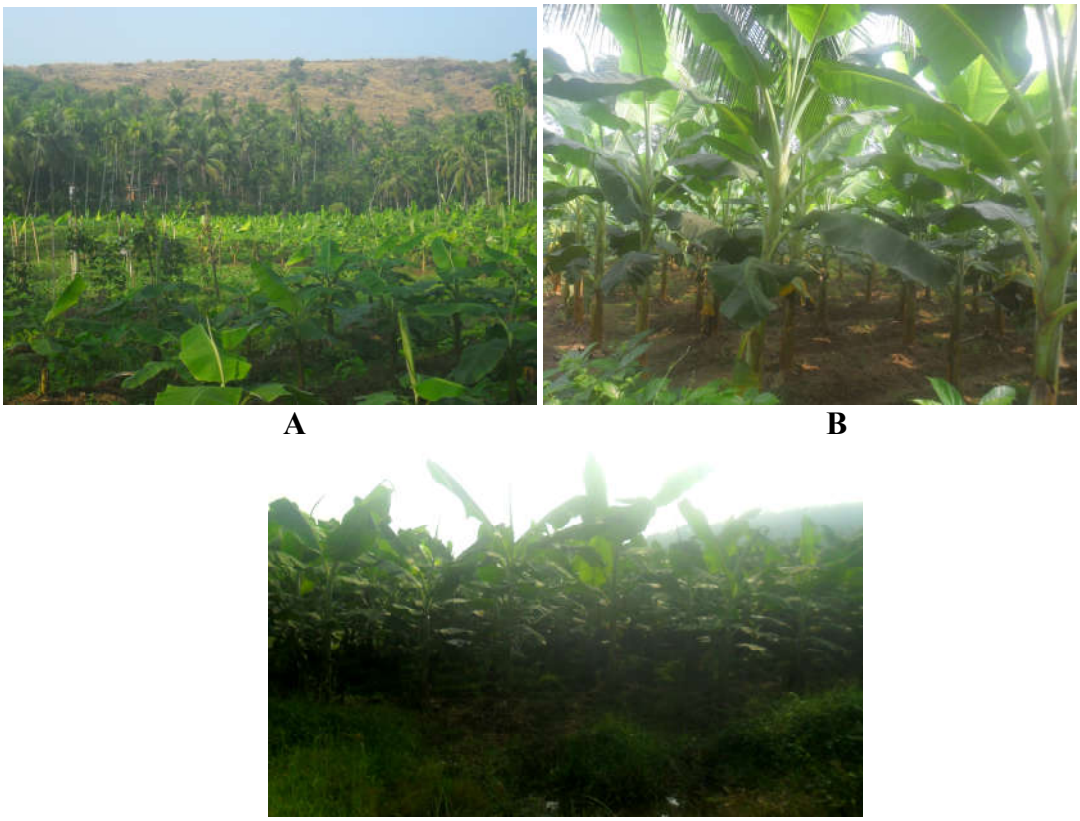


Plate 1.3. Damages caused by banana pseudostem weevil (A) premature falling of the banana plant (B) gummy exudation from the holes made by the adult weevil (C) & (D) excessive tunneling inside the pseudostem



A **B**

C

Plate 1.4. Collection Areas (A) Annassery (B) Vellalassery (C) Chathamangalam of Kozhikode District.

1.3.4. Estimation of LD_{50} and LD_{90}

Adults of banana weevil exposed to wide range of concentrations of carbosulfan by topical application and a narrow range of concentrations, yielding between 10% and 90% mortality in 24 hr was used to determine LD_{50} and LD_{90} using a log dosage probit mortality regression line using statistical analysis by Finney (1971). Repeated the bioassays three times using different batches of insect.

1.3.5. Biochemical assays

The biochemical assays were conducted to quantify the different detoxifying enzymes such as glutathione-s-transferase, cytochrome monooxygenase and also acetyl choline esterase. The enzyme assays were

conducted in tissues like gut, reproductive organ, fat body and also the whole insect. Insects were cut laterally and tergum was removed. Alimentary canal, fat body and reproductive organs of adults were taken out separately. They were transferred to eppendorf tubes kept on ice. Tissues from 10 insects were pooled every time to get sufficient quantity. The adult insects were treated with different subethal concentrations like 10%, 50% and 80% of lethal dose (LD_{50}) for 24 hr, 48 hr and 72 hr.

1.3.5.1. Total soluble protein assay (Modified Lowry method, 1990)

Reagents required:

- Dissolved 20 gm sodium carbonate in 260 ml water, 0.4 gm cupric sulfate (5x hydrated) in 20 ml water, and 0.2 gm sodium potassium tartrate in 20 ml water. Mixed all three solutions to prepare the copper reagent.
- Prepared 100 ml of a 1% solution (1 gm/100 ml) of sodium dodecyl sulfate (SDS).
- Prepared a 1 M solution of NaOH (4 gm/100 ml).
- For the 2x Lowry concentrate mix 3 parts copper reagent with 1 part SDS and 1 part NaOH. Solution is stable for 2-3 weeks. Warmed the solution to 37º C if a white precipitate formed, and discarded if there is a black precipitate. For better results, the three stock solutions mixed just before use.
- Prepared 0.2 N Folin reagent by mixing 10 ml 2 N Folin reagent with 90 ml water. Kept in an amber bottle, the dilution was stable for several months.
- Protein standard: 5 mg bovine serum albumin in 100 ml distilled water

Methodology:

- Prepared 80 microliters of different sample dilution with distilled water. Triplicate samples were taken for the assay.
- Prepared standards from 200 µg/ml bovine serum albumin by adding different volumes of distilled water to bring volume to 80 microliters.
- Added 80 microliters of 2x Lowry concentrate, mix thoroughly, and incubate at room temperature for 10 min.
- Added 40 microliters 0.2 N Folin reagent very quickly, and immediately. Complete mixing of the reagent must be accomplished quickly to avoid decomposition of the reagent before it reacts with protein. Incubate for 30 min more at room temperature.
- Used microplates to read the absorbances at 750 nm.

1.3.5.2. Glutathione-S-transferase assay (Habig *et al*., 1974)

Reagents required:

- Glutathione-(GSH) (1mM): dissolve 3.073 mg of GSH in 10 ml of distilled water.
- 1-chloro-2, 4-dinitrobenzene (CDNB) (30mM) in 100% alcohol: dissolve 151.912mg of CDNB in 25 ml of 100% ethanol.
- Phosphate buffer: 0.1M pH 7.0

Methodology

- Taken triplicates of 100 µl phosphate buffer in separate wells of micro plate reader.

- Added 10µl of CDNB, 10µl of tissue homogenate and 70µl of distilled water to the wells.
- Triplicates of blanks should be used which contains 80µl of distilled water, 100 µl of phosphate buffer and 10µl of CDNB.
- The reaction mixture was incubated at 37ºC for 5 min
- Started the reaction by addition of 10µl of reduced glutathione to each well.
- The increase in activity measured for 10 min at 340 nm. Values are expressed as micromoles of reduced glutathione and CDNB conjugate formed/min/µg protein.

1.3.5.3. Monooxygenase titration assay (Brogden *et al*., 1997)

Reagents required:

- 3% hydrogen peroxide.
- 0.625 M potassium phosphate buffer, pH 7.2.
- 0.01g 3, 3′, 5, 5′- tetramethyl benzidine in 5ml methanol.
- 0.25 M sodium acetate buffer pH 5.0.

Methodology:

2 X 10 µl replicates of homogenate were taken in separate wells of a microtitre plate. 40 µl of 0.625 M potassium phosphate buffer (pH 7.2) is added to each replicate. 5 ml methanol solution of tetramethylbenzidine (TMB) was mixed with 15 ml of 0.25 M sodium acetate buffer (pH 5.0) and 100 µl of the above mixture is added to each replicate. 12.5 µl of 3% hydrogen peroxide is added to each replicate and the mixture was left for 2 hours at room temperature and absorbance was read at 630nm. Controls were run with 10 µl of buffer in place of the insect homogenate.

1.3.5.4. Acetylcholinesterase assay

Reagents required:

- Acetylthiocholine iodide (ACTI) solution 0.01M: dissolve 0.273 g of ACTI in 100 ml of distilled water and store in an amber bottle. The solution is kept in refrigerator.
- Dithiobis 2 nitro benzoic acid (DTNB) solution (0.01M): dissolve 0.396g of DTNB in 100 ml of sodium phosphate buffer.
- Phosphate buffer (pH 7.0, 0.1M)

Methodology

- Taken triplicates of 50µl of insect homogenate and place in separate wells of microtitre plate.
- Added 10µl of DTNB and 15µl of acetylcholine iodide to each well.
- Added 1.43 ml of sodium phosphate buffer to each well.
- For keeping the blank solution, added 10µl of DTNB and 15µl of acetylcholine iodide to 1.48 ml of phosphate buffer.
- Recorded the increase in absorbance at 412 nm for 10 min against the blank.

1.3.6. Degradation of carbosulfan by enzymes extracted from various tissues of *Odoiporus longicollis*

1.3.6.1. Preparation of enzyme extract from insect tissues for incubation

Fat body, alimentary canal, reproductive organs and whole body were homogenized using a hand glass homogenizer and made to a final volume of 5 ml each. The diluted enzyme extracts were taken in eppendorf tube and cold

centrifuged at 10000 rpm for 10 min. After centrifugation supernatant were taken out into another set of eppendorf tubes and kept frozen. This extract was further diluted as required.

1.3.6.2. Assessment of enzyme activity

Flat-bottomed glass vials (5 ml capacity) with bakelite screw caps were used for incubation at 37ºC. Different volumes of the enzyme extracts (0.1, 0.2, 0.5 ml) were taken in glass vials and 0.5 ml of carbosulfan (100 µg/ 1ml) was added in each and made up to 1.0 ml with phosphate buffer. Incubations were carried out in shaker water bath set at 37ºC. Different enzyme concentrations of the different tissues were incubated for 60 min.

1.3.7. HPLC analysis of carbosulfan and the break down products

HPLC analysis of the various samples was done using a High Performance Liquid Chromatograph (Shimadzu 10 AVP) equipped with an UV/Visible detector (Shimadzu SPD-10AVP). The detection of carbosulfan was done at λ_{max}= 272 nm, using a C-18 column (Luna 5µ C18 (2) 100 A, Phenomenex) of 250 x 4.6 mm dimension and 5 µm particle size in an isocratic run. Methanol at a flow rate of 1 ml min^{-1} was used as the mobile phase for the analysis. The separation was done at ambient temperature. The standards and samples were delivered via 20 µl injection loop using a 25 µl capacity Hamilton microliter syringe. A run time of 10 min was given for each run. Shimadzu Class-*VP* software (Chromatography Data System) on PC was used for integration and computation of signals.

1.3.8. Statistical Analysis

Statistical analysis was performed on the data observed at each level using statistical package SPSS 20.0.

1.4. RESULTS

1.4.1. Estimation of LD_{50}

The adults of banana stem weevil collected were treated with different concentrations of carbosulfan and percentage mortality after 24 hr was recorded. Table 1.1 shows the different concentrations in µg/µl and mortality of the adult insect in percentages recorded for the bioassay. The concentrations applied were 0.7µg/µl, 0.8µg/µl, 0.85 µg/µl, 0.9 µg/µl, 0.95 µg/µl, 0.98 µg/µl, and 1.0 µg/µl and the percentage mortality observed were 25, 37.5, 39, 40, 50, 70, and 99 respectively. LD_{50} of carbosulfan against *Odoiporus longicollis* was 0.876 and LD_{90} obtained was 1.117. (Table 1. 2)

Table 1.1: Data on percentage mortality observed against the treatment with different concentrations of carbosulfan on the adult *Odoiporus longicollis*

CONCENTRATION(µg/µl)	MORTALITY (%)
0.7	25
0.8	37.5
0.85	39
0.9	40
0.95	50
0.98	70
1.0	99

Table 1.2: Data on 24 hr LD_{50} (µg/µl) of carbosulfan treated against the adult *Odoiporus longicollis* and its associated statistics

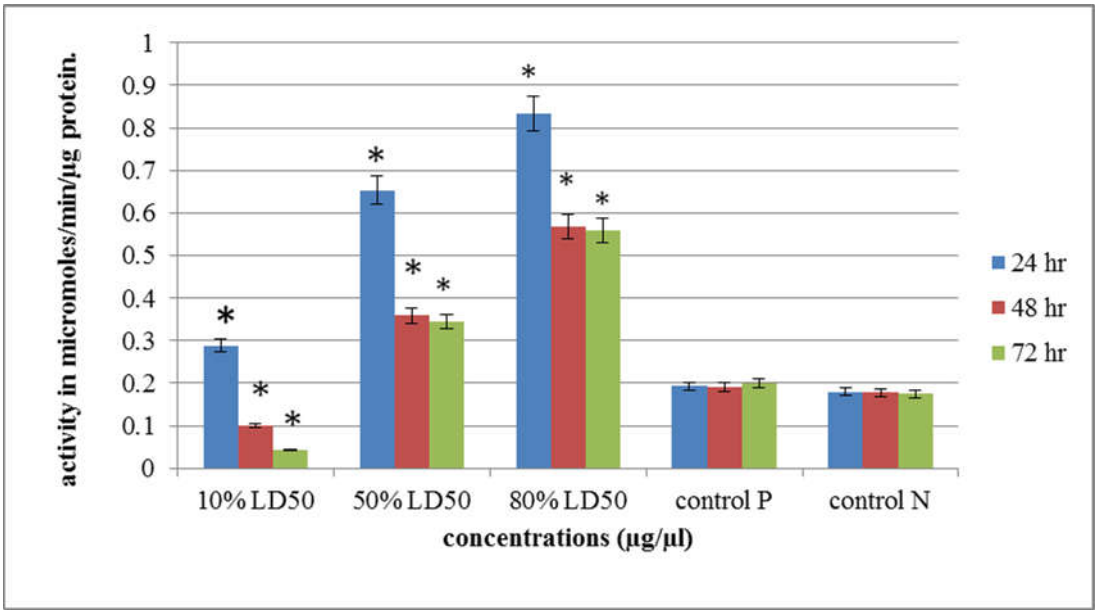
24 hr Lethal Dose		Lower - Upper limit	Regression equation	Degree of freedom	Chi square	Significance
LD_{50}	0.876	0.694 – 1.013	Y=10.643x+8.9286	5	56.786	$p<0.05$
LD_{90}	1.117	0.993 – 2.533	Y=10.643x+8.9286	5	56.786	$p<0.05$

1.4.2. Biochemical assays

1.4.2.1. Glutathione-S-transferase (GST) activity:

GSTs are group of detoxifying enzymes which catalyse the conjugation of the reduced form of glutathione (GSH) to electrophile xenobiotic substrates (Habig *et al*., 1974). Glutathione-S-transferases are a family of multifunctional proteins involved in cellular detoxification of deleterious electrophilic xenobiotics such as anti-cancer drugs, herbicides, pesticides, chemical carcinogens and environmental pollutants (Hayes and Pulford, 1995; Pérez-López *et al*., 2002; Hayes *et al*., 2005).

Figure 1.1 shows the data on (GST) activity of adult insect over different time duration. At a sublethal concentration of 10% of LD_{50} application, GST activity was 0.288±0.001, 0.100±0.001, and 0.043±0.001 for 24 hr, 48 hr and 72 hr respectively. GST activities on 50% of LD50 application were 0.654±0.001, 0.359±0.001, 0.345±0.001 for different time interval of 24 hr, 48 hr and 72 hr respectively. When treated with 80% of LD_{50}, the GST activity obtained was 0.833±0.01, 0.569±0.001, 0.559±0.001 for different time intervals of 24 hr, 48hr and 72 hr respectively. The data obtained against positive and negative control for 24 hr of exposure were 0.193±0.001 and 0.180±0.001, 48 hr of exposure were 0.191±0.001 and 0.178±0.001 and for 72 hr exposure were 0.199±0.001 and 0.175±0.001.



*Level of significance < 0.01

Figure 1.1: GST activity of adult banana weevil whole body on treatment with different sublethal concentrations of carbosulfan on different time intervals

Table 1.3& 1.4 provided the data on statistical analysis of GST activity of whole body of *Odoiporus longicollis* on application of 10% LD_{50}, 50% LD_{50}, 80% of LD_{50}, during 24 hr, 48 hr and 72 hr. In all the samples the p value was less than 0.0001. The F values obtained were 232826.971, 92216.118, and 157423.636 for 24hr, 48 hr and 72 hr respectively. Hence the results were highly significant and exist significant changes in the treated and control groups.

Table 1.3: Data on statistical analysis of GST activity of whole body of adult banana weevil on application of different sublethal concentrations of carbosulfan and different time durations.

Duration (hr)	Concentration (µg/µl)	Mean enzyme activity ± SD (µ mol/min/µg protein)	One - way ANOVA
24	0.1	.28767± .001	F = 232826.971 p < .0001 The result is significant at p < .0001
	0.4	.65400 ± .001	
	0.7	.83300 ± .001	
	Control P	.19333 ± .001	
	Control N	.18000 ± .001	
48	0.1	.10033 ± .001	F = 92216.118 p < .0001 The result is significant at p < .0001
	0.4	.35933 ± .001	
	0.7	.56900 ± .001	
	Control P	.19100 ± .001	
	Control N	.17800 ± .001	
72	0.1	.04300 ± .001	F = 157423.636 p < .0001 The result is significant at p < .0001
	0.4	.34500 ± .001	
	0.7	.55900 ± .001	
	Control P	.19867 ± .001	
	Control N	.17467 ± .001	

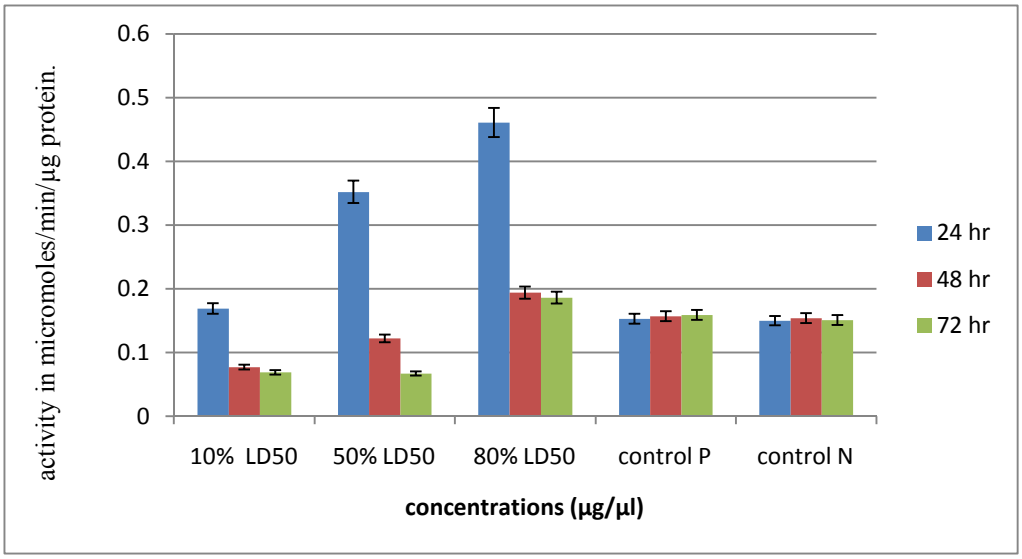
Table 1.4: Multiple comparison of GST activity of whole body of adult banana weevil on application of different sublethal concentrations of carbosulfan and different time durations.

Duration (hr)	Concentration (% of LD_{50} in µg/µl)		Mean Difference	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
24	10%	50%	-.36633*	.000869	.000	-.36919	-.36347
		80%	-.54533*	.000869	.000	-.54819	-.54247
		Control P	.09533*	.000869	.000	.09247	.09819
		Control N	.10767*	.000869	.000	.10481	.11053
	50%	10%	.36633*	.000869	.000	.36347	.36919
		80%	-.17900*	.000869	.000	-.18186	-.17614
		Control P	.46167*	.000869	.000	.45881	.46453
		Control N	.47400*	.000869	.000	.47114	.47686
	80%	10%	.54533*	.000869	.000	.54247	.54819
		50%	.17900*	.000869	.000	.17614	.18186
		Control P	.64067*	.000869	.000	.63781	.64353
		Control N	.65300*	.000869	.000	.65014	.65586
	Control P	10%	-.09533*	.000869	.000	-.09819	-.09247
		50%	-.46167*	.000869	.000	-.46453	-.45881
		80%	-.64067*	.000869	.000	-.64353	-.63781
		Control N	.01233*	.000869	.000	.00947	.01519
	Control N	10%	-.10767*	.000869	.000	-.11053	-.10481
		50%	-.47400*	.000869	.000	-.47686	-.47114
		80%	-.65300*	.000869	.000	-.65586	-.65014
		Control P	-.01233*	.000869	.000	-.01519	-.00947
48	10%	50%	-.25700*	.000869	.000	-.25986	-.25414
		80%	-.46767*	.000869	.000	-.47053	-.46481
		Control P	-.09167*	.000869	.000	-.09453	-.08881
		Control N	-.07767*	.000869	.000	-.08053	-.07481
	50%	10%	.25700*	.000869	.000	.25414	.25986
		80%	-.21067*	.000869	.000	-.21353	-.20781
		Control P	.16533*	.000869	.000	.16247	.16819
		Control N	.17933*	.000869	.000	.17647	.18219
	80%	10%	.46767*	.000869	.000	.46481	.47053
		50%	.21067*	.000869	.000	.20781	.21353
		Control P	.37600*	.000869	.000	.37314	.37886
		Control N	.39000*	.000869	.000	.38714	.39286
	Control P	10%	.09167*	.000869	.000	.08881	.09453
		50%	-.16533*	.000869	.000	-.16819	-.16247
		80%	-.37600*	.000869	.000	-.37886	-.37314

		Control N	.01400*	.000869	.000	.01114	.01686
	Control N	10%	.07767*	.000869	.000	.07481	.08053
		50%	-.17933*	.000869	.000	-.18219	-.17647
		80%	-.39000*	.000869	.000	-.39286	-.38714
		Control P	-.01400*	.000869	.000	-.01686	-.01114
72	10%	50%	-.25700*	.000869	.000	-.25986	-.25414
		80%	-.46767*	.000869	.000	-.47053	-.46481
		Control P	-.09167*	.000869	.000	-.09453	-.08881
		Control N	-.07767*	.000869	.000	-.08053	-.07481
	50%	10%	.25700*	.000869	.000	.25414	.25986
		80%	-.21067*	.000869	.000	-.21353	-.20781
		Control P	.16533*	.000869	.000	.16247	.16819
		Control N	.17933*	.000869	.000	.17647	.18219
	80%	10%	.46767*	.000869	.000	.46481	.47053
		50%	.21067*	.000869	.000	.20781	.21353
		Control P	.37600*	.000869	.000	.37314	.37886
		Control N	.39000*	.000869	.000	.38714	.39286
	Control P	10%	.09167*	.000869	.000	.08881	.09453
		50%	-.16533*	.000869	.000	-.16819	-.16247
		80%	-.37600*	.000869	.000	-.37886	-.37314
		Control N	.01400*	.000869	.000	.01114	.01686
	Control N	10%	.07767*	.000869	.000	.07481	.08053
		50%	-.17933*	.000869	.000	-.18219	-.17647
		80%	-.39000*	.000869	.000	-.39286	-.38714
		Control P	-.01400*	.000869	.000	-.01686	-.01114

*The mean difference is significant at 0.05 level

Data on GST activity of adult fat body when treated with different sublethal concentrations on different time intervals are provided in Figure 1.2. At a concentration of 10% of LD_{50} application, the GST activity obtained were 0.169±0.017, 0.077±0.001, 0.069±0.001 for 24 hr, 48hr and 72 hr exposure respectively. A value of 0.352±0.001, 0.122±0.001 and 0.067±0.001 obtained for the GST activity against the treatment with 50% of LD_{50} application and 0.461±0.001, 0.194±0.001 and 0.186±0.001 obtained during 80% of LD_{50} application respectively for 24 hr, 48hr and 72 hr of exposure. The GST activity on positive and negative controls observed are 0.153±0.001 and 0.150±0.001 for 24 hr exposure, 0.157±0.001 and 0.154±0.001 for 48 hr of exposure and 0.159±0.001 and 0.151±0.001 for 72 hr exposure.



*Level of significance < 0.01

Figure 1.2: GST activity of fat body of adult insect on application of different sublethal concentrations of carbosulfan and time of exposure

Table 1.5 & 1.6 provides the data on statistical analysis of GST activity of fat body of adult banana weevil on application of different sub lethal concentrations during 24 hr, 48 hr and 72 hr. The F values obtained were 1017.814, 6573.192, and 17879.071 for 24 hr, 48 hr and 72 hr. The result is highly significant with the p value less than 0.0001. There were changes in the control and treated groups.

Table 1.5: Data on statistical analysis of GST activity of fat body tissue on different sublethal concentration and time exposure.

Duration (hr)	Concentration (µg/µl)	Mean enzyme activity ± SD (µ mol/min/µg protein)	One - way ANOVA
24	0.1	.16933 ± .017	F = 1017.814 p < .0001 The result is significant at p < .0001
	0.4	.35200 ± .001	
	0.7	.46100 ± .001	
	Control P	.15333 ± .001	
	Control N	.15033 ± .001	
48	0.1	.07667 ± .001	F = 6573.192 p < .0001 The result is significant at p < .0001
	0.4	.12233 ± .001	
	0.7	.19400 ± .001	
	Control P	.15667 ±.001	
	Control N	.15367 ± .001	
72	0.1	.06867 ± .001	F = 17879.071 p < .0001 The result is significant at p < .0001
	0.4	.06733 ± .001	
	0.7	.18600 ± .001	
	Control P	.15867 ± .001	
	Control N	.15067 ± .001	

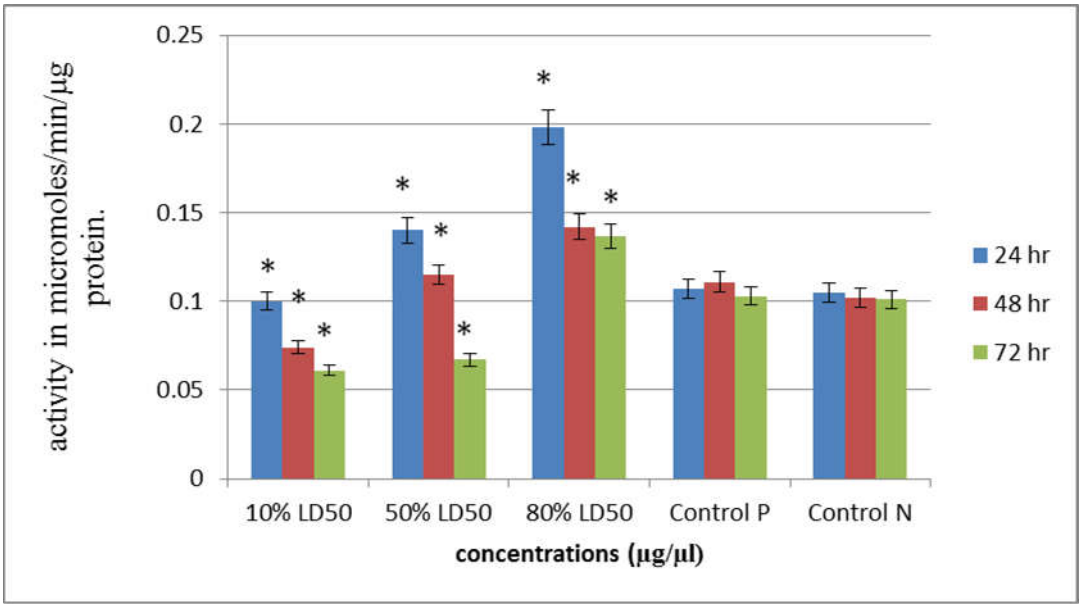
Table 1.6: Multiple comparison of GST activity of fat body tissue of adult banana weevil on different sublethal concentrations and time exposure.

Duration (hr)	Concentration (% of LD_{50} in µg/µl)		Mean Difference	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
24	10%	50%	-.17467*	.006250	.000	-.19524	-.15410
		80%	-.28367*	.006250	.000	-.30424	-.26310
		Control P	.02500*	.006250	.017	.00443	.04557
		Control N	.02800*	.006250	.008	.00743	.04857
	50%	10%	.17467*	.006250	.000	.15410	.19524
		80%	-.10900*	.006250	.000	-.12957	-.08843
		Control P	.19967*	.006250	.000	.17910	.22024
		Control N	.20267*	.006250	.000	.18210	.22324
	80%	10%	.28367*	.006250	.000	.26310	.30424
		50%	.10900*	.006250	.000	.08843	.12957
		Control P	.30867*	.006250	.000	.28810	.32924
		Control N	.31167*	.006250	.000	.29110	.33224
	Control P	10%	-.02500*	.006250	.017	-.04557	-.00443
		50%	-.19967*	.006250	.000	-.22024	-.17910
		80%	-.30867*	.006250	.000	-.32924	-.28810

		Control N	.00300	.006250	.988	-.01757	.02357
	Control N	10%	-.02800*	.006250	.008	-.04857	-.00743
		50%	-.20267*	.006250	.000	-.22324	-.18210
		80%	-.31167*	.006250	.000	-.33224	-.29110
		Control P	-.00300	.006250	.988	-.02357	.01757
48	10%	50%	-.04567*	.000760	.000	-.04817	-.04317
		80%	-.11633*	.000760	.000	-.11883	-.11383
		Control P	-.08000*	.000760	.000	-.08250	-.07750
		Control N	-.07700*	.000760	.000	-.07950	-.07450
	50%	10%	.04567*	.000760	.000	.04317	.04817
		80%	-.07067*	.000760	.000	-.07317	-.06817
		Control P	-.03433*	.000760	.000	-.03683	-.03183
		Control N	-.03133*	.000760	.000	-.03383	-.02883
	80%	10%	.11633*	.000760	.000	.11383	.11883
		50%	.07067*	.000760	.000	.06817	.07317
		Control P	.03633*	.000760	.000	.03383	.03883
		Control N	.03933*	.000760	.000	.03683	.04183
	Control P	10%	.08000*	.000760	.000	.07750	.08250
		50%	.03433*	.000760	.000	.03183	.03683
		80%	-.03633*	.000760	.000	-.03883	-.03383
		Control N	.00300*	.000760	.018	.00050	.00550
	Control N	10%	.07700*	.000760	.000	.07450	.07950
		50%	.03133*	.000760	.000	.02883	.03383
		80%	-.03933*	.000760	.000	-.04183	-.03683
		Control P	-.00300*	.000760	.018	-.00550	-.00050
72	10%	50%	-.00767*	.000558	.000	-.00950	-.00583
		80%	-.11833*	.000558	.000	-.12017	-.11650
		Control P	-.09000*	.000558	.000	-.09184	-.08816
		Control N	-.08200*	.000558	.000	-.08384	-.08016
	50%	10%	.00767*	.000558	.000	.00583	.00950
		80%	-.11067*	.000558	.000	-.11250	-.10883
		Control P	-.08233*	.000558	.000	-.08417	-.08050
		Control N	-.07433*	.000558	.000	-.07617	-.07250
	80%	10%	.11833*	.000558	.000	.11650	.12017
		50%	.11067*	.000558	.000	.10883	.11250
		Control P	.02833*	.000558	.000	.02650	.03017
		Control N	.03633*	.000558	.000	.03450	.03817
	Control P	10%	.09000*	.000558	.000	.08816	.09184
		50%	.08233*	.000558	.000	.08050	.08417
		80%	-.02833*	.000558	.000	-.03017	-.02650
		Control N	.00800*	.000558	.000	.00616	.00984
	Control N	10%	.08200*	.000558	.000	.08016	.08384
		50%	.07433*	.000558	.000	.07250	.07617
		80%	-.03633*	.000558	.000	-.03817	-.03450
		Control P	-.00800*	.000558	.000	-.00984	-.00616

*The mean difference is significant at 0.05 level

When treated with different sublethal concentrations of carbosulfan the GST activity of reproductive organ of adult insects for different concentrations and time intervals (Figure 1.3). 0.1±0.001, 0.074±0.001, 0.061±0.001 are the GST activity obtained during 10% of LD_{50} application, for 24 hr, 48 hr and 72 hr respectively. 0.140±0.001, 0.115±0.001, 0.067±0.001 are the GST activity obtained during 50% of LD_{50} application. 0.198±0.001, 0.142±0.001, 0.137±0.001 are the GST activity obtained during 80% of LD_{50} application, for 24 hr, 48 hr and 72 hr respectively. GST activities for positive and negative controls are 0.107±0.001 and 0.105±0.001 for 24 hr of exposure, 0.111±0.001 and 0.102±0.001 for 48 hr of exposure and 0.103±0.001 and 0.101±0.001 for 72 hr of exposure respectively.



*Level of significance < 0.01

Figure 1.3: GST activity of reproductive organs of adult insect on different time interval exposure during different concentrations of carbosulfan application

Table 1.7 & 1.8 provides the data on GST activity statisitical analysis of the reproductive organs of the adult weevil. The p values obtained were

less than 0.0001 indicating the highly significant data. The F values were 15161.300, 5413.700, and 8478.800 for 24 hr, 48 hr and 72 hr respectively.

Table 1.7: Data on statistical analysis of GST activity in Reproductive organ on different sublethal concentrations and time durations.

Duration (hr)	Concentration (µg/µl)	Mean enzyme activity ± SD (µ mol/min/µg protein)	One - way ANOVA
24	0.1	.10033 ± .001	F = 15161.300 p < .0001 The result is significant at p < .0001
	0.4	.13967 ± .001	
	0.7	.19767 ± .001	
	Control P	.10667 ± .001	
	Control N	.10467 ± .001	
48	0.1	.07367 ± .001	F = 5413.700 p < .0001 The result is significant at p < .0001
	0.4	.11467 ± .001	
	0.7	.14167 ± .001	
	Control P	.11133 ± .001	
	Control N	.10167 ± .001	
72	0.1	.06133 ± .001	F = 8478.800 p < .0001 The result is significant at p < .0001
	0.4	.06667 ± .001	
	0.7	.13667 ± .001	
	Control P	.10267 ± .001	
	Control N	.10167 ± .001	

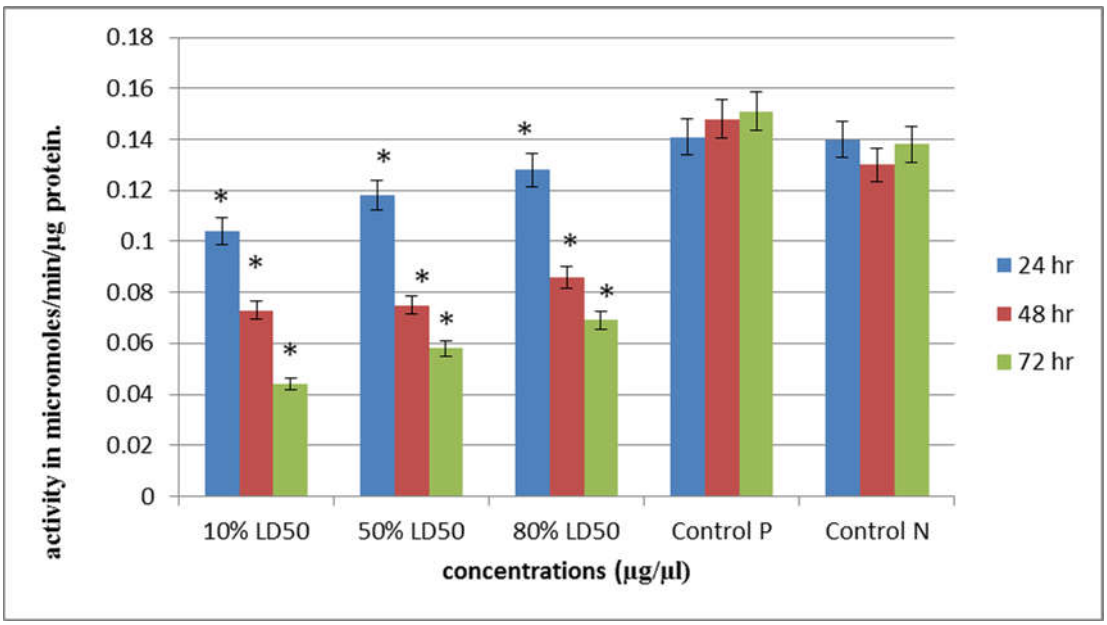
Table 1.8: Multiple comparison of GST activity of reproductive organ of adult banana weevil on different sublethal concentration and time exposure.

Duration (hr)	Concentration (% of LD_{50} in µg/µl)		Mean Difference	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
24	10%	50%	-.03933*	.000471	.000	-.04088	-.03778
		80%	-.09733*	.000471	.000	-.09888	-.09578
		Control P	-.00633*	.000471	.000	-.00788	-.00478
		Control N	-.00433*	.000471	.000	-.00588	-.00278
	50%	10%	.03933*	.000471	.000	.03778	.04088
		80%	-.05800*	.000471	.000	-.05955	-.05645
		Control P	.03300*	.000471	.000	.03145	.03455
		Control N	.03500*	.000471	.000	.03345	.03655
	80%	10%	.09733*	.000471	.000	.09578	.09888
		50%	.05800*	.000471	.000	.05645	.05955
		Control P	.09100*	.000471	.000	.08945	.09255
		Control N	.09300*	.000471	.000	.09145	.09455
	Control P	10%	.00633*	.000471	.000	.00478	.00788
		50%	-.03300*	.000471	.000	-.03455	-.03145
		80%	-.09100*	.000471	.000	-.09255	-.08945
		Control N	.00200*	.000471	.012	.00045	.00355
	Control N	10%	.00433*	.000471	.000	.00278	.00588
		50%	-.03500*	.000471	.000	-.03655	-.03345
		80%	-.09300*	.000471	.000	-.09455	-.09145
		Control P	-.00200*	.000471	.012	-.00355	-.00045
48	10%	50%	-.04100*	.000471	.000	-.04255	-.03945
		80%	-.06800*	.000471	.000	-.06955	-.06645
		Control P	-.03767*	.000471	.000	-.03922	-.03612
		Control N	-.02800*	.000471	.000	-.02955	-.02645
	50%	10%	.04100*	.000471	.000	.03945	.04255
		80%	-.02700*	.000471	.000	-.02855	-.02545
		Control P	.00333*	.000471	.000	.00178	.00488
		Control N	.01300*	.000471	.000	.01145	.01455
	80%	10%	.06800*	.000471	.000	.06645	.06955
		50%	.02700*	.000471	.000	.02545	.02855
		Control P	.03033*	.000471	.000	.02878	.03188
		Control N	.04000*	.000471	.000	.03845	.04155
	Control P	10%	.03767*	.000471	.000	.03612	.03922
		50%	-.00333*	.000471	.000	-.00488	-.00178
		80%	-.03033*	.000471	.000	-.03188	-.02878
		Control N	.00967*	.000471	.000	.00812	.01122
	Control N	10%	.02800*	.000471	.000	.02645	.02955
		50%	-.01300*	.000471	.000	-.01455	-.01145

		80%	-.04000*	.000471	.000	-.04155	-.03845
		Control P	-.00967*	.000471	.000	-.01122	-.00812
72	10%	50%	-.00533*	.000471	.000	-.00688	-.00378
		80%	-.07533*	.000471	.000	-.07688	-.07378
		Control P	-.04133*	.000471	.000	-.04288	-.03978
		Control N	-.04033*	.000471	.000	-.04188	-.03878
	50%	10%	.00533*	.000471	.000	.00378	.00688
		80%	-.07000*	.000471	.000	-.07155	-.06845
		Control P	-.03600*	.000471	.000	-.03755	-.03445
		Control N	-.03500*	.000471	.000	-.03655	-.03345
	80%	10%	.07533*	.000471	.000	.07378	.07688
		50%	.07000*	.000471	.000	.06845	.07155
		Control P	.03400*	.000471	.000	.03245	.03555
		Control N	.03500*	.000471	.000	.03345	.03655
	Control P	10%	.04133*	.000471	.000	.03978	.04288
		50%	.03600*	.000471	.000	.03445	.03755
		80%	-.03400*	.000471	.000	-.03555	-.03245
		Control N	.00100	.000471	.283	-.00055	.00255
	Control N	10%	.04033*	.000471	.000	.03878	.04188
		50%	.03500*	.000471	.000	.03345	.03655
		80%	-.03500*	.000471	.000	-.03655	-.03345
		Control P	-.00100	.000471	.283	-.00255	.00055

*The mean difference is significant at 0.05 level

GST activity of gut of adult insect over different time interval and sub lethal doses of carbosulfan is provided in figure 1.4. GST activity obtained during 10% of LD_{50} application were 0.104±0.001, 0.073±0.001, 0.044±0.001 for 24 hr, 48 hr and 72 hr of exposure respectively. GST activity obtained on 50% of LD_{50} application were 0.118±0.001, 0.075±0.001, 0.058±0.001 for 24 hr, 48 hr and 72 hr and for treatment with 80% of LD_{50} were 0.128±0.001, 0.086±0.001, 0.069±0.001 for 24 hr, 48 hr and 72 hr respectively. GST activity obtained for positive and negative controls obtained are 0.141±0.001 and 0.140±0.001 for 24 hr of exposure, 0.148±0.001 and 0.13±0.001 for 48 hr of exposure and 0.151±0.001 and 0.138±0.001 for 72 hr of exposure respectively.



*Level of significance < 0.01

Figure 1.4: GST activity of gut of adult insect on different time of exposure treated with different sublethal concentrations of carbosulfan.

Table 1.9 & 1.10 shows the statistical analysis of GST activitiy of adult banana weevil gut tissue sample. During different concentrations of sublethal doses of carbosulfan at different time intervals in gut tissue shows the p value less than 0.0001.The F value obtained are 2818.375, 10462.500, 21781.200 for 24 hr, 48 hr and 72 hr. The result is significant in each group and there was significant changes between control and treated samples.

Table 1.9: Data on statistical analysis of GST activity in Gut tissue on application of different sublethal concentration and different time durations.

Duration (hr)	Concentration (µg/µl)	Mean enzyme activity± SD (µ mol/min/µg protein)	One-way ANOVA
24	0.1	.10367 ± .001	F = 2818.375 p < .0001 The result is significant at p < .0001
	0.4	.11767 ± .001	
	0.7	.12767± .001	
	Control P	.14133 ± .001	
	Control N	.14000 ± .001	
48	0.1	.07333 ± .001	F = 10462.500 p < .0001 The result is significant at p < .0001
	0.4	.07533 ± .001	
	0.7	.08567 ± .001	
	Control P	.14767 ± .001	
	Control N	.12967 ± .001	
72	0.1	.04367 ± .001	F = 21781.200 p < .0001 The result is significant at p < .0001
	0.4	.05767 ± .001	
	0.7	.06933 ± .001	
	Control P	.15133 ± .001	
	Control N	.13767 ± .001	

Table 1.10: Multiple comparison of GST activity of gut tissue of adult banana weevil on different sublethal concentration and time exposure.

Duration (hr)	Concentration (% of LD_{50} in µg/µl)		Mean Difference	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
24	10%	50%	-.01400*	.000422	.000	-.01539	-.01261
		80%	-.02400*	.000422	.000	-.02539	-.02261
		Control P	-.03767*	.000422	.000	-.03905	-.03628
		Control N	-.03633*	.000422	.000	-.03772	-.03495
	50%	10%	.01400*	.000422	.000	.01261	.01539
		80%	-.01000*	.000422	.000	-.01139	-.00861
		Control P	-.02367*	.000422	.000	-.02505	-.02228
		Control N	-.02233*	.000422	.000	-.02372	-.02095
	80%	10%	.02400*	.000422	.000	.02261	.02539
		50%	.01000*	.000422	.000	.00861	.01139

		Control P	-.01367*	.000422	.000	-.01505	-.01228
		Control N	-.01233*	.000422	.000	-.01372	-.01095
	Control P	10%	.03767*	.000422	.000	.03628	.03905
		50%	.02367*	.000422	.000	.02228	.02505
		80%	.01367*	.000422	.000	.01228	.01505
		Control N	.00133	.000422	.061	-.00005	.00272
	Control N	10%	.03633*	.000422	.000	.03495	.03772
		50%	.02233*	.000422	.000	.02095	.02372
		80%	.01233*	.000422	.000	.01095	.01372
		Control P	-.00133	.000422	.061	-.00272	.00005
48	10%	50%	-.00200*	.000471	.012	-.00355	-.00045
		80%	-.01233*	.000471	.000	-.01388	-.01078
		Control P	-.07433*	.000471	.000	-.07588	-.07278
		Control N	-.05633*	.000471	.000	-.05788	-.05478
	50%	10%	.00200*	.000471	.012	.00045	.00355
		80%	-.01033*	.000471	.000	-.01188	-.00878
		Control P	-.07233*	.000471	.000	-.07388	-.07078
		Control N	-.05433*	.000471	.000	-.05588	-.05278
	80%	10%	.01233*	.000471	.000	.01078	.01388
		50%	.01033*	.000471	.000	.00878	.01188
		Control P	-.06200*	.000471	.000	-.06355	-.06045
		Control N	-.04400*	.000471	.000	-.04555	-.04245
	Control P	10%	.07433*	.000471	.000	.07278	.07588
		50%	.07233*	.000471	.000	.07078	.07388
		80%	.06200*	.000471	.000	.06045	.06355
		Control N	.01800*	.000471	.000	.01645	.01955
	Control N	10%	.05633*	.000471	.000	.05478	.05788
		50%	.05433*	.000471	.000	.05278	.05588
		80%	.04400*	.000471	.000	.04245	.04555
		Control P	-.01800*	.000471	.000	-.01955	-.01645
72	10%	50%	-.01400*	.000471	.000	-.01555	-.01245
		80%	-.02467*	.000471	.000	-.02622	-.02312
		Control P	-.10767*	.000471	.000	-.10922	-.10612
		Control N	-.09400*	.000471	.000	-.09555	-.09245
	50%	10%	.01400*	.000471	.000	.01245	.01555
		80%	-.01067*	.000471	.000	-.01222	-.00912
		Control P	-.09367*	.000471	.000	-.09522	-.09212
		Control N	-.08000*	.000471	.000	-.08155	-.07845
	80%	10%	.02467*	.000471	.000	.02312	.02622

		50%	.01067*	.000471	.000	.00912	.01222
		Control P	-.08300*	.000471	.000	-.08455	-.08145
		Control N	-.06933*	.000471	.000	-.07088	-.06778
	Control P	10%	.10767*	.000471	.000	.10612	.10922
		50%	.09367*	.000471	.000	.09212	.09522
		80%	.08300*	.000471	.000	.08145	.08455
		Control N	.01367*	.000471	.000	.01212	.01522
	Control N	10%	.09400*	.000471	.000	.09245	.09555
		50%	.08000*	.000471	.000	.07845	.08155
		80%	.06933*	.000471	.000	.06778	.07088
		Control P	-.01367*	.000471	.000	-.01522	-.01212

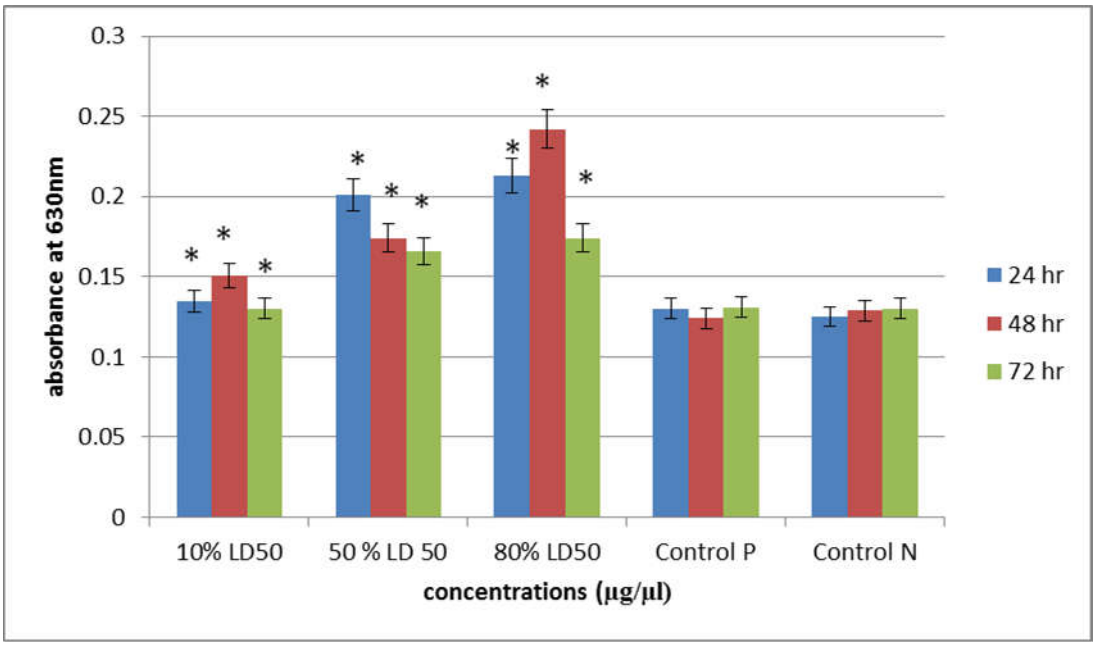
*The mean difference is significant at 0.05 level

1.4.2.2. Monooxygenase activity:

Mixed function oxygenase (MFO) system include a group of enzymes that play a critical role in xenobiotic detoxification by carrying out a series of oxidation reactions whereby relatively insoluble compounds are converted into water soluble metabolites which may be further conjugated and excreted (Bend and James, 1978; Lech *et al*., 1982; Payne, 1984). Insect Cytochrome P450 can be detected in a wide range of tissues. Highest monooxygenase activities are usually associated with the mid gut, fat bodies and malpighian tubules (Hodgson, 1983).

The monooxygenase activity of the whole body of the adult insects, fat body, gut and reproductive organs were analysed. Figure 1.5 provides the activity of monooxygenase in whole body of adult insects.0.135±0.001, 0.151±0.001, 0.13±0.001 were the absorbance obtained on applying 10% of LD_{50} (0.1µg/µl) of carbosulfan on adult insects over 24 hr, 48 hr, 72 hr of exposure respectively. 0.201±0.001, 0.174±0.001, 0.166±0.001 were the absorbance obtained on applying 50% of LD_{50} (0.4µg/µl) of carbosulfan on adult insects over 24 hr, 48 hr, 72 hr of exposure and 0.213±0.001,

0.242±0.001, 0.174±0.001 were the absorbance obtained on applying 80% of LD_{50} (0.7µg/µl) of carbosulfan on adult insects over 24 hr, 48 hr, 72 hr of exposure respectively. 0.13±0.001, 0.124±0.001, 0.131±0.001 were the monooxygenase activity of positive control over different time duration of 24 hr, 48 hr, 72 hr of exposure and 0.125±0.001, 0.129±0.001, 0.13±0.001 were the monooxygenase activity of negative control over 24 hr, 48 hr, 72 hr of exposure respectively.



*Level of significance < 0.01

Figure 1.5: Monooxygenase activity of whole body of adult insects of *Odoiporus longicollis* treated with different sublethal concentrations of carbosulfan at different time intervals

Table 1.11 & 1.12 shows the data on staisitical analysis of MFO in the wholebody of adult banana weevil during 24 hr, 48 hr and 72 hr. The F value obtained for each group was 20515.250, 20869.200, and 3002.000. The p value is very less than 0.0001 indicating the significant changes in the control and treated groups.

Table 1.11: Data on statistical analysis MFO activity of whole body tissue on application of different sublethal concentrations and time intervals in adult banana weevil, *Odoiporus longicollis.*

Duration (hr)	Concentration (µg/µl)	Mean enzyme activity ± SD (absorbance at 630 nm)	One - way ANOVA
24	0.1	.13467 ± .001	F = 20515.250 p < .0001 The result is significant at p < .0001
	0.4	.20133 ± .001	
	0.7	.21267 ± .001	
	Control P	.13000 ± .001	
	Control N	.12433 ± .001	
48	0.1	.15067 ± .001	F = 20869.200 p < .0001 The result is significant at p < .0001
	0.4	.17333 ± .001	
	0.7	.24267 ± .001	
	Control P	.12433 ± .001	
	Control N	.12867 ± .001	
72	0.1	.13033 ± .001	F = 3002.000 p < .0001 The result is significant at p < .0001
	0.4	.16567 ± .001	
	0.7	.17367 ± .001	
	Control P	.13100 ± .001	
	Control N	.13033 ± .001	

Table 1.12: Multiple comparison of MFO activity of adult whole body of banana weevil on different sublethal concentration and time exposure.

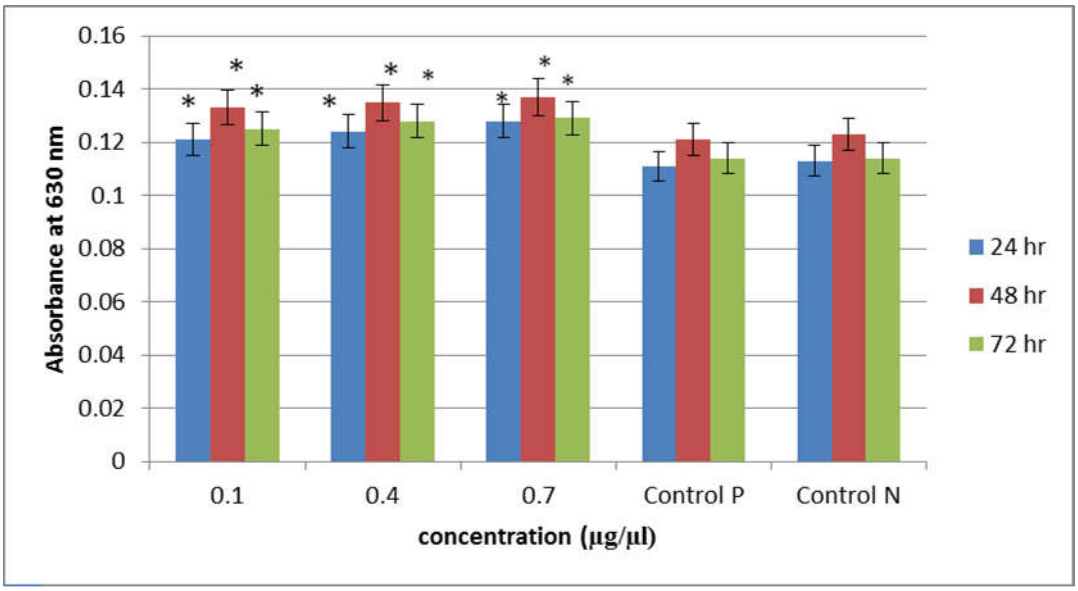
Duration (hr)	Concentration (% of LD_{50} in µg/µl)		Mean Difference	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
24	10%	50%	-.06667*	.000422	.000	-.06805	-.06528
		80%	-.07800*	.000422	.000	-.07939	-.07661
		Control P	.00467*	.000422	.000	.00328	.00605
		Control N	.01033*	.000422	.000	.00895	.01172
	50%	10%	.06667*	.000422	.000	.06528	.06805
		80%	-.01133*	.000422	.000	-.01272	-.00995
		Control P	.07133*	.000422	.000	.06995	.07272
		Control N	.07700*	.000422	.000	.07561	.07839
	80%	10%	.07800*	.000422	.000	.07661	.07939
		50%	.01133*	.000422	.000	.00995	.01272
		Control P	.08267*	.000422	.000	.08128	.08405
		Control N	.08833*	.000422	.000	.08695	.08972
	Control P	10%	-.00467*	.000422	.000	-.00605	-.00328
		50%	-.07133*	.000422	.000	-.07272	-.06995
		80%	-.08267*	.000422	.000	-.08405	-.08128
		Control N	.00567*	.000422	.000	.00428	.00705
	Control N	10%	-.01033*	.000422	.000	-.01172	-.00895
		50%	-.07700*	.000422	.000	-.07839	-.07561
		80%	-.08833*	.000422	.000	-.08972	-.08695
		Control P	-.00567*	.000422	.000	-.00705	-.00428
48	10%	50%	-.02267*	.000471	.000	-.02422	-.02112
		80%	-.09200*	.000471	.000	-.09355	-.09045
		Control P	.02633*	.000471	.000	.02478	.02788
		Control N	.02200*	.000471	.000	.02045	.02355
	50%	10%	.02267*	.000471	.000	.02112	.02422
		80%	-.06933*	.000471	.000	-.07088	-.06778
		Control P	.04900*	.000471	.000	.04745	.05055
		Control N	.04467*	.000471	.000	.04312	.04622
	80%	10%	.09200*	.000471	.000	.09045	.09355
		50%	.06933*	.000471	.000	.06778	.07088
		Control P	.11833*	.000471	.000	.11678	.11988

		Control N	.11400*	.000471	.000	.11245	.11555
	Control P	10%	-.02633*	.000471	.000	-.02788	-.02478
		50%	-.04900*	.000471	.000	-.05055	-.04745
		80%	-.11833*	.000471	.000	-.11988	-.11678
		Control N	-.00433*	.000471	.000	-.00588	-.00278
	Control N	10%	-.02200*	.000471	.000	-.02355	-.02045
		50%	-.04467*	.000471	.000	-.04622	-.04312
		80%	-.11400*	.000471	.000	-.11555	-.11245
		Control P	.00433*	.000471	.000	.00278	.00588
72	10%	50%	-.03533*	.000558	.000	-.03717	-.03350
		80%	-.04333*	.000558	.000	-.04517	-.04150
		Control P	-.00067	.000558	.754	-.00250	.00117
		Control N	.00000	.000558	1.000	-.00184	.00184
	50%	10%	.03533*	.000558	.000	.03350	.03717
		80%	-.00800*	.000558	.000	-.00984	-.00616
		Control P	.03467*	.000558	.000	.03283	.03650
		Control N	.03533*	.000558	.000	.03350	.03717
	80%	10%	.04333*	.000558	.000	.04150	.04517
		50%	.00800*	.000558	.000	.00616	.00984
		Control P	.04267*	.000558	.000	.04083	.04450
		Control N	.04333*	.000558	.000	.04150	.04517
	Control P	10%	.00067	.000558	.754	-.00117	.00250
		50%	-.03467*	.000558	.000	-.03650	-.03283
		80%	-.04267*	.000558	.000	-.04450	-.04083
		Control N	.00067	.000558	.754	-.00117	.00250
	Control N	10%	.00000	.000558	1.000	-.00184	.00184
		50%	-.03533*	.000558	.000	-.03717	-.03350
		80%	-.04333*	.000558	.000	-.04517	-.04150
		Control P	-.00067	.000558	.754	-.00250	.00117

*The mean difference is significant at 0.05 level

Figure 1.6 shows the activity of monooxygenase in the fat body of adult insects *Odoiporus longicollis*. 0.121±0.001, 0.133±0.006, 0.125±0.002 are the absorbance obtained on treatment with 10% of LD_{50} (0.1µg/µl) of carbosulfan on fat body of adult insects for 24hr, 48hr and 72hr respectively. Value of 0.124±0.001, 0.135±0.001, 0.128±0.001 obtained on the treatment with a sublethal dose of 50% of LD_{50} (0.4µg/µl) of carbosulfan on fat body of adult insects over 24 hr, 48 hr and 72 hr and 0.128±0.001, 0.137±0.001and

0.129±0.001 are the values obtained on applying 80% of LD_{50} (0.7µg/µl) of carbosulfan on adult insects over 24 hr, 48 hr and 72 hr respectively. 0.121±0.001, 0.13±0.001and 0.125±0.001 are the monooxygenase activity of positive control and 0.120±0.001, 0.128±0.001, 0.123±0.001 are the monooxygenase activity of negative control over time interval of 24 hr, 48 hr and 72 hr respectively.



*Level of significance < 0.01

Figure 1.6: Monooxygenase activity of fat body of adult of *Odoiporus longicollis* on application of different sublethal concentrations of carbosulfan and time intervals.

The data analysis by one–way ANOVA results shows that the MFO activity in the fat body tissue on application of different sub lethal concentrations of carbosulfan and time interval in adult banana weevil, *Odoiporus longicollis* is significant with p value less than 0.0001. The F value obtained for each group was 458.700, 475.20 and 1309.500. The significant changes between control and treated groups were shown in the table1.13 & 1.14.

Table 1.13: Data on statistical analysis of MFO activity in fat body tissue on application of different sublethal concentrations of carbosulfan and time interval in adult banana weevil, *Odoiporus longicollis.*

Duration (hr)	Concentration (µg/µl)	Mean enzyme activity ± SD (absorbance at 630 nm)	One - way ANOVA
24	0.1	.12053 ± .001	F = 458.700 p < .0001 The result is significant at p < .0001
	0.4	.12433 ± .001	
	0.7	.12767 ± .001	
	Control P	.11133 ± .001	
	Control N	.11267 ± .001	
48	0.1	.13267 ± .001	F = 475.20 p < .0001 The result is significant at p < .0001
	0.4	.13467 ± .001	
	0.7	.13733 ± .001	
	Control P	.12133 ± .001	
	Control N	.12267 ± .001	
72	0.1	.12500 ± .001	F = 1309.500 p < .0001 The result is significant at p < .0001
	0.4	.12800 ± .001	
	0.7	.12900 ± .001	
	Control P	.11367 ± .001	
	Control N	.11367 ± .001	

Table 1.14: Multiple comparison of MFO activity of fat body of adult banana weevil on different sublethal concentration and time exposure.

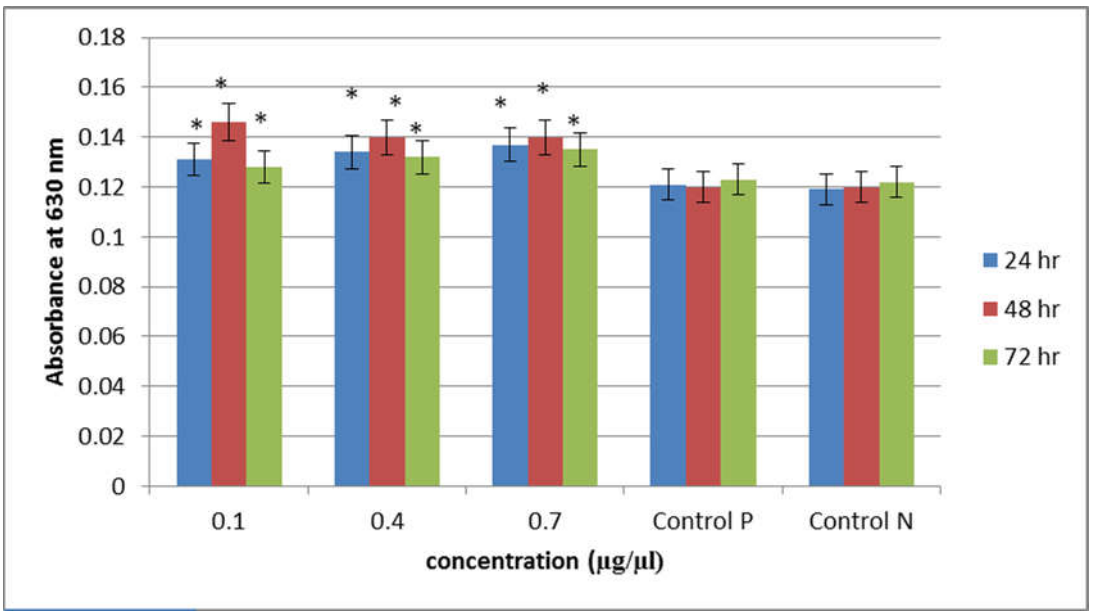
Duration (hr)	Concentration (% of LD_{50} in µg/µl)		Mean Difference	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
24	10%	50%	-.00400*	.000471	.000	-.00555	-.00245
		80%	-.00733*	.000471	.000	-.00888	-.00578
		Control P	.00900*	.000471	.000	.00745	.01055
		Control N	.00767*	.000471	.000	.00612	.00922
	50%	10%	.00400*	.000471	.000	.00245	.00555
		80%	-.00333*	.000471	.000	-.00488	-.00178
		Control P	.01300*	.000471	.000	.01145	.01455
		Control N	.01167*	.000471	.000	.01012	.01322

	80%	10%	.00733*	.000471	.000	.00578	.00888
		50%	.00333*	.000471	.000	.00178	.00488
		Control P	.01633*	.000471	.000	.01478	.01788
		Control N	.01500*	.000471	.000	.01345	.01655
	Control P	10%	-.00900*	.000471	.000	-.01055	-.00745
		50%	-.01300*	.000471	.000	-.01455	-.01145
		80%	-.01633*	.000471	.000	-.01788	-.01478
		Control N	-.00133	.000471	.102	-.00288	.00022
	Control N	10%	-.00767*	.000471	.000	-.00922	-.00612
		50%	-.01167*	.000471	.000	-.01322	-.01012
		80%	-.01500*	.000471	.000	-.01655	-.01345
		Control P	.00133	.000471	.102	-.00022	.00288
48	10%	50%	-.00200*	.000471	.012	-.00355	-.00045
		80%	-.00467*	.000471	.000	-.00622	-.00312
		Control P	.01133*	.000471	.000	.00978	.01288
		Control N	.01000*	.000471	.000	.00845	.01155
	50%	10%	.00200*	.000471	.012	.00045	.00355
		80%	-.00267*	.000471	.002	-.00422	-.00112
		Control P	.01333*	.000471	.000	.01178	.01488
		Control N	.01200*	.000471	.000	.01045	.01355
	80%	10%	.00467*	.000471	.000	.00312	.00622
		50%	.00267*	.000471	.002	.00112	.00422
		Control P	.01600*	.000471	.000	.01445	.01755
		Control N	.01467*	.000471	.000	.01312	.01622
	Control P	10%	-.01133*	.000471	.000	-.01288	-.00978
		50%	-.01333*	.000471	.000	-.01488	-.01178
		80%	-.01600*	.000471	.000	-.01755	-.01445
		Control N	-.00133	.000471	.102	-.00288	.00022
	Control N	10%	-.01000*	.000471	.000	-.01155	-.00845
		50%	-.01200*	.000471	.000	-.01355	-.01045
		80%	-.01467*	.000471	.000	-.01622	-.01312
		Control P	.00133	.000471	.102	-.00022	.00288
72	10%	50%	-.00300*	.000298	.000	-.00398	-.00202
		80%	-.00400*	.000298	.000	-.00498	-.00302
		Control P	.01133*	.000298	.000	.01035	.01231
		Control N	.01133*	.000298	.000	.01035	.01231
	50%	10%	.00300*	.000298	.000	.00202	.00398
		80%	-.00100*	.000298	.045	-.00198	-.00002
		Control P	.01433*	.000298	.000	.01335	.01531

		Control N	.01433*	.000298	.000	.01335	.01531
	80%	10%	.00400*	.000298	.000	.00302	.00498
		50%	.00100*	.000298	.045	.00002	.00198
		Control P	.01533*	.000298	.000	.01435	.01631
		Control N	.01533*	.000298	.000	.01435	.01631
	Control P	10%	-.01133*	.000298	.000	-.01231	-.01035
		50%	-.01433*	.000298	.000	-.01531	-.01335
		80%	-.01533*	.000298	.000	-.01631	-.01435
		Control N	.00000	.000298	1.000	-.00098	.00098
	Control N	10%	-.01133*	.000298	.000	-.01231	-.01035
		50%	-.01433*	.000298	.000	-.01531	-.01335
		80%	-.01533*	.000298	.000	-.01631	-.01435
		Control P	.00000	.000298	1.000	-.00098	.00098

*The mean difference is significant at 0.05 level

Figure 1.7 provides the activity of the enzyme monooxygenase in the reproductive organ of adult insects when treated with different sublethal concentrations of carbosulfan on different time intervals. 0.131±0.001, 0.146±0.001, 0.128±0.001 are the absorbance obtained on applying 10% of LD_{50} (0.1µg/µl) of carbosulfan on reproductive organ of adult insects over 24 hr, 48 hr and 72 hr duration respectively. Monooxygenase activity of 0.134±0.001, 0.14±0.001 and 0.132±0.001 are obtained for the treatment with 50% of LD_{50} (0.4µg/µl) of carbosulfan on reproductive organ of adult insects over 24 hr, 48 hr and 72 hr respectively. 0.137±0.001, 0.14±0.001 and 0.135±0.001 are the values obtained after applying 80% of LD_{50} (0.7µg/µl) of carbosulfan on reproductive organ of adult insects over 24hr, 48hr and 72hr respectively. 0.121±0.001, 0.120± 0.001 and 0.123± 0.001 are the values of the positive control and 0.119±0.001, 0.120±0.001 and 0.122±0.001 are the values of negative control over 24 hr, 48 hr and 72 hr respectively.



*Level of significance < 0.01

Figure 1.7: Monooxygenase activity of reproductive organ of adult insects of *Odoiporus longicollis* treated with sublethal doses of carbosulfan on different duration of time.

The data analysis of MFO activity in adult insects of *Odoiporus longicollis* treated with sublethal doses of carbosulfan and different duration of time was shown in table 1.15. One- Way ANOVA result shows that the P value is less than 0.0001. The F values obtained were 730.00, 677.600, and 278.700. The result is significant in different sublethal concentrations. There is significant change between treated and control groups (Table 1.15 & 1.16).

Table 1.15: Data on statistical analysis of MFO activity in reproductive organ tissue in adult insects of *Odoiporus longicollis* treated with sublethal doses of carbosulfan and different duration of time

Duration (hr)	Concentration (µg/µl)	Mean enzyme activity± SD (absorbance at 630 nm)	One - way ANOVA
24	0.1	.13133 ± .001	F = 730.000 p < .0001 The result is significant at p < .0001
	0.4	.13400 ± .001	
	0.7	.13667 ± .001	
	Control P	.12133 ± .001	
	Control N	.11853 ± .001	
48	0.1	.14567 ± .001	F = 677.600 p < .0001 The result is significant at p < .0001
	0.4	.14100 ± .001	
	0.7	.14047 ± .001	
	Control P	.12033 ± .001	
	Control N	.12033 ± .001	
72	0.1	.12767 ± .001	F = 278.700 p < .0001 The result is significant at p < .0001
	0.4	.13233 ± .001	
	0.7	.13467 ± .001	
	Control P	.12267 ± .001	
	Control N	.12233 ± .001	

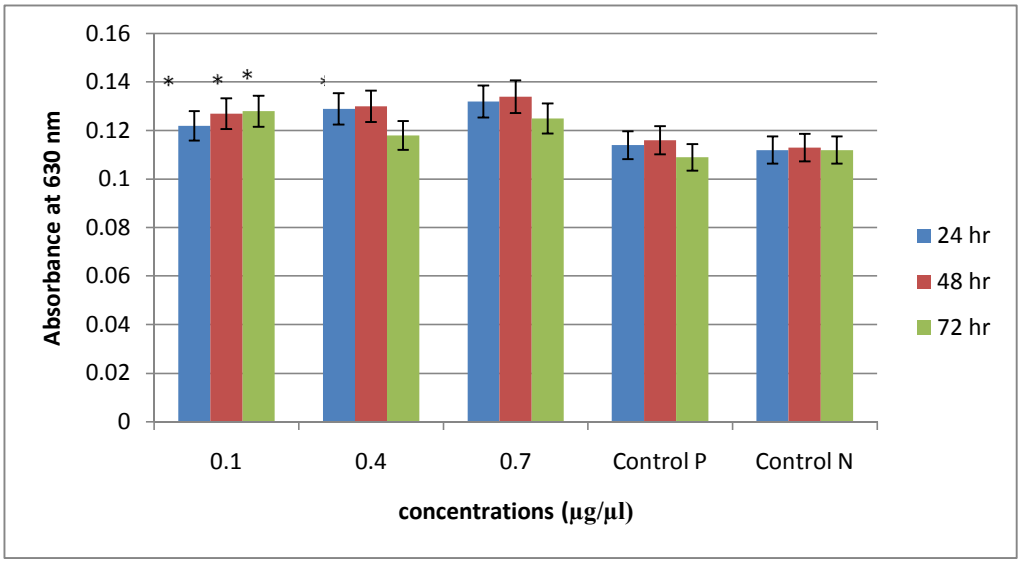
Table 1.16: Multiple comparison of MFO activity of adult reproductive organ of banana weevil on different sublethal concentration and time exposure.

Duration (hr)	Concentration (% of LD_{50} in µg/µl)		Mean Difference	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
24	10%	50%	-.00267*	.000422	.001	-.00405	-.00128
		80%	-.00533*	.000422	.000	-.00672	-.00395
		Control P	.01000*	.000422	.000	.00861	.01139
		Control N	.01300*	.000422	.000	.01161	.01439
	50%	10%	.00267*	.000422	.001	.00128	.00405
		80%	-.00267*	.000422	.001	-.00405	-.00128
		Control P	.01267*	.000422	.000	.01128	.01405
		Control N	.01567*	.000422	.000	.01428	.01705
	80%	10%	.00533*	.000422	.000	.00395	.00672
		50%	.00267*	.000422	.001	.00128	.00405
		Control P	.01533*	.000422	.000	.01395	.01672
		Control N	.01833*	.000422	.000	.01695	.01972
	Control P	10%	-.01000*	.000422	.000	-.01139	-.00861
		50%	-.01267*	.000422	.000	-.01405	-.01128
		80%	-.01533*	.000422	.000	-.01672	-.01395
		Control N	.00300*	.000422	.000	.00161	.00439
	Control N	10%	-.01300*	.000422	.000	-.01439	-.01161
		50%	-.01567*	.000422	.000	-.01705	-.01428
		80%	-.01833*	.000422	.000	-.01972	-.01695
		Control P	-.00300*	.000422	.000	-.00439	-.00161
48	10%	50%	.00467*	.000667	.000	.00247	.00686
		80%	.00500*	.000667	.000	.00281	.00719
		Control P	.02533*	.000667	.000	.02314	.02753
		Control N	.02533*	.000667	.000	.02314	.02753
	50%	10%	-.00467*	.000667	.000	-.00686	-.00247
		80%	.00033	.000667	.986	-.00186	.00253
		Control P	.02067*	.000667	.000	.01847	.02286
		Control N	.02067*	.000667	.000	.01847	.02286
	80%	10%	-.00500*	.000667	.000	-.00719	-.00281
		50%	-.00033	.000667	.986	-.00253	.00186
		Control P	.02033*	.000667	.000	.01814	.02253
		Control N	.02033*	.000667	.000	.01814	.02253
	Control P	10%	-.02533*	.000667	.000	-.02753	-.02314
		50%	-.02067*	.000667	.000	-.02286	-.01847
		80%	-.02033*	.000667	.000	-.02253	-.01814
		Control N	.00000	.000667	1.000	-.00219	.00219
	Control N	10%	-.02533*	.000667	.000	-.02753	-.02314
		50%	-.02067*	.000667	.000	-.02286	-.01847
		80%	-.02033*	.000667	.000	-.02253	-.01814
		Control P	.00000	.000667	1.000	-.00219	.00219

72	10%	50%	-.00467*	.000471	.000	-.00622	-.00312
		80%	-.00700*	.000471	.000	-.00855	-.00545
		Control P	.00500*	.000471	.000	.00345	.00655
		Control N	.00533*	.000471	.000	.00378	.00688
	50%	10%	.00467*	.000471	.000	.00312	.00622
		80%	-.00233*	.000471	.004	-.00388	-.00078
		Control P	.00967*	.000471	.000	.00812	.01122
		Control N	.01000*	.000471	.000	.00845	.01155
	80%	10%	.00700*	.000471	.000	.00545	.00855
		50%	.00233*	.000471	.004	.00078	.00388
		Control P	.01200*	.000471	.000	.01045	.01355
		Control N	.01233*	.000471	.000	.01078	.01388
	Control P	10%	-.00500*	.000471	.000	-.00655	-.00345
		50%	-.00967*	.000471	.000	-.01122	-.00812
		80%	-.01200*	.000471	.000	-.01355	-.01045
		Control N	.00033	.000471	.950	-.00122	.00188
	Control N	10%	-.00533*	.000471	.000	-.00688	-.00378
		50%	-.01000*	.000471	.000	-.01155	-.00845
		80%	-.01233*	.000471	.000	-.01388	-.01078
		Control P	-.00033	.000471	.950	-.00188	.00122

*The mean difference is significant at 0.05 level

Monooxygenase activity on gut of the banana weevil, *Odoiporus longicollis* treated against different sublethal doses of carbosulfan on different duration of time is provided in figure 1.8. Values of 0.122±0.007, 0.127±0.002, 0.128±0.002 are the MFO activity of adult gut samples obtained on the application of 10% (0.1µg/µl) of LD_{50} and the values of 0.129±0.002, 0.13±0.005, 0.118±0.001 are the MFO activity of adult gut samples obtained on the application of 50% (0.4µg/µl) of LD_{50} and the values of 0.132±0.004, 0.134±0.002, 0.125±0.002 are the MFO activity of adult gut samples obtained on the application of 80% (0.7µg/µl) of LD_{50} for 24hr, 48hr and 72hr respectively. MFO activity of positive control are 0.114±0.004, 0.116±0.002, 0.109±0.004 and MFO activity of negative control are 112±0.003, 0.113±0.003, 0.112±0.001 for different duration of 24 hr, 48 hr and 72 hr respectively.



*Level of significance < 0.01

Figure 1.8: Monooxygenase activity of gut of adult insects of *Odoiporus longicollis* treated with different sublethal doses of carbosulfan on different duration of time

Table 1.17 & 1.18 shows the data on statistical analysis of MFO activity due to different sub lethal concentrations and time exposure. The F values obtained are 706.800, 487.812 and 759.625. The p value is less than 0.0001 ($p<0.0001$) indicating the significant changes in treated and sample groups.

Table 1.17: Data on statistical analysis MFO activity of Gut tissue in adult insects of *Odoiporus longicollis* treated with sublethal doses of carbosulfan and different duration of time

Duration (hr)	Concentration (µg/µl)	Mean enzyme activity ± SD (absorbance at 630 nm)	One - way ANOVA
24	0.1	.12233 ± .001	F = 706.800 p < .0001 The result is significant at p < .0001
	0.4	.12867 ± .001	
	0.7	.13233 ± .001	
	Control P	.11367 ± .001	
	Control N	.11233 ± .001	
48	0.1	.12733 ± .001	F = 487.812 p < .0001 The result is significant at p < .0001
	0.4	.13047 ± .001	
	0.7	.13367 ± .001	
	Control P	.11567 ± .001	
	Control N	.11267 ± .001	
72	0.1	.12767 ± .001	F = 759.625 p < .0001 The result is significant at p < .0001
	0.4	.11767 ± .001	
	0.7	.12533 ± .001	
	Control P	.10867 ± .001	
	Control N	.11200 ± .001	

Table 1.18: Multiple comparison of MFO activity of adult gut tissue of banana weevil on different sublethal concentration and time exposure.

Duration (hr)	Concentration (% of LD_{50} in µg/µl)		Mean Difference	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
24	10%	50%	-.00633*	.000471	.000	-.00788	-.00478
		80%	-.01000*	.000471	.000	-.01155	-.00845
		Control P	.00867*	.000471	.000	.00712	.01022
		Control N	.01000*	.000471	.000	.00845	.01155
	50%	10%	.00633*	.000471	.000	.00478	.00788
		80%	-.00367*	.000471	.000	-.00522	-.00212
		Control P	.01500*	.000471	.000	.01345	.01655
		Control N	.01633*	.000471	.000	.01478	.01788
	80%	10%	.01000*	.000471	.000	.00845	.01155
		50%	.00367*	.000471	.000	.00212	.00522
		Control P	.01867*	.000471	.000	.01712	.02022
		Control N	.02000*	.000471	.000	.01845	.02155
	Control P	10%	-.00867*	.000471	.000	-.01022	-.00712
		50%	-.01500*	.000471	.000	-.01655	-.01345

		80%	-.01867*	.000471	.000	-.02022	-.01712
		Control N	.00133	.000471	.102	-.00022	.00288
	Control N	10%	-.01000*	.000471	.000	-.01155	-.00845
		50%	-.01633*	.000471	.000	-.01788	-.01478
		80%	-.02000*	.000471	.000	-.02155	-.01845
		Control P	-.00133	.000471	.102	-.00288	.00022
48	10%	50%	-.00333*	.000596	.002	-.00530	-.00137
		80%	-.00633*	.000596	.000	-.00830	-.00437
		Control P	.01167*	.000596	.000	.00970	.01363
		Control N	.01467*	.000596	.000	.01270	.01663
	50%	10%	.00333*	.000596	.002	.00137	.00530
		80%	-.00300*	.000596	.004	-.00496	-.00104
		Control P	.01500*	.000596	.000	.01304	.01696
		Control N	.01800*	.000596	.000	.01604	.01996
	80%	10%	.00633*	.000596	.000	.00437	.00830
		50%	.00300*	.000596	.004	.00104	.00496
		Control P	.01800*	.000596	.000	.01604	.01996
		Control N	.02100*	.000596	.000	.01904	.02296
	Control P	10%	-.01167*	.000596	.000	-.01363	-.00970
		50%	-.01500*	.000596	.000	-.01696	-.01304
		80%	-.01800*	.000596	.000	-.01996	-.01604
		Control N	.00300*	.000596	.004	.00104	.00496
	Control N	10%	-.01467*	.000596	.000	-.01663	-.01270
		50%	-.01800*	.000596	.000	-.01996	-.01604
		80%	-.02100*	.000596	.000	-.02296	-.01904
		Control P	-.00300*	.000596	.004	-.00496	-.00104
72	10%	50%	.01000*	.000422	.000	.00861	.01139
		80%	.00233*	.000422	.002	.00095	.00372
		Control P	.01900*	.000422	.000	.01761	.02039
		Control N	.01567*	.000422	.000	.01428	.01705
	50%	10%	-.01000*	.000422	.000	-.01139	-.00861
		80%	-.00767*	.000422	.000	-.00905	-.00628
		Control P	.00900*	.000422	.000	.00761	.01039
		Control N	.00567*	.000422	.000	.00428	.00705
	80%	10%	-.00233*	.000422	.002	-.00372	-.00095
		50%	.00767*	.000422	.000	.00628	.00905
		Control P	.01667*	.000422	.000	.01528	.01805
		Control N	.01333*	.000422	.000	.01195	.01472
	Control P	10%	-.01900*	.000422	.000	-.02039	-.01761
		50%	-.00900*	.000422	.000	-.01039	-.00761
		80%	-.01667*	.000422	.000	-.01805	-.01528
		Control N	-.00333*	.000422	.000	-.00472	-.00195
	Control N	10%	-.01567*	.000422	.000	-.01705	-.01428
		50%	-.00567*	.000422	.000	-.00705	-.00428
		80%	-.01333*	.000422	.000	-.01472	-.01195
		Control P	.00333*	.000422	.000	.00195	.00472

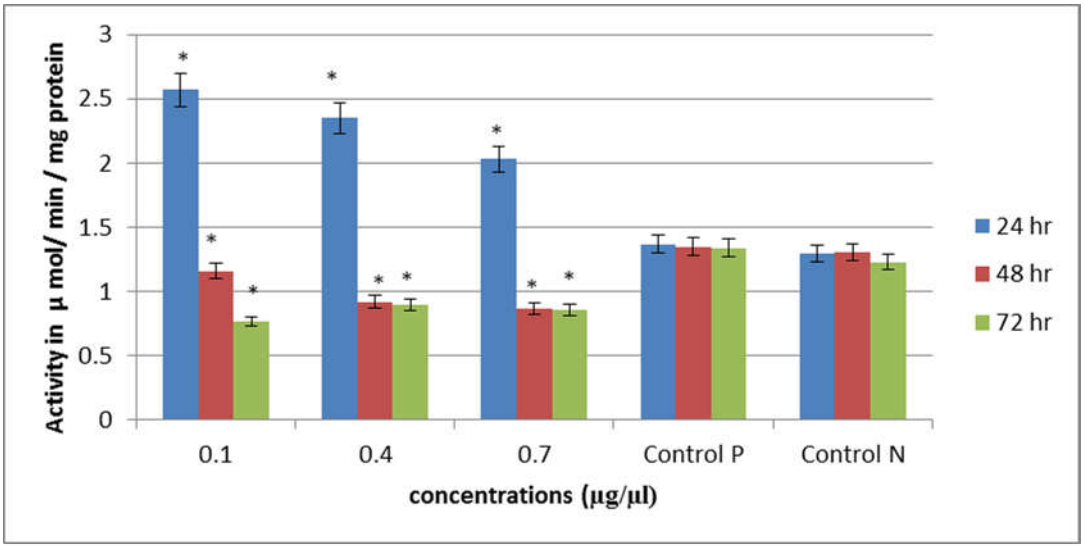
*The mean difference is significant at 0.05 level

1.4.2.3. Acetylcholinesterase activity:

The toxicity of organophosphorus and carbamate esters to animals is attributed to their ability to inhibit acetylcholine esterase, which is a class of enzymes that catalyzes the hydrolysis of the neurotransmitting agent

acetylcholine (AChE). Its inhibition causes death, so irreversible inhibitors have been developed as insecticides such as organophosphates and carbamates (Aldridge, 1952).

Acetylcholinesterase activity on the whole body of the target insect tested after treatment with sublethal concentrations of carbosulfan at various time intervals are provided in figure 1.9. Acetylcholine esterase activity (µ mol/ min / mg protein) in adult insects on application of 10% (0.1µg/µl) of insecticide LD_{50} are 2.57±0.001, 1.161±0.001 and 0.766±0.001; and that on application of 50% (0.4µg/µl) of insecticide LD_{50} are 2.35±0.001, 0.921±0.001 and 0.901±0.001 and the acetyl choline esterase activity (µ mol/ min/mg protein) on application with 80% of LD_{50} are 2.03±0.001, 0.866±0.001 and 0.859±0.001 for 24 hr, 48 hr, 72 hr time interval respectively. Positive control values obtained were 1.37±0.001, 1.35±0.001, 1.34±0.001 for 24 hr, 48 hr and 72 hr respectively. Negative control values obtained were 1.3±0.001, 1.31±0.001, 1.23±0.001 for 24 hr, 48 hr and 72 hr respectively.



*Level of significance < 0.01

Figure 1.9: Acetyl choline esterase activity of the whole body of adult insect after treatment with different sublethal concentrations of carbosulfan at different time duration.

The data of statistical analysis shows there is significan changes in the acetyl choline esterase activity between the different sublethal concentrations and control groups. The p value is less than 0.0001. The F values obtained are 36664.000, 10607.959 and 2730.564 (Table 1.19 & 1.20).

Table 1.19: Data on statistical analysis of AChE activity in whole body tissue of adult banana weevil insect after treatment with different sublethal concentrations of carbosulfan at different time duration

Duration (hr)	Concentration (µg/µl)	Mean enzyme activity ± SD (µ mol/min/mg protein)	One - way ANOVA
24	0.1	2.56667 ± .001	F = 36664.000 p < .0001 The result is significant at p < .0001
	0.4	2.34667 ± .001	
	0.7	2.03333 ± .001	
	Control P	1.36667 ± .001	
	Control N	1.30000 ± .001	
48	0.1	1.16100 ± .001	F = 10607.959 p < .0001 The result is significant at p < .0001
	0.4	.92067 ± .001	
	0.7	.86500 ±.001	
	Control P	1.34667 ± .001	
	Control N	1.31333 ± .001	
72	0.1	.76233 ± .001	F = 2730.564 p < .0001 The result is significant at p < .0001
	0.4	.90100 ± .001	
	0.7	.85733 ± .001	
	Control P	1.3500 ± .001	
	Control N	1.24667 ± .001	

Table 1.20: Multiple comparison of AChE activity of whole body tissue of adult banana weevil insect after treatment with different sublethal concentrations of carbosulfan at different time duration

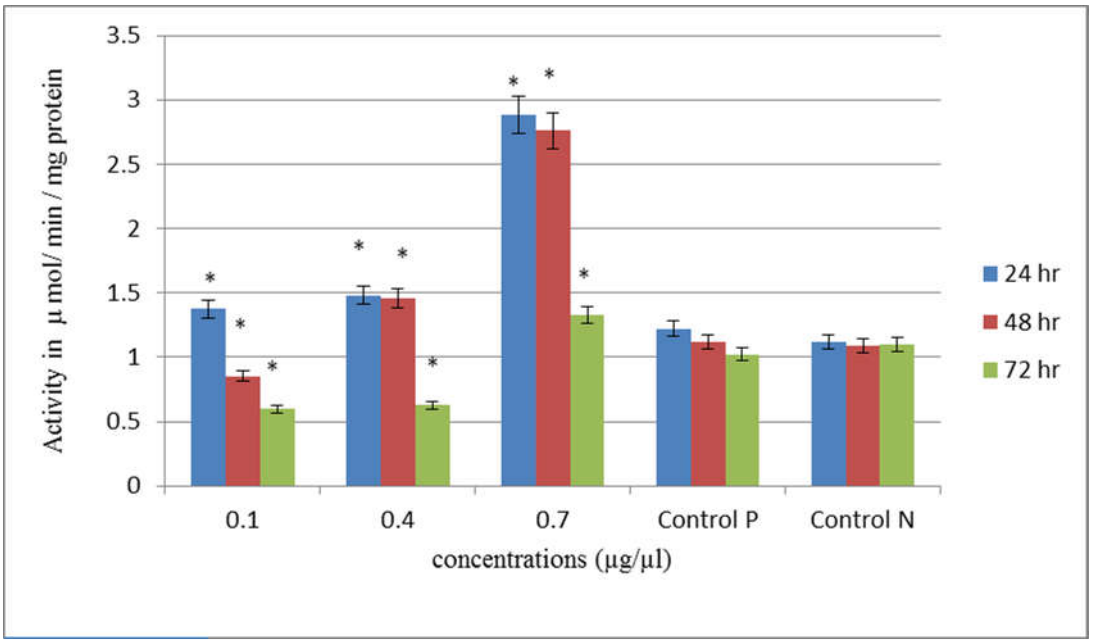
Duration (hr)	Concentration (% of LD_{50} in µg/µl)		Mean Difference	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
24	10%	50%	.220000*	.004216	.000	.21061	.22939
		80%	.533333*	.004216	.000	.52394	.54273
		Control P	1.200000*	.004216	.000	1.19061	1.20939
		Control N	1.266667*	.004216	.000	1.25727	1.27606
	50%	10%	-.220000*	.004216	.000	-.22939	-.21061
		80%	.313333*	.004216	.000	.30394	.32273
		Control P	.980000*	.004216	.000	.97061	.98939
		Control N	1.046667*	.004216	.000	1.03727	1.05606
	80%	10%	-.533333*	.004216	.000	-.54273	-.52394
		50%	-.313333*	.004216	.000	-.32273	-.30394
		Control P	.666667*	.004216	.000	.65727	.67606
		Control N	.733333*	.004216	.000	.72394	.74273
	Control P	10%	-1.200000*	.004216	.000	-1.20939	-1.19061
		50%	-.980000*	.004216	.000	-.98939	-.97061
		80%	-.666667*	.004216	.000	-.67606	-.65727
		Control N	.066667*	.004216	.000	.05727	.07606
	Control N	10%	-1.266667*	.004216	.000	-1.27606	-1.25727
		50%	-1.046667*	.004216	.000	-1.05606	-1.03727
		80%	-.733333*	.004216	.000	-.74273	-.72394
		Control P	-.066667*	.004216	.000	-.07606	-.05727
48	10%	50%	.240333*	.003033	.000	.23358	.24709
		80%	.296000*	.003033	.000	.28924	.30276
		Control P	-.185667*	.003033	.000	-.19242	-.17891
		Control N	-.152333*	.003033	.000	-.15909	-.14558
	50%	10%	-.240333*	.003033	.000	-.24709	-.23358
		80%	.055667*	.003033	.000	.04891	.06242
		Control P	-.426000*	.003033	.000	-.43276	-.41924
		Control N	-.392667*	.003033	.000	-.39942	-.38591
	80%	10%	-.296000*	.003033	.000	-.30276	-.28924
		50%	-.055667*	.003033	.000	-.06242	-.04891
		Control P	-.481667*	.003033	.000	-.48842	-.47491

		Control N	-.448333*	.003033	.000	-.45509	-.44158
	Control P	10%	.185667*	.003033	.000	.17891	.19242
		50%	.426000*	.003033	.000	.41924	.43276
		80%	.481667*	.003033	.000	.47491	.48842
		Control N	.033333*	.003033	.000	.02658	.04009
	Control N	10%	.152333*	.003033	.000	.14558	.15909
		50%	.392667*	.003033	.000	.38591	.39942
		80%	.448333*	.003033	.000	.44158	.45509
		Control P	-.033333*	.003033	.000	-.04009	-.02658
72	10%	50%	-.138667*	.006995	.000	-.15425	-.12308
		80%	-.095000*	.006995	.000	-.11059	-.07941
		Control P	-.587667*	.006995	.000	-.60325	-.57208
		Control N	-.484333*	.006995	.000	-.49992	-.46875
	50%	10%	.138667*	.006995	.000	.12308	.15425
		80%	.043667*	.006995	.000	.02808	.05925
		Control P	-.449000*	.006995	.000	-.46459	-.43341
		Control N	-.345667*	.006995	.000	-.36125	-.33008
	80%	10%	.095000*	.006995	.000	.07941	.11059
		50%	-.043667*	.006995	.000	-.05925	-.02808
		Control P	-.492667*	.006995	.000	-.50825	-.47708
		Control N	-.389333*	.006995	.000	-.40492	-.37375
	Control P	10%	.587667*	.006995	.000	.57208	.60325
		50%	.449000*	.006995	.000	.43341	.46459
		80%	.492667*	.006995	.000	.47708	.50825
		Control N	.103333*	.006995	.000	.08775	.11892
	Control N	10%	.484333*	.006995	.000	.46875	.49992
		50%	.345667*	.006995	.000	.33008	.36125
		80%	.389333*	.006995	.000	.37375	.40492
		Control P	-.103333*	.006995	.000	-.11892	-.08775

*The mean difference is significant at 0.05 level

Figure 1.10 provides the activity of AChE on fat body of the target insect. Acetyl choline esterase activity (μ mol/ min / mg protein) in the fat body of the adult insects on application of 10% (0.1μg/μl) of insecticide LD_{50} were 1.376±0.001, 0.853±0.001 and 0.596±0.001; and that on application of 50% (0.4μg/μl) of insecticide LD_{50} were 1.482±0.001, 1.46±0.001 and 0.625±0.001 and the acetyl choline esterase activity (μ mol/ min / mg protein) on application with 80% of LD_{50} are 2.884±0.001, 02.76±0.001 and

1.33±0.001 for 24 hr, 48 hr, 72 hr time interval respectively. Positive control values obtained were 1.22±0.001, 1.12±0.001, 1.02±0.001 for 24 hr, 48 hr and 72 hr respectively. Negative control values were 1.12±0.001, 1.09±0.001, 1.1±0.001 for 24 hr, 48 hr and 72 hr respectively.



*Level of significance < 0.01

Figure 1.10: Acetyl choline esterase activity of the fat body of adult insect after treatment with different sublethal concentrations of carbosulfan at different time duration.

The data analysis of AChE activity of fat body tissue in adult banana weevil after treatment with different sublethal concentrations of carbosulfan and time shows the significant changes between the treated and control groups. The p value obtained is less than 0.0001 ($p < .0001$) in all groups. The F values obtained were 40563.935, 10607.959 and 18117.382 (Table 1.21). Multiple comparison of AChE activity of fat body tissue of *Odoiporus longicollis* after treatment with different sublethal concentrations of carbosulfan for different time durations is provided in table 1.22. The values of mean differences are significant at 0.05 level.

Table 1.21: Data on statistical analysis AChE activity of fat body tissue in adult insect after treatment with different sublethal concentrations of carbosulfan at different time duration

Duration (hr)	Concentration (µg/µl)	Mean enzyme activity ± SD (µ mol/min/mg protein)	One - way ANOVA
24	0.1	1.37600 ± .001	F = 40563.935 p < .0001 The result is significant at p < .0001
	0.4	1.48237 ± .001	
	0.7	2.88400 ± .001	
	Control P	1.22333 ± .001	
	Control N	1.12000 ± .001	
48	0.1	.85300 ±.001	F = 10607.959 p < .0001 The result is significant at p < .0001
	0.4	1.46333 ± .001	
	0.7	2.76467 ± .001	
	Control P	1.12333 ± .001	
	Control N	1.08667 ± .001	
72	0.1	.59600 ±.001	F = 18117.382 p < .0001 The result is significant at p < .0001
	0.4	.62500 ± .001	
	0.7	1.33733 ± .001	
	Control P	1.02000 ± .001	
	Control N	1.10367 ± .001	

Table 1.22: Multiple comparison of AChE activity of fat body tissue of adult banana weevil insect after treatment with different sublethal concentrations of carbosulfan at different time duration.

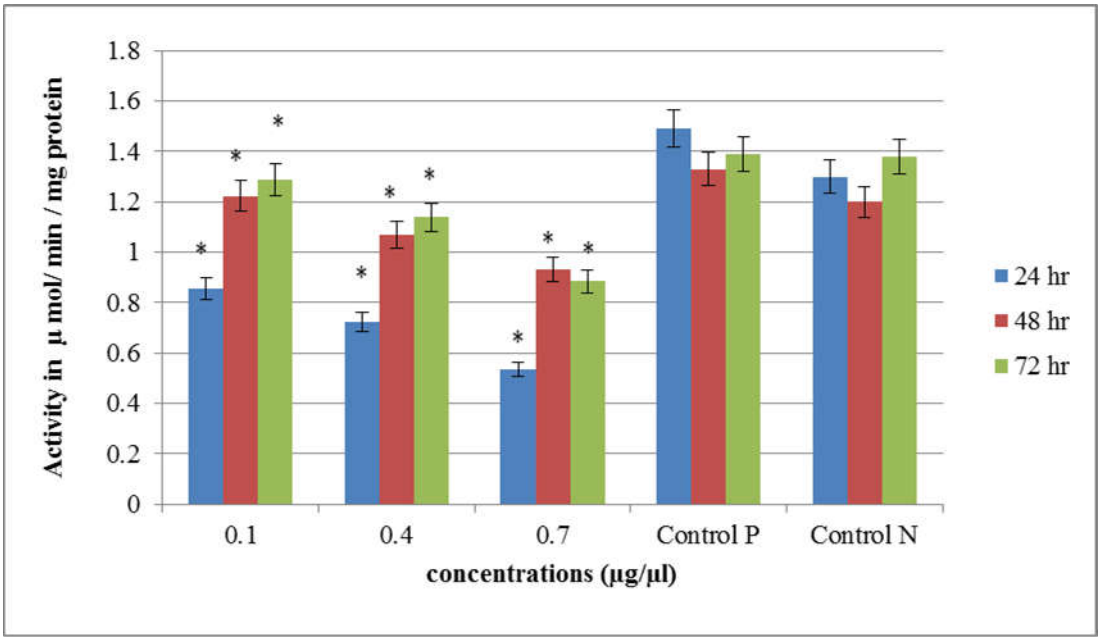
Duration (hr)	Concentration (% of LD_{50} in µg/µl)		Mean Difference	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
24	10%	50%	.523333*	.002181	.000	.51847	.52819
		80%	.781000*	.002181	.000	.77614	.78586
		Control P	.152667*	.002181	.000	.14781	.15753
		Control N	.256000*	.002181	.000	.25114	.26086
	50%	10%	-.523333*	.002181	.000	-.52819	-.51847
		80%	.257667*	.002181	.000	.25281	.26253
		Control P	-.370667*	.002181	.000	-.37553	-.36581
		Control N	-.267333*	.002181	.000	-.27219	-.26247
	80%	10%	-.781000*	.002181	.000	-.78586	-.77614
		50%	-.257667*	.002181	.000	-.26253	-.25281

		Control P	-.628333*	.002181	.000	-.63319	-.62347
		Control N	-.525000*	.002181	.000	-.52986	-.52014
	Control P	10%	-.152667*	.002181	.000	-.15753	-.14781
		50%	.370667*	.002181	.000	.36581	.37553
		80%	.628333*	.002181	.000	.62347	.63319
		Control N	.103333*	.002181	.000	.09847	.10819
	Control N	10%	-.256000*	.002181	.000	-.26086	-.25114
		50%	.267333*	.002181	.000	.26247	.27219
		80%	.525000*	.002181	.000	.52014	.52986
		Control P	-.103333*	.002181	.000	-.10819	-.09847
48	10%	50%	.019667*	.003676	.000	.01148	.02786
		80%	.858333*	.003676	.000	.85014	.86652
		Control P	.359667*	.003676	.000	.35148	.36786
		Control N	.396333*	.003676	.000	.38814	.40452
	50%	10%	-.019667*	.003676	.000	-.02786	-.01148
		80%	.838667*	.003676	.000	.83048	.84686
		Control P	.340000*	.003676	.000	.33181	.34819
		Control N	.376667*	.003676	.000	.36848	.38486
	80%	10%	-.858333*	.003676	.000	-.86652	-.85014
		50%	-.838667*	.003676	.000	-.84686	-.83048
		Control P	-.498667*	.003676	.000	-.50686	-.49048
		Control N	-.462000*	.003676	.000	-.47019	-.45381
	Control P	10%	-.359667*	.003676	.000	-.36786	-.35148
		50%	-.340000*	.003676	.000	-.34819	-.33181
		80%	.498667*	.003676	.000	.49048	.50686
		Control N	.036667*	.003676	.000	.02848	.04486
	Control N	10%	-.396333*	.003676	.000	-.40452	-.38814
		50%	-.376667*	.003676	.000	-.38486	-.36848
		80%	.462000*	.003676	.000	.45381	.47019
		Control P	-.036667*	.003676	.000	-.04486	-.02848
72	10%	50%	-.120333*	.004222	.000	-.12974	-.11093
		80%	1.430000*	.004222	.000	1.42059	1.43941
		Control P	1.740000*	.004222	.000	1.73059	1.74941
		Control N	1.660000*	.004222	.000	1.65059	1.66941
	50%	10%	.120333*	.004222	.000	.11093	.12974
		80%	1.550333*	.004222	.000	1.54093	1.55974
		Control P	1.860333*	.004222	.000	1.85093	1.86974
		Control N	1.780333*	.004222	.000	1.77093	1.78974
	80%	10%	-1.430000*	.004222	.000	-1.43941	-1.42059
		50%	-1.550333*	.004222	.000	-1.55974	-1.54093
		Control P	.310000*	.004222	.000	.30059	.31941
		Control N	.230000*	.004222	.000	.22059	.23941
	Control P	10%	-1.740000*	.004222	.000	-1.74941	-1.73059
		50%	-1.860333*	.004222	.000	-1.86974	-1.85093
		80%	-.310000*	.004222	.000	-.31941	-.30059
		Control N	-.080000*	.004222	.000	-.08941	-.07059

	Control N	10%	-1.660000*	.004222	.000	-1.66941	-1.65059
		50%	-1.780333*	.004222	.000	-1.78974	-1.77093
		80%	-.230000*	.004222	.000	-.23941	-.22059
		Control P	.080000*	.004222	.000	.07059	.08941

*. The mean difference is significant at the 0.05 level.

Activity of AChE on reproductive organ of adult insects is provided in figure 1.11. AChE activities for 10% of lethal dose application on the reproductive organ of insect were 0.854±0.001, 1.223±0.001 and 1.288±0.001; and that of 50% lethal dose application are 0.723±0.001, 1.07±0.001 and 1.139±0.001 and AChE activity for 80% lethal dose application are 0.535±0.001, 0.932±0.001 and 0.884±0.001 for the different time duration of 24 hr, 48 hr, and 72 hr respectively. Positive control values for different time durations of 24 hr, 48 hr and 72 hr were 1.49±0.001, 1.33±0.001, 1.39±0.001 and that of negative controls values were 1.3±0.001, 1.2±0.001, and 1.38±0.001 respectively.



*Level of significance < 0.01

Figure 1.11: Acetyl choline esterase activity of the reproductive organ of adult insect after treatment with different sublethal concentrations of carbosulfan at different time duration.

The statistical analysis of AChE activity in banana weevil shows that difference in between the treated and control groups are significant with a p value less than 0.0001 ($p<0.0001$). The F values are 35300.239, 572.158 and 9694.184 (Table 1.23 & 1.24).

Table 1.23: Data on statistical analysis of AChE activity of Reproductive organ tissue in adult banana weevil insect after treatment with different sublethal concentrations of carbosulfan at different time duration.

Duration (hr)	Concentration (µg/µl)	Mean enzyme activity ± SD (µ mol/min/mg protein)	One - way ANOVA
24	0.1	.85367 ± .001	F = 35300.239 p < .0001 The result is significant at p < .0001
	0.4	.72347 ± .001	
	0.7	.53467 ± .001	
	Control P	1.48667 ± .001	
	Control N	1.29667 ± .001	
48	0.1	1.22267 ± .001	F = 572.158 p < .0001 The result is significant at p < .0001
	0.4	1.07000 ± .001	
	0.7	.93200 ±.001	
	Control P	1.33000 ± .001	
	Control N	1.22333 ± .001	
72	0.1	1.28750 ± .001	F = 9694.184 p < .0001 The result is significant at p < .0001
	0.4	1.13867 ± .001	
	0.7	.88400 ±.001	
	Control P	1.38667 ± .001	
	Control N	1.38333 ± .001	

Table 1.24: Multiple comparison of AChE activity of Reproductive organ tissue in adult banana weevil insect after treatment with different sublethal concentrations of carbosulfan at different time duration.

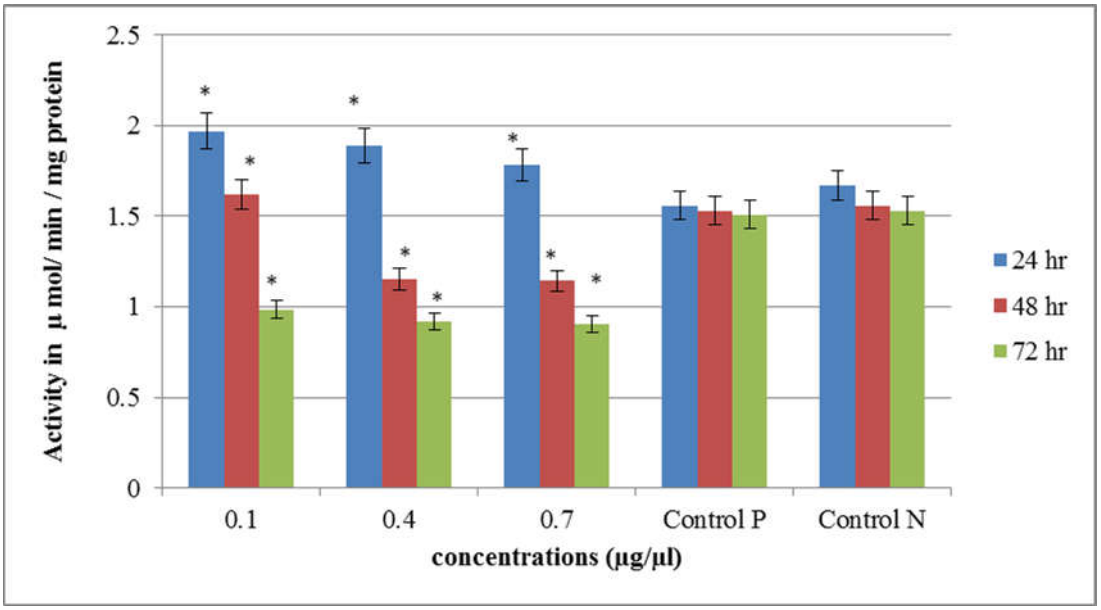
Duration (hr)	Concentration (% of LD_{50} in µg/µl)		Mean Difference	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
24	10%	50%	.130000*	.003004	.000	.12331	.13669
		80%	.319000*	.003004	.000	.31231	.32569
		Control P	-.633000*	.003004	.000	-.63969	-.62631
		Control N	-.443000*	.003004	.000	-.44969	-.43631
	50%	10%	-.130000*	.003004	.000	-.13669	-.12331

		80%	.189000*	.003004	.000	.18231	.19569
		Control P	-.763000*	.003004	.000	-.76969	-.75631
		Control N	-.573000*	.003004	.000	-.57969	-.56631
	80%	10%	-.319000*	.003004	.000	-.32569	-.31231
		50%	-.189000*	.003004	.000	-.19569	-.18231
		Control P	-.952000*	.003004	.000	-.95869	-.94531
		Control N	-.762000*	.003004	.000	-.76869	-.75531
	Control P	10%	.633000*	.003004	.000	.62631	.63969
		50%	.763000*	.003004	.000	.75631	.76969
		80%	.952000*	.003004	.000	.94531	.95869
		Control N	.190000*	.003004	.000	.18331	.19669
	Control N	10%	.443000*	.003004	.000	.43631	.44969
		50%	.573000*	.003004	.000	.56631	.57969
		80%	.762000*	.003004	.000	.75531	.76869
		Control P	-.190000*	.003004	.000	-.19669	-.18331
48	10%	50%	.151667*	.009214	.000	.13114	.17220
		80%	.290667*	.009214	.000	.27014	.31120
		Control P	-.108333*	.009214	.000	-.12886	-.08780
		Control N	-.001667	.009214	.860	-.02220	.01886
	50%	10%	-.151667*	.009214	.000	-.17220	-.13114
		80%	.139000*	.009214	.000	.11847	.15953
		Control P	-.260000*	.009214	.000	-.28053	-.23947
		Control N	-.153333*	.009214	.000	-.17386	-.13280
	80%	10%	-.290667*	.009214	.000	-.31120	-.27014
		50%	-.139000*	.009214	.000	-.15953	-.11847
		Control P	-.399000*	.009214	.000	-.41953	-.37847
		Control N	-.292333*	.009214	.000	-.31286	-.27180
	Control P	10%	.108333*	.009214	.000	.08780	.12886
		50%	.260000*	.009214	.000	.23947	.28053
		80%	.399000*	.009214	.000	.37847	.41953
		Control N	.106667*	.009214	.000	.08614	.12720
	Control N	10%	.001667	.009214	.860	-.01886	.02220
		50%	.153333*	.009214	.000	.13280	.17386
		80%	.292333*	.009214	.000	.27180	.31286
		Control P	-.106667*	.009214	.000	-.12720	-.08614
72	10%	50%	.148333*	.003033	.000	.14158	.15509
		80%	.403000*	.003033	.000	.39624	.40976
		Control P	-.099667*	.003033	.000	-.10642	-.09291

		Control N	-.096333*	.003033	.000	-.10309	-.08958
	50%	10%	-.148333*	.003033	.000	-.15509	-.14158
		80%	.254667*	.003033	.000	.24791	.26142
		Control P	-.248000*	.003033	.000	-.25476	-.24124
		Control N	-.244667*	.003033	.000	-.25142	-.23791
	80%	10%	-.403000*	.003033	.000	-.40976	-.39624
		50%	-.254667*	.003033	.000	-.26142	-.24791
		Control P	-.502667*	.003033	.000	-.50942	-.49591
		Control N	-.499333*	.003033	.000	-.50609	-.49258
	Control P	10%	.099667*	.003033	.000	.09291	.10642
		50%	.248000*	.003033	.000	.24124	.25476
		80%	.502667*	.003033	.000	.49591	.50942
		Control N	.003333	.003033	.298	-.00342	.01009
	Control N	10%	.096333*	.003033	.000	.08958	.10309
		50%	.244667*	.003033	.000	.23791	.25142
		80%	.499333*	.003033	.000	.49258	.50609
		Control P	-.003333	.003033	.298	-.01009	.00342

*. The mean difference is significant at the 0.05 level.

The data on the figure 1.12 shows the activity of AchE on gut of the adult insects when treated with different sub lethal concentrations of carbosulfan for different time intervals. Acetyl choline esterase activity (µ mol/ min / mg protein) in the gut of the adult insects on application of 10% (0.1µg/µl) of insecticide LD_{50} are 1.97±0.001, 1.62±0.001 and 0.985±0.001; and that on application of 50% (0.4µg/µl) of insecticide LD_{50} are 1.89±0.001, 1.152±0.001 and 0.916±0.001 and the acetyl choline esterase activity (µ mol/ min/mg protein) on application with 80% (0.8µg/µl) of LD_{50} are 1.782±0.001, 1.142±0.001 and 0.906±0.001 for 24 hr, 48 hr, 72 hr time intervals respectively. Positive control values were 1.56±0.001, 1.53±0.001, and 1.51±0.001 for 24 hr, 48 hr and 72 hr respectively. Negative control values were 1.67±0.001, 1.56±0.001 and 1.53±0.001 for 24 hr, 48 hr and 72 hr respectively.



*Level of significance < 0.01

Figure 1.12: Acetyl choline esterase activity of the gut of adult insect after treatment with different sublethal concentrations of carbosulfan at different time duration

Table 1.25 & 1.26 shows the statistical analysis of AChE activity of adult banana weevil gut tissue samples. The p value obtained is less than 0.001 ($p < 0.0001$). The F values are 35300.239, 3510.226 and 22561.152. The result is significant in all the time duration.

Table 1.25: Data on statistical analysis of AChE activity in Gut of adult banana weevil after treatment with different sublethal concentrations of carbosulfan at different time duration

Duration (hr)	Concentration (µg/µl)	Mean enzyme activity + SD (µ mol/min/mg protein)	One - way ANOVA
24	0.1	1.97000 + .001	F = 35300.239 p < .0001 The result is significant at p < .0001
	0.4	1.88667 + .001	
	0.7	1.78200 + .001	
	Control P	1.55667 + .001	
	Control N	1.66667 + .001	
48	0.1	1.62000 + .001	F = 3510.226 p < .0001 The result is significant at p < .0001
	0.4	1.15200 + .001	
	0.7	1.14233 + .001	
	Control P	1.53333 + .001	
	Control N	1.56000 + .001	
72	0.1	.98500 + .001	F = 22561.152 p < .0001 The result is significant at p < .0001
	0.4	.91600 + .001	
	0.7	.90567 + .001	
	Control P	1.51467 + .001	
	Control N	1.52667 + .001	

Table 1.26: Multiple comparison of AChE activity of Gut of adult banana weevil after treatment with different sublethal concentrations of carbosulfan at different time duration

Duration (hr)	Concentration (% of LD_{50} in µg/µl)		Mean Difference	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
24	10%	50%	.083333*	.005177	.000	.07180	.09487
		80%	.187000*	.005177	.000	.17547	.19853
		Control P	.413333*	.005177	.000	.40180	.42487
		Control N	.303333*	.005177	.000	.29180	.31487
	50%	10%	-.083333*	.005177	.000	-.09487	-.07180
		80%	.103667*	.005177	.000	.09213	.11520
		Control P	.330000*	.005177	.000	.31847	.34153
		Control N	.220000*	.005177	.000	.20847	.23153

	80%	10%	-.187000*	.005177	.000	-.19853	-.17547
		50%	-.103667*	.005177	.000	-.11520	-.09213
		Control P	.226333*	.005177	.000	.21480	.23787
		Control N	.116333*	.005177	.000	.10480	.12787
	Control P	10%	-.413333*	.005177	.000	-.42487	-.40180
		50%	-.330000*	.005177	.000	-.34153	-.31847
		80%	-.226333*	.005177	.000	-.23787	-.21480
		Control N	-.110000*	.005177	.000	-.12153	-.09847
	Control N	10%	-.303333*	.005177	.000	-.31487	-.29180
		50%	-.220000*	.005177	.000	-.23153	-.20847
		80%	-.116333*	.005177	.000	-.12787	-.10480
		Control P	.110000*	.005177	.000	.09847	.12153
48	10%	50%	.468000*	.005594	.000	.45554	.48046
		80%	.477667*	.005594	.000	.46520	.49013
		Control P	.086667*	.005594	.000	.07420	.09913
		Control N	.060000*	.005594	.000	.04754	.07246
	50%	10%	-.468000*	.005594	.000	-.48046	-.45554
		80%	.009667	.005594	.115	-.00280	.02213
		Control P	-.381333*	.005594	.000	-.39380	-.36887
		Control N	-.408000*	.005594	.000	-.42046	-.39554
	80%	10%	-.477667*	.005594	.000	-.49013	-.46520
		50%	-.009667	.005594	.115	-.02213	.00280
		Control P	-.391000*	.005594	.000	-.40346	-.37854
		Control N	-.417667*	.005594	.000	-.43013	-.40520
	Control P	10%	-.086667*	.005594	.000	-.09913	-.07420
		50%	.381333*	.005594	.000	.36887	.39380
		80%	.391000*	.005594	.000	.37854	.40346
		Control N	-.026667*	.005594	.001	-.03913	-.01420
	Control N	10%	-.060000*	.005594	.000	-.07246	-.04754
		50%	.408000*	.005594	.000	.39554	.42046
		80%	.417667*	.005594	.000	.40520	.43013
		Control P	.026667*	.005594	.001	.01420	.03913
72	10%	50%	.069000*	.003033	.000	.06224	.07576
		80%	.080333*	.003033	.000	.07358	.08709
		Control P	-.530667*	.003033	.000	-.53742	-.52391
		Control N	-.540667*	.003033	.000	-.54742	-.53391
	50%	10%	-.069000*	.003033	.000	-.07576	-.06224
		80%	.011333*	.003033	.004	.00458	.01809
		Control P	-.599667*	.003033	.000	-.60642	-.59291

		Control N	-.609667*	.003033	.000	-.61642	-.60291
	80%	10%	-.080333*	.003033	.000	-.08709	-.07358
		50%	-.011333*	.003033	.004	-.01809	-.00458
		Control P	-.611000*	.003033	.000	-.61776	-.60424
		Control N	-.621000*	.003033	.000	-.62776	-.61424
	Control P	10%	.530667*	.003033	.000	.52391	.53742
		50%	.599667*	.003033	.000	.59291	.60642
		80%	.611000*	.003033	.000	.60424	.61776
		Control N	-.010000*	.003033	.008	-.01676	-.00324
	Control N	10%	.540667*	.003033	.000	.53391	.54742
		50%	.609667*	.003033	.000	.60291	.61642
		80%	.621000*	.003033	.000	.61424	.62776
		Control P	.010000*	.003033	.008	.00324	.01676

*. The mean difference is significant at the 0.05 level.

1.4.3. HPLC analysis of carbosulfan degradation products

Enzymes extracted from gut, fat body and reproductive organ of adult banana stem weevil, *Odoiporus longicollis* were analyzed for their activity on carbosulfan at different enzyme concentration during 60 min of time incubation. The degraded carbosulfan and the products were scanned to detect them by HPLC.

Carbosulfan standard was analysed for the comparison with the samples. The retention time and absorbance units were recorded during scanning of samples taken after degradation of carbosulfan with different concentrations of enzymes extracted from different tissues were provided in table 1.27- 1.29. The retention time of standard carbosulfan is 5.209 min (Fig. 1.13). All the samples taken for analysis showed additional peaks other than that of carbosulfan peak indicating the presence of additional degradation products and hence the presence of degrading enzymes in the insect tissue extracts (Fig. 1.13-1.25).

Samples taken from incubations with 0.1ml of adult gut enzyme extract showed 6 additional peaks (Fig. 1.14). Four peaks are formed before carbosulfan standard peak and other two peaks after it. The retention times of

peaks formed are3.324 min, 3.458 min, 3.714 min, 4.621 min, 5.386 min, and 6.9 min. Of this highest area percentage (43.168%) obtained for the peak of 3.714 min. The retention time of the peaks formed are obtained as 3.314 min, 3.439 min, 3.727 min, 5.415 min, 6.97 min, and 1.649 min when 0.2 ml of adult gut enzyme extract is analysed (Fig. 1.15). The highest area percentage (55.998%) obtained for the peak at 3.727 min. At 0.5 ml enzyme application, the retention time observed for the different peaks formed are 1.590 min, 3.302 min, 3.714 min and 5.408 min (Fig. 1.16).The highest area percentage (47.798%) observed for the peak at 3.714 min. The retention time observed for the different peaks of the control gut samples are 0.360min, 1.850 min, 2.513 min, 3.413 min, and 3.844 min (Fig.1.17). The highest area percentage of 42.783 % was obtained for the retention time at 1.850 min (Fig. 1.14, 1.15, 1.16, 1.17).

Samples taken from incubations with adult fat body enzyme extract (0.1ml) samples showed three peaks at the retention times of 3.33 min, 3.721 min and 5.417 min (Fig. 1.18). Of this highest area percentage (68.693%) obtained for the peak at 3.721 min. There were 6 additional peaks formed on the analysis of 0.2ml fat body enzyme extract incubation. The retentiontime of the peaks observed are 3.316 min, 3.475 min, 3.727 min, 5.429 min, 5.944 min and 7.002 min (Fig. 1.19). Of this highest area percentage (66.729 %) obtained for the peak at 3.727 min. Analysis of 0.5 ml of enzyme fat body extract, the retention time of the peaks formed are 3.290 min, 3.455 min, 3.709 min, 5.399 min, 5.955 min and 6.952 min (Fig. 1.20).The highest area percentage (56.82%) obtained for the peak at 3.709 min peak. For the control samples, the retention time of the peaksformed are 2.113 min, 2.815 min, 3.388 min, 3.589min, 3.733 min and 3.836 min. Of these, the highest area percentage (42.104 %) obtained for the peak at 2.815 min (Fig. 1.18- 1.21).

Samples taken from incubations with 0.1 ml of adult reproductive organ enzyme extract showed 3 peaks at the retention times of 3.28min, 3.714 min and 5.4 min (Fig. 1.22).The highest area percentage (70.649 %) obtained

for the peak at 3.714 min. On application of 0.2 ml of enzyme extract, 3.28 min, 3.722 min, 5.403 min, and 6.955 min are the retention time of the peaks formed. Of this highest area percentage (56.779 %) obtained for the peak at 3.722 min (Fig.1.23). Analysis of 0.5 ml of enzyme extract the retention time observed for the different peaks formed are 1.896 min, 3.272 min, 3.712 min, 5.396 min and 5.948 min (Fig.1.24).The highest areapercentage (57.246 %) obtained for the peak at 3.712 min. For the control samples the retention time observed for the different peaks formed are 2.375 min, 2.795 min, 3.392 min, 3.585 min, 3.747 min, and 4.182 min. Of this highest area percentage (39.185 %) obtained for the peak at 2.795 min (Fig. 1.22-1.25).

Table 1.27: The retention time (min) and absorbance units (mAU) recorded during the scanning of samples taken after degradation of carbosulfan with different concentration of enzyme extracted from adult gut

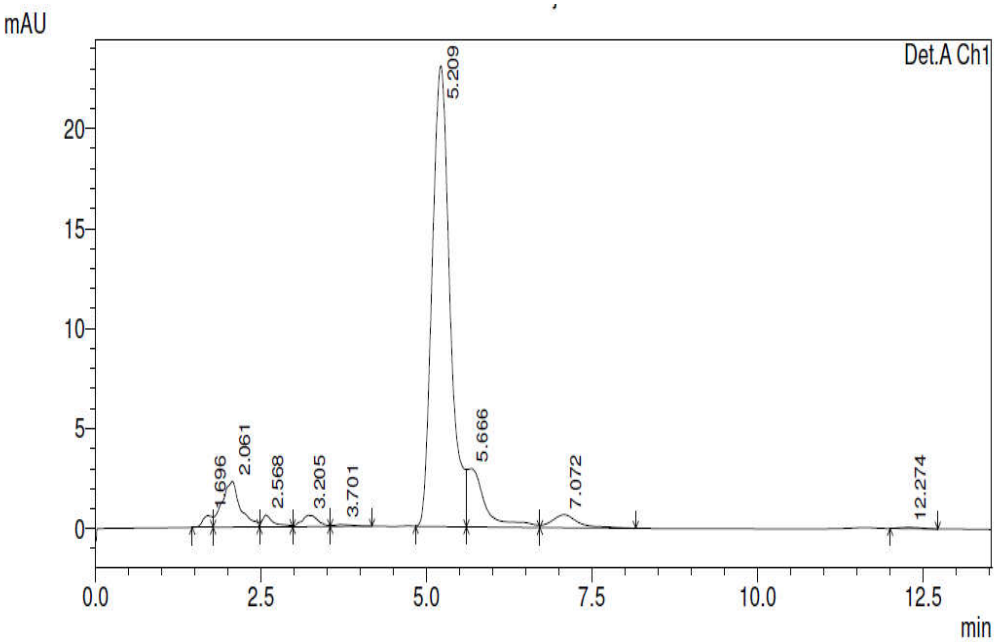
Source of enzyme		Retention time (min)	Absorbance (mAU)
gut	0.1 ml	3.324 3.458 3.714 4.621 5.386 6.9	11 4 29 4 6 9
	0.2ml	3.314 3.439 3.727 5.415 6.97 1.649	12 8 29 9 2 2
	0.5 ml	1.590 3.302 3.714 5.408	30 45 145 30
	Control	0.360 1.850 2.513 3.413 3.844	.8 3.2 2.6 1.4 .8
Carbosulfan		5.209	23

Table 1.28: The retention time (min) and absorbance units (mAU) recorded during the scanning of samples taken after degradation of carbosulfan with different concentration of enzyme extracted from adult fat body

Source of enzyme		Retention time (min)	Absorbance (mAU)
Fat body	0.1 ml	3.33 3.721 5.417	20 150 30
	0.2ml	3.316 3.475 3.727 5.429 5.944 7.002	60 20 170 30 10 10
	0.5 ml	3.290 3.455 3.709 5.399 5.955 6.952	30 10 170 30 10 10
	Control	2.113 2.815 3.388 3.589 3.733 3.836	.15 9.5 2 3 2 1.5
Carbosulfan		5.209	23

Table 1.29: The retention time (min) and absorbance units (mAU) recorded during the scanning of samples taken after degradation of carbosulfan with different concentration of enzyme extracted from adult reproductive organ.

Source of enzyme		Retention time (min)	Absorbance (mAU)
Reproductive organ	0.1 ml	3.28 3.714 5.4	20 170 40
	0.2ml	3.28 3.722 5.403 6.955	30 140 30 20
	0.5 ml	1.896 3.272 3.712 5.396 5.948	10 40 160 30 5
	control	2.375 2.795 3.392 3.585 3.747 4.182	1.5 6.5 2.5 2 2.5 .5
Carbosulfan		5.209	23

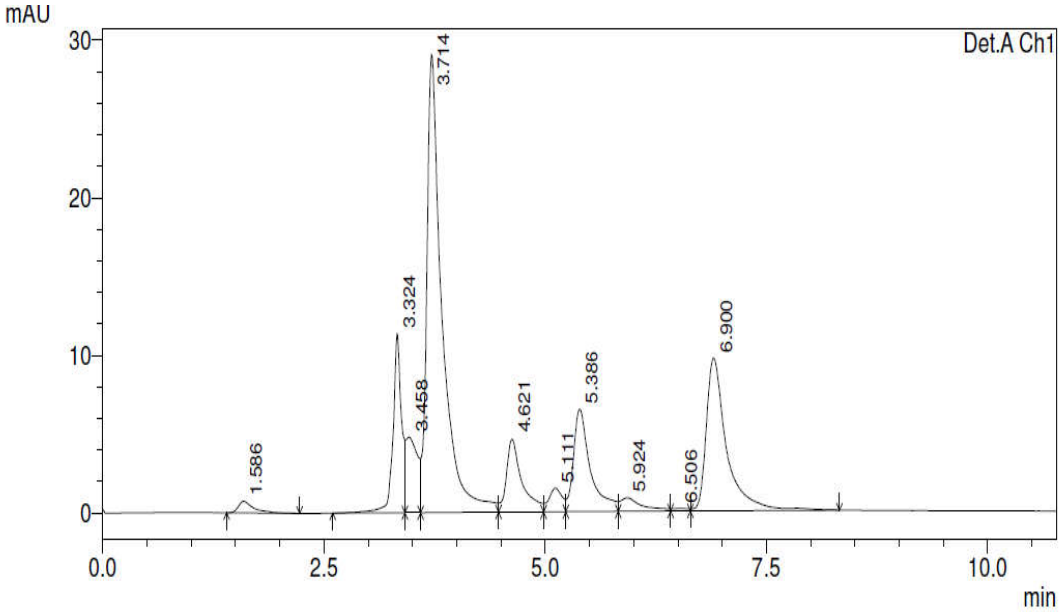


PeakTable

UV Detector Ch1 280nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	1.696	5789	572	1.013	1.862
2	2.061	43627	2291	7.633	7.454
3	2.568	7047	585	1.233	1.903
4	3.205	9610	556	1.681	1.809
5	3.701	1880	101	0.329	0.328
6	5.209	429268	23018	75.109	74.887
7	5.666	55227	2901	9.663	9.439
8	7.072	17657	645	3.089	2.098
9	12.274	1426	68	0.249	0.220
Total		571530	30737	100.000	100.000

Fig 1.13: HPLC of carbosulfan standard

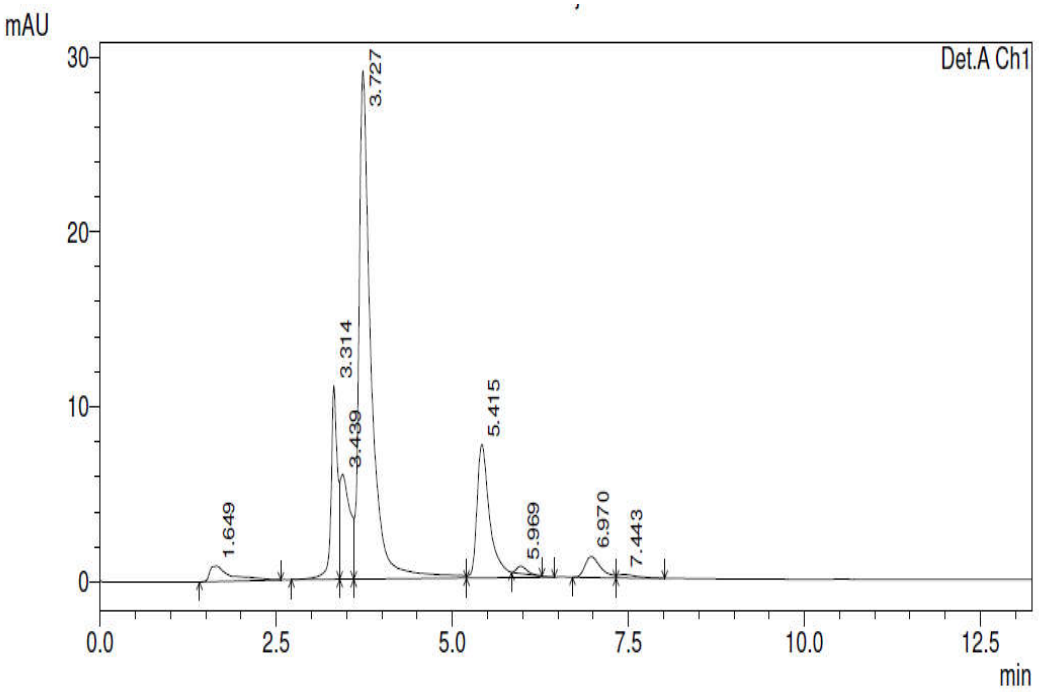


PeakTable

UV Detector Ch1 280nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	1.586	8687	724	1.076	1.045
2	3.324	76592	11375	9.489	16.426
3	3.458	44252	4781	5.482	6.904
4	3.714	348437	29059	43.168	41.963
5	4.621	56096	4617	6.950	6.668
6	5.111	15551	1494	1.927	2.158
7	5.386	86256	6501	10.686	9.388
8	5.924	14726	855	1.824	1.235
9	6.506	2003	159	0.248	0.229
10	6.900	154557	9684	19.148	13.984
Total		807156	69249	100.000	100.000

Fig 1.14: HPLC of carbosulfan degradation products after activity of enzyme extracted from adult insect gut (0.1 ml, 60 min incubation).

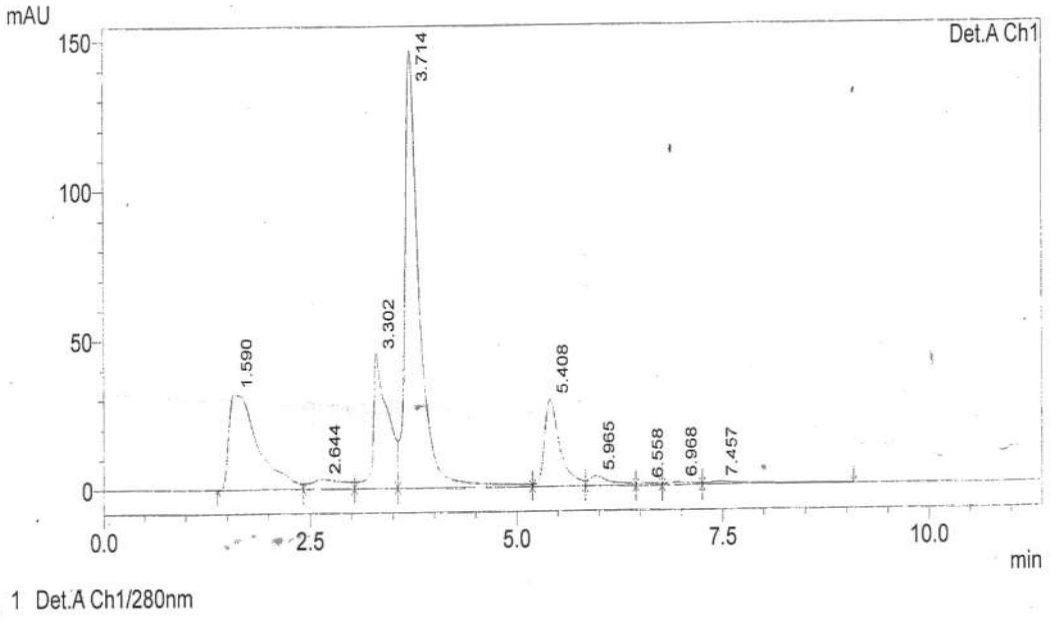


PeakTable

UV Detector Ch1 280nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	1.649	18323	876	2.952	1.554
2	3.314	70233	11061	11.316	19.619
3	3.439	57576	5990	9.276	10.624
4	3.727	347561	29032	55.998	51.495
5	5.415	100599	7616	16.208	13.508
6	5.969	4137	411	0.667	0.729
7	6.970	18673	1194	3.009	2.118
8	7.443	3561	199	0.574	0.352
Total		620661	56378	100.000	100.000

Fig 1.15: HPLC of carbosulfan degradation products after activity of enzyme extracted from adult insect gut (0.2 ml, 60 min incubation).

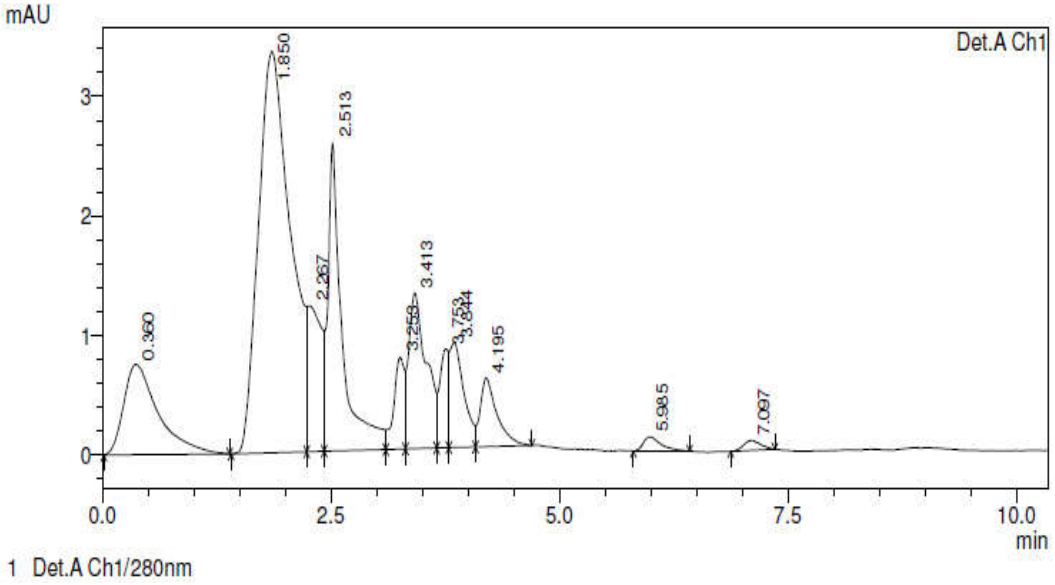


PeakTable

UV Detector Ch1 280nm

Peak#	Ret. Time	Area	Height	Area %
1	1.590	787031	32366	21.436
2	2.644	92361	3204	2.516
3	3.302	552138	45418	15.039
4	3.714	1754882	146368	47.798
5	5.408	377560	29323	10.284
6	5.965	54432	3176	1.483
7	6.558	8503	514	0.232
8	6.968	16575	865	0.451
9	7.457	27998	849	0.763
Total		3671481	262084	100.000

Fig 1.16: HPLC of carbosulfan degradation products after activity of enzyme extracted from adult insect gut (0.5 ml, 60 min incubation).

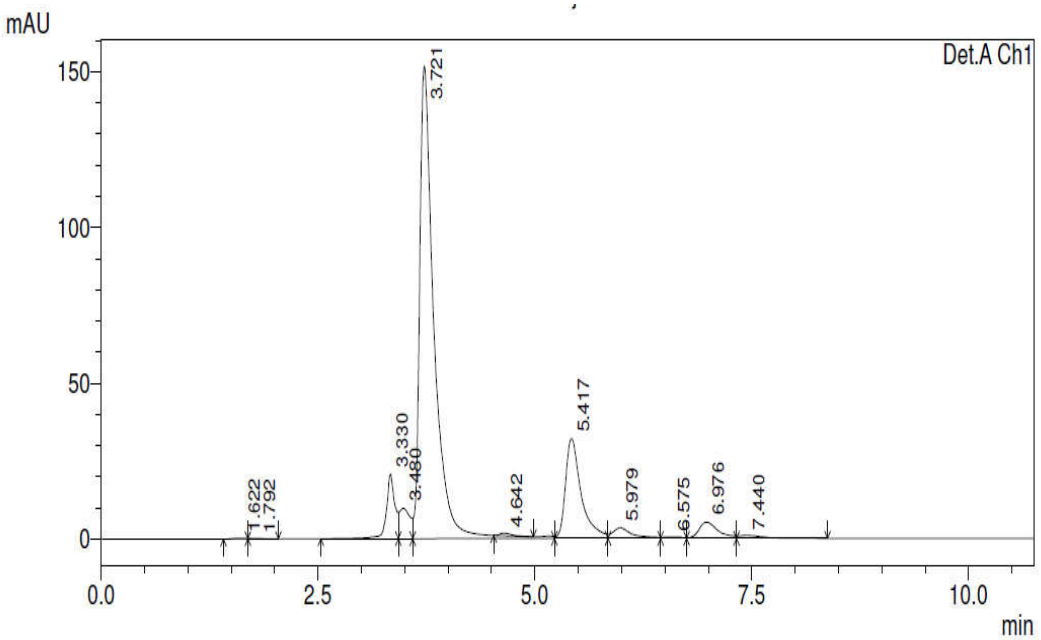


PeakTable

UV Detector Ch1 280nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	0.360	19866	755	10.379	6.061
2	1.850	81895	3356	42.783	26.935
3	2.267	13010	1218	6.797	9.775
4	2.513	27938	2576	14.595	20.677
5	3.253	6279	769	3.281	6.173
6	3.413	17522	1301	9.154	10.445
7	3.753	5615	828	2.934	6.647
8	3.844	9343	879	4.881	7.056
9	4.195	7268	575	3.797	4.614
10	5.985	1620	119	0.846	0.952
11	7.097	1060	83	0.554	0.666
Total		191417	12461	100.000	100.000

Figs 1.17: HPLC of adult weevil gut enzyme extract (control).

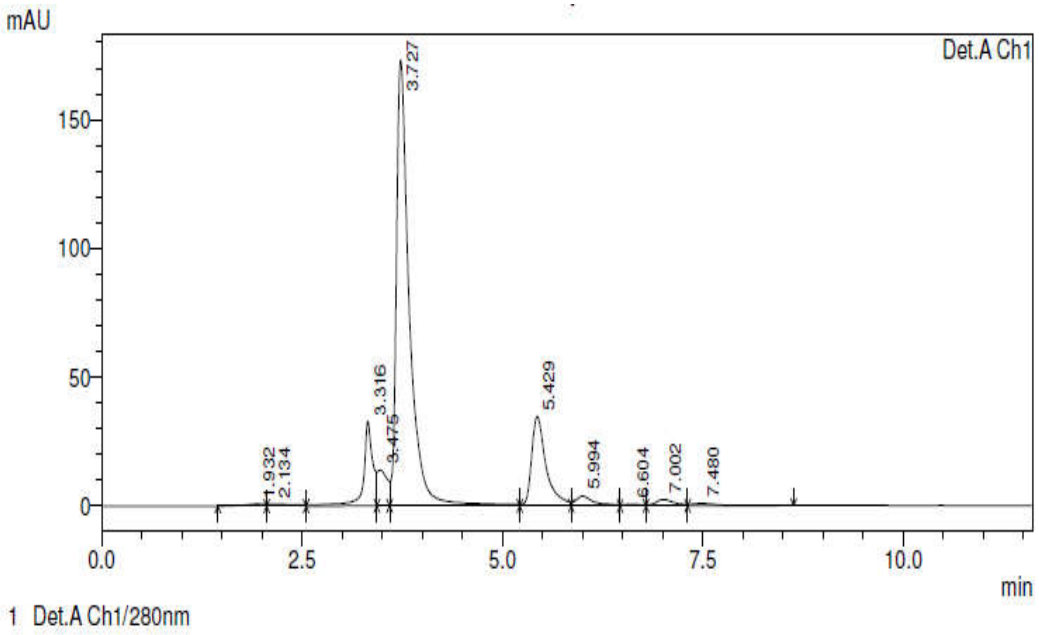


PeakTable

UV Detector Ch1 280nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	1.622	1847	185	0.073	0.082
2	1.792	1615	130	0.064	0.058
3	3.330	140112	20773	5.546	9.217
4	3.480	83375	9738	3.300	4.321
5	3.721	1735580	151667	68.693	67.294
6	4.642	8126	893	0.322	0.396
7	5.417	401171	32078	15.878	14.233
8	5.979	51821	3389	2.051	1.503
9	6.575	5836	392	0.231	0.174
10	6.976	79117	5206	3.131	2.310
11	7.440	17976	929	0.711	0.412
Total		2526578	225379	100.000	100.000

Fig1.18: HPLC of carbosulfan degradation products after activity of enzyme extracted from adult insect fat body (0.1 ml, 60 min incubation).

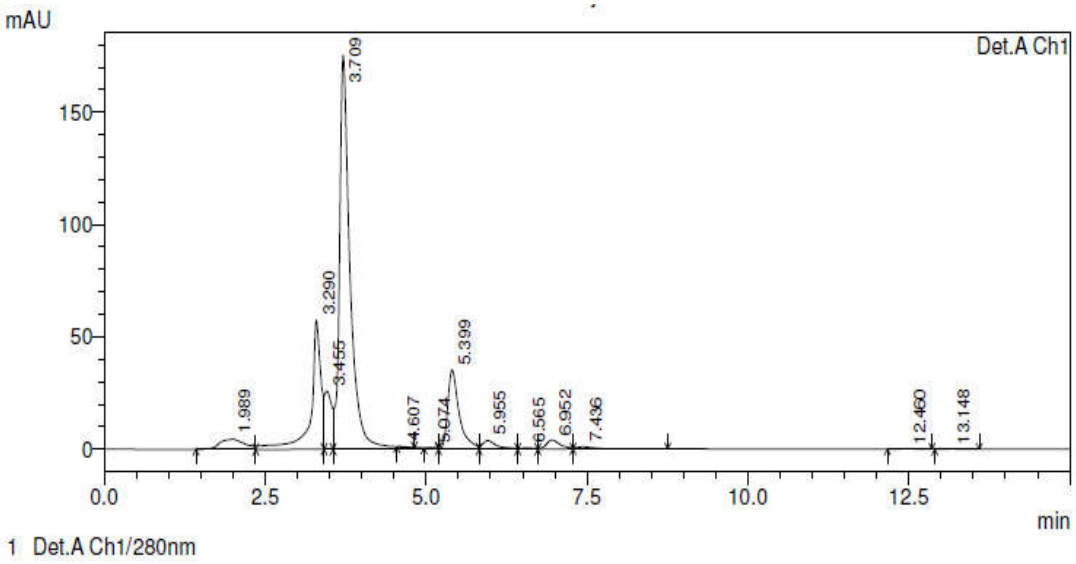


Peak Table

UV Detector Ch1 280nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	1.932	13282	713	0.456	0.271
2	2.134	16373	645	0.562	0.245
3	3.316	267766	32906	9.191	12.487
4	3.475	115818	13911	3.975	5.279
5	3.727	1944012	173128	66.729	65.699
6	5.429	433667	34726	14.886	13.178
7	5.994	57652	3715	1.979	1.410
8	6.604	7821	485	0.268	0.184
9	7.002	37290	2382	1.280	0.904
10	7.480	19630	904	0.674	0.343
Total		2913310	263515	100.000	100.000

Fig 1.19: HPLC of carbosulfan degradation products after activity of enzyme extracted from adult insect fat body (0.2 ml, 60 min incubation).

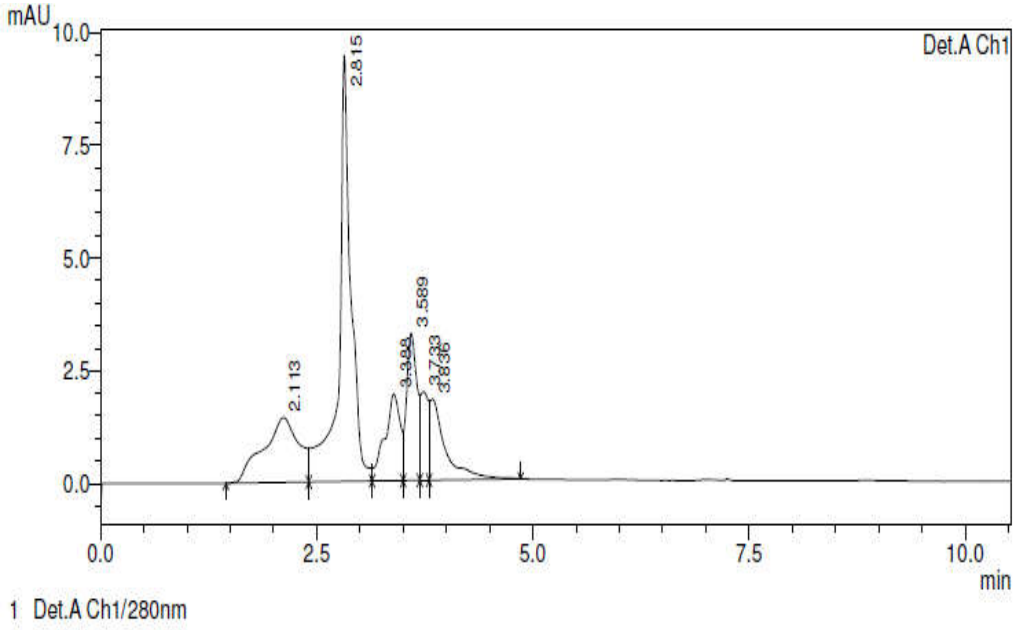


PeakTable

UV Detector Ch1 280nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	1.989	121291	4514	3.405	1.464
2	3.290	626548	57470	17.591	18.636
3	3.455	193407	25902	5.430	8.399
4	3.709	2023768	175432	56.820	56.888
5	4.607	1149	147	0.032	0.048
6	5.074	1013	150	0.028	0.049
7	5.399	439388	35278	12.336	11.440
8	5.955	58548	3814	1.644	1.237
9	6.565	7628	488	0.214	0.158
10	6.952	61816	4014	1.736	1.302
11	7.436	23662	996	0.664	0.323
12	12.460	1818	90	0.051	0.029
13	13.148	1671	85	0.047	0.028
Total		3561707	308381	100.000	100.000

Fig 1.20: HPLC of carbosulfan degradation products after activity of enzyme extracted from adult insect fat body (0.5 ml, 60 min incubation).

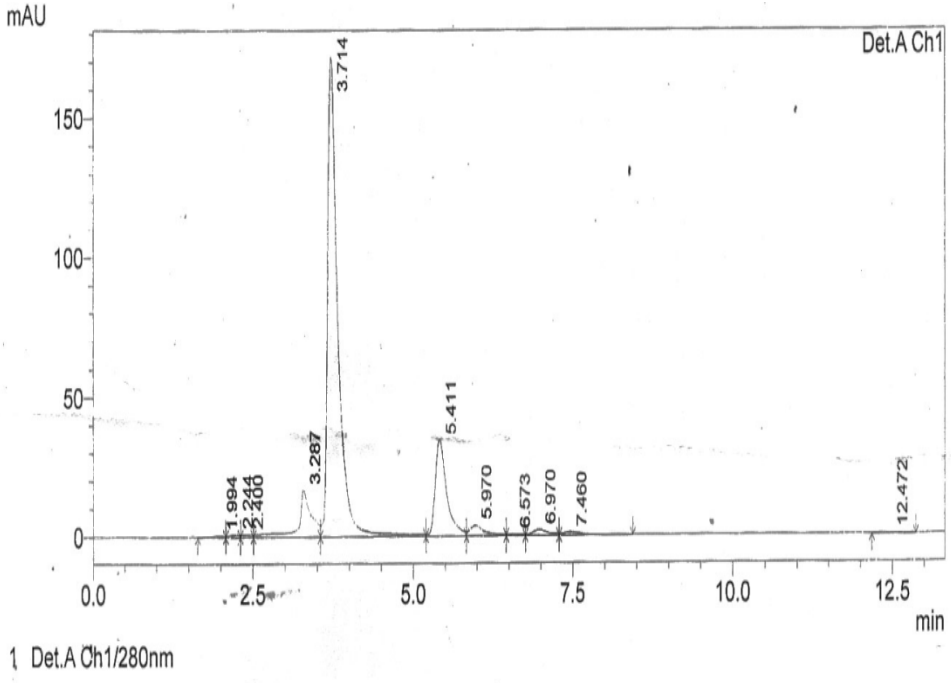


PeakTable

UV Detector Ch1 280nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	2.113	41275	1437	18.770	7.243
2	2.815	92587	9446	42.104	47.599
3	3.388	23829	1930	10.836	9.724
4	3.589	27287	3268	12.409	16.469
5	3.733	12248	1966	5.570	9.909
6	3.836	22675	1797	10.311	9.056
Total		219901	19844	100.000	100.000

Fig 1.21: HPLC of adult weevil fat body enzyme extracts (control).

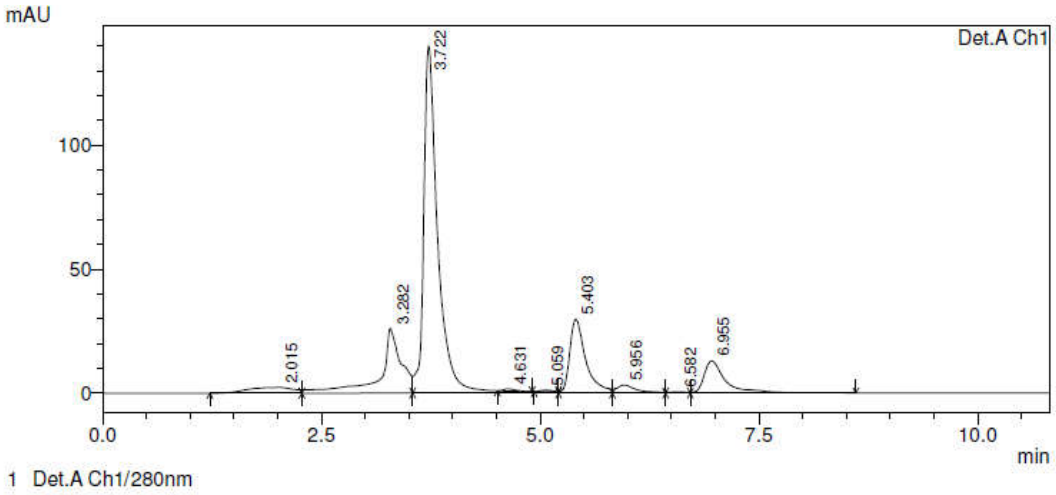


PeakTable

UV Detector Ch1 280nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	1.994	6641	451	0.247	0.195
2	2.244	8642	744	0.322	0.321
3	2.400	9046	790	0.337	0.340
4	3.287	227925	16935	8.489	7.301
5	3.714	1896904	171642	70.649	73.999
6	5.411	426698	34533	15.892	14.888
7	5.970	55348	3649	2.061	1.573
8	6.573	5384	356	0.201	0.153
9	6.970	29799	1926	1.110	0.830
10	7.460	17172	856	0.640	0.369
11	12.472	1413	69	0.053	0.030
Total		2684972	231951	100.000	100.000

Fig 1.22: HPLC of carbosulfan degradation products after activity of enzyme extracted from adult insect reproductive organ (0.1ml, 60 min incubation).

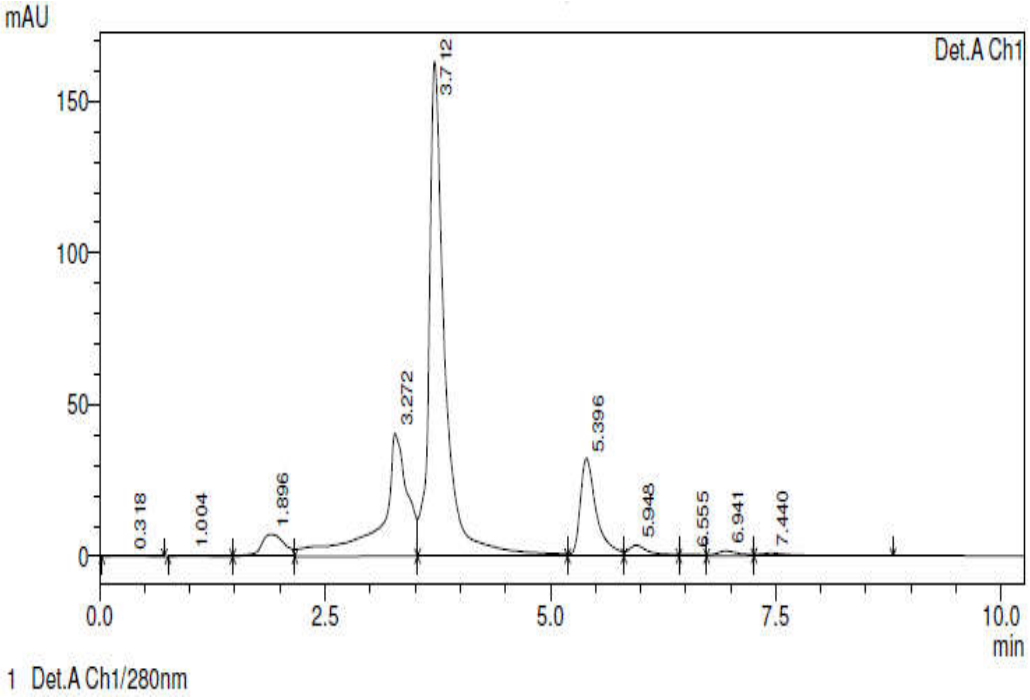


PeakTable

UV Detector Ch1 280nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	2.015	84884	2344	3.063	1.083
2	3.282	440930	26101	15.909	12.062
3	3.722	1573707	140028	56.779	64.712
4	4.631	8083	879	0.292	0.406
5	5.059	5144	640	0.186	0.296
6	5.403	378960	29800	13.673	13.772
7	5.956	50871	3230	1.835	1.493
8	6.582	6147	416	0.222	0.192
9	6.955	222899	12947	8.042	5.984
Total		2771625	216385	100.000	100.000

Fig 1.23: HPLC of carbosulfan degradation products after activity of enzyme extracted from adult insect reproductive organ (0.2ml, 60 min incubation).

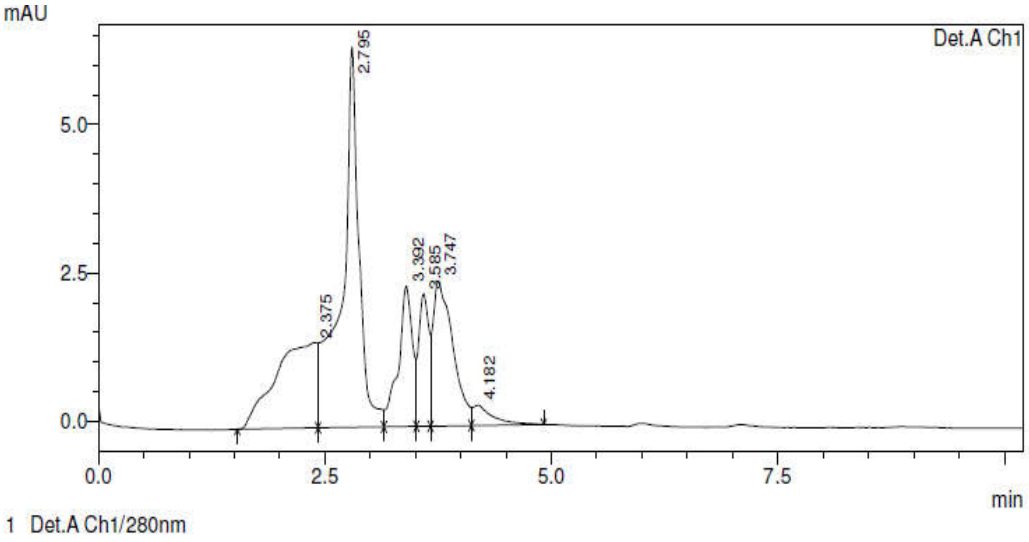


PeakTable

UV Detector Ch1 280nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	0.318	1856	92	0.054	0.037
2	1.004	1624	81	0.047	0.033
3	1.896	130129	7187	3.766	2.878
4	3.272	824977	40509	23.878	16.222
5	3.712	1977863	163138	57.246	65.331
6	5.396	403655	32172	11.683	12.884
7	5.948	56423	3542	1.633	1.418
8	6.555	7788	478	0.225	0.192
9	6.941	27504	1602	0.796	0.641
10	7.440	23188	910	0.671	0.364
Total		3455007	249710	100.000	100.000

Fig 1.24: HPLC of carbosulfan degradation products after activity of enzyme extracted from adult insect reproductive organ (0.5ml, 60 min incubation).



PeakTable

UV Detector Ch1 280nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	2.375	46317	1433	21.337	9.419
2	2.795	85060	6411	39.185	42.129
3	3.392	25957	2364	11.958	15.532
4	3.585	17162	2230	7.906	14.651
5	3.747	37296	2441	17.181	16.037
6	4.182	5281	340	2.433	2.232
Total		217075	15218	100.000	100.000

Fig 1.25: HPLC of adult weevil reproductive organ enzyme extracts (control).

1.5. DISCUSSION

Pesticides and toxicants always exert a systemic effect on both target and non target organisms, which could be identified by studying the mobilization of different key substrates and by following the activity of important enzymes. Chemical control of insect pests is the most dominant approach at present. Toxicants in sub lethal dosages produced changes in the biochemical response of organisms leading to a change in physiological functions (Simpson and Raubenheimer, 1993; Srivastava, 1962 and Singh *et al*., 1988).

Monitoring key biochemical factors lead to the identification of the various effects produced by toxicants (Rao *et al*., 1984). When toxic chemical pesticides were used in the management of injurious insect pests, the various biochemical factors of the pests were modified leading to the death of the organisms or to changes in the organisms preventing its reproductive capabilities (Nath *et al*., 1997 and Nath and Kumar, 1999). The results obtained in the present investigation clearly indicate that various concentrations of the carbosufan have exerted intensive toxic effect at the tissue level in the *Odoiporus longicollis*.

Detoxification mechanism is an important mode of resistance in almost all insects. The detoxification enzyme analysis helps in the estimation of resistance mechanism in insects. Glutathione-s-transferase (GSTs) and P450 mediated monooxygenase (MFOs) are important detoxifying enzymes involved in resistance mechanism in carbamates and organophosphate insecticides. In insects, AChE has mainly been studied in relation to insecticide resistance because the enzyme is the target of organophosphate and carbamate insecticides and its insensitivity to insecticides is one of the major factors accounting for resistance; so these enzymes are used as reliable

markers to assess the impact of toxic compounds on insects (Kudom *et al*., 2011).

GST enzymes catalyze the conjugation of reduced glutathione (GSH) with exogenous (drugs, pesticides, and other pollutants) and endogenous toxic compounds (intracellular metabolites) with electrophilic functional groups to render them less toxic, more water soluble, more easily degraded and excreted (Halliwell and Gutteridge, 1989; Leaver and George, 1998; Armstrong, 1997; Wang and Ballatori, 1998; Gadagbui and James, 2000; Kostaropoulos *et al*., 2001; Oost *et al*., 2003). They help to protect cells from oxidative stress and chemical toxicants by aiding the excretion of electrophilic and lipophilic compounds from the cell (Hayes and Pulford, 1995). It is mainly found in the hepatic cytosolic and microsomal fractions, which catalyses the conjugation of reduced glutathione (GSH) with a wide variety of electrophilic xenobiotics and other endogenous compounds, which include carcinogens as well as various compounds that are the products of oxidative stress including oxidised DNA and lipid (Hayes and Strange, 1995). The GSTs often act as a secondary resistance mechanism in conjunction with a P450 or esterase based resistance mechanism (Hemingway *et al*., 1991). Several studies have shown that insecticide-resistant insects have elevated levels of Glutathione-S-Transferase (GST) activity in crude homogenates, which suggests GSTs role in resistance (Grant *et al*., 1991). In addition to the role in detoxification and excretion of xenobiotics, GSTs are also concerned with the protection of cells from peroxidative damages and participates in the transport of endogenous compounds such as steroids, bilirubin and haem (Smith *et al*., 1989; Armstrong, 1991).

The present study investigated that GST activity was increased after the treatment of the insect with sub lethal concentration of carbosulfan. Results demonstrate that GSTs of the banana weevil are involved in the

detoxification of carbosulfan. Induced GST activity indicates the role of this enzyme in protection against the toxicity of xenobiotic-induced lipid peroxidation. Glutathione can act either to detoxify activated oxygen species such as H_2O_2 or to reduce lipid peroxides themselves. Antioxidants such as reduced glutathione (GSH) are central to cellular defense against oxidative stress. The analysis of the results obtained from the present study on GST activity in banana weevil shows that, GSTs were involved in carbosulfan detoxification and resistance development.

It is observed in the present study that GST enzymes activity is dose dependent and time dependent. On application of different concentrations of lethal dose of carbosulfan on adult insect whole body, the GST activity was significantly increased with dose dependent manner during 24hr, 48 hr and 72 hr of exposure. The GST activity due to 10% of lethal dose showed a decrease in the activity in 48 hr and 72 hr exposure, whereas 50% and 80% lethal dose exhibited an elevation in activity compared to control samples (Fig.1.1. & Table 1.3, 1.4)

GST activity in fat body showed a significant difference in the activity compared to control samples. On 24 hr of exposure, activity was high in all the three samples (10% LD_{50}, 50% LD_{50}, and 80% LD_{50}) compared to control. For 10% LD_{50} and 50% LD_{50} application; GST activity decreased over 48hr and 72hr time exposure compared to control samples. But there is a slight increase in the activity for 80% LD_{50} application during 48hr and 72hr time exposure compared to control samples. The increase in the GST activity on application of the different sub lethal concentrations was dose dependent and time dependent (Fig 1.2 & Table 1.5, 1.6).

GST activity of reproductive organ also showed an increase in the activity compared to control on 80% LD_{50} application of carbosulfan for 24hr, 48hr and 72hr compared to control insects. But the GST activity was reduced

on 10% LD_{50} application of carbosulfan for all the time exposure. For 50% of LD_{50} application 24hr and 48hr samples showed an elevated activity whereas 72hr sample showed a reduction in activity compared to control (Fig. 1.3 & Table 1.7, 1.8).

There is a reduced GST activity in the gut tissue compared to all the other tissue. GST enzyme got inhibited in the samples exposed to different concentrations of LD_{50} and different time intervals compared to control samples in the insect gut (Fig. 1.4 & Table 1.9, 1.10). There was a reduction in GST activity in different time intervals. This shows that there is a reduction in detoxifying activity in insects over time. Depletion of glutathione may reduce the cellular ability to destroy free radicals and reactive oxygen species, so that it raises the general oxidative potential in the cells. As GST is involved in xenobiotic detoxification and excretion of xenobiotics and their metabolites, its increased activity in fat body may indicate development of a defensive mechanism to cope with reactive toxicant. The high increase in GST activity in the fat body may be attributed to its function as the most effective site for the detoxification of xenobiotics.

P450s are a group of important stress response-related genes that play significant roles in several physiological processes, including hormone metabolism, the adaptation to natural and synthetic toxins, and insecticide detoxification. As we know, over expression of the gene coding of the P450 clades (CYP4, CYP6, and CYP9), contribute considerably to insecticide-resistance (Li *et al*., 2007; Bass *et al*., 2014). Insect Cytochrome P450 can be detected in a wide range of tissues. Highest monooxygenase activities are usually associated with the mid gut, fat bodies and malpighian tubules (Hodgson, 1983).

In the present study the MFO activity is significantly increased compared to control samples in adult insect whole body extract (Fig. 1.5 &

Table 1.11, 1.12). For 0.1µg/µl application of insecticide 48hrs showed the highest activity. There is a reduction in MFO activity over time during 50% application of LD_{50}. At 48 hrs the activity was maximum at 0.7µg/µl application of carbosulfan. All the MFO values were higher than the control values in whole body samples.The data on the monooxygenase activity of fat body of the insect suggests that there is an increased detoxification in banana weevil (Fig 1.6 & 1.13, 1.14). All the samples showed a high activity at 48hr of insecticide application. There is a significant increase of enzyme activity in all the samples of reproductive organ tissue extract (Fig. 1.7 & 1.15, 1.16). Here also 48hr of incubation samples showed higher activity. Studies on the gut tissue samples also suggest the role of gut in detoxification of insecticide. The sample applied with the 10% of LD_{50} of Carbosulfan shows that the MFO activity increased over time duration. For concentrations of 50% and 80% of LD_{50} carbosulfan application, MFO levels increased at 24hr and 48hr and reduced at 72 hr of exposure duration. The maximum activity was at 48 hr compared to controlgroup (Fig. 1.8 & Table 1.17, 1.18).

The increased MFO levels in the entire treated samples suggest its role in detoxification in adult banana stem weevil. These findings indicate that insects can adapt to the stress induced by carbosulfan by activating their detoxifying enzymes. Elevated levels of MFO in the present study suggest that the enzymatic detoxifications due to MFO are responsible for the development of resistance in insects.

Acetylcholinesterase (AChE) is the target of both organophosphate and carbamate insecticides. It is responsible for neurotransmitter degradation at the cholinergic nerve synapse. In the adult whole body samples at 24hr exposure, the AChE activity is increased on application of different sub lethal concentrations compared to control samples. But the AChE activity is inhibited at 48 hr and 72 hr time interval (Fig. 1.19 & Table 1.19, 1.20).

Compared to other tissues, fat body showed the highest AChE activity (Fig. 1.20 & Table 1.21, 1.22). This may suggest the involvement of metabolic resistance mechanism in this insect population. In reproductive organs, AChE activity is inhibited for all the samples compared to control samples (Fig.1.21 & Table 1.23, 1.24). In insect gut samples, only the 24hr incubation samples showed an increased level whereas others got inhibited compared to control samples (Fig.1.22 & Table 1.24, 1.25).

The target site for carbamate insecticides is acetylcholinesterase, neurotransmitter. These insecticides block the nerve transmission and leads to the death of the insect. Increased level of the enzyme shows that it is less sensitive to insecticide leading to resistance. A major type of antiChE insecticide resistance is selection for mutations conferring reduced OP and/or MC sensitivity, first noted in spider mites (Smissaert, 1964) with well over 20 examples in insects involving at least 14 specific identified mutations (Oakeshott *et al*., 2010; Villatte *et al*., 2000; Fournier *et al*.,1993 and Fournier, 2005).

The present study to detect the presence of insecticide degrading enzymes in different insect tissues such as gut, reproductive organ and fat body of banana weevil indicated that there are enzymes in these tissues that are involved in the degradation of carbosulfan. Looking at the number of degradation products (presumed from the number of peaks appeared in HPLC analysis) it appears that fat body and gut from adults consists of higher quantity of the detoxifying enzyme (Table 1.27, 1.28, 1.29 & Fig. 13-25). The results obtained shows that there are certain enzymes in the insect tissue that can degrade the carbosulfan insecticide. The standard carbosulfan shows a retention time of 5.209 min. When the adult gut enzyme extract was used, there were about six peaks. The peak at 3.7 min is having a highest concentration indicating the presence of highest enzyme activity. The other

peaks were smaller indicating the lower concentration of the product (Fig. 1.13-1.17 & Table 1.27).

HPLC analysis of the samples taken from fat body showed three to six additional peaks in insecticide application. The peak at 3.7 min is having a highest concentration indicating the presence of highest enzyme activity. The other peaks were smaller indicating the lower concentration of the product (Table 1.28 & Fig. 1.18-1.21).

Samples taken from incubations with adult reproductive organ have three to five peaks. The highest activity was at 3.7 min (Table 1.29 & Fig. 1.22-1.25). The results provide strong indication that all the three tissues contained enzyme that can degrade carbosulfan insecticide.

Statistical analysis of data indicates the significant change between the control and treated groups. All the sub lethal concentrations showed significant difference to the control samples. One- way ANOVA results shows the p value less than 0.0001 ($p<0.0001$). In multiple comparison statistical analysis data, each samples showed significant difference at different time duration. The significance in almost all the samples was 0.000 ($p<0.0001$) (Table 1.3- 1.26).

The activity profile over time and concentration give further indication that fat body and gut had higher titre of the enzyme compared to the reproductive organ. Insect fat body which is comparable to the vertebrate liver-adipose tissue combined, is the place were enzymes are synthesized and from where it is released into the haemolymph. It is also true that the activity of enzymes such as those involved in detoxification, takes place in the haemolymph were ingested materials including xenobiotics get accumulated before they are taken up by fat body. Therefore it is not surprising that fat body also is actively involved in detoxifying xenobiotics.

Degradation of carbosulfan and other insecticides have been studied in several pest species of insects. Carla *et al*. (2007) analysed, identified and confirmed carbosulfan and seven of its main metabolites as carbofuran, 3-hydroxycarbofuran, 3-ketocarbofuran, 3-hydroxy-7-phenol carbofuran, 3-keto-7-phenolcarbofuran, 7-phenolcarbofuran, dibutylamine. Field degradation studies show that carbofuran, 3-hydroxycarbofuran, and dibutylamine are the main degradation products formed in the carbosulfan degradation in environment. The present study shows the presence of hydrolytic enzymes in the tissue samples that can degrade the carbosulfan into their intermediate compounds. The nature of the products are to be determined using standard libraries.

CHAPTER 2

EFFECT OF CARBOSULFAN ON THE MID GUT AND PROVENTRICULUS OF ADULT BANANA PSEUDOSTEM WEEVIL

2.1. INTRODUCTION

The morphology of insect alimentary canal varies according to the variety of food consumed by them. The alimentary canal of insects is a long narrow or coiled tube which extends from the mouth to the anus, with three main sections having different embryonic origin: the foregut or stomodaeum; the midgut or mesenteron, consisting of the ventriculus and gastric caeca; and the hindgut or proctodaeum (Uvarov, 1966; Borror and De Long, 1969; Belkin, 1976; Maranhao, 1976). Midgut is an important part of digestive tract where the digestive enzyme action takes place. Among the three parts of alimentary canal, midgut is the main location for enzyme production, digestion and secretion (Anderson and Harvey, 1966; Humbert, 1979; Chapman, 1998). The digestive and absorptive cells are the most predominant epithelial cells in insect midgut and are responsible for processing of the diet by actively involving in enzyme production and secretion, as well absorption of nutrients (Billingsley and Lehane, 1996; Zyl and Linde, 2000). Many studies carried out on the midgut region reveal that any kind of alteration on this region may directly affect the growth and development of insects as a result of changes occurred in their different physiological processes (Mordue and Blackwell, 1993; Nisbet *et al*., 1993; De Sousa *et al*., 2013).

The digestive tract of insects functions as an effective barrier against different pathogens and chemicals. Any damage in cellular and tissue level may lead to histological disorders and act as direct indicator of toxicity. Histopathological modifications are the outcome of adverse biochemical and physiological changes in an organism, which implies the rapport of histopathology with physiological and biochemical biomarkers (Hinton *et al*., 1992). Insects under stress produce certain serious changes in cellular level

that can be studied by light microscopic examinations. Structural deformations in cells can be easily studied by light microscopy.

Histomorphology of alimentary canal

The alimentary canal of banana weevil is a coiled tube measuring 39-42mm in length. It is divided into foregut, mid gut and hind gut. There is a constriction in between each division. Midgut is the longest portion of the alimentary canal characterized by numerous regenerative crypts.

Fore gut

Anterior division of the alimentary canal is the fore gut. It includes oesophagus, crop, proventriculus and stomodeal valve. The histological studies reveal the presence of intima, epithelium, basement membrane and musculature in the insect fore gut. The intima is divided into highly cuticularised inner intima and less cuticularised outer intima. Thin and syncytial epithelium is present external to the intima. Outside the intima, a layer of outer circular muscles and inner longitudinal muscles are present. Oesophagus is well developed extending throughout the rostrum. From the posterior end of oesophagus highly muscular chamber, crop arises. At the terminal part of the foregut highly muscularised and sclerotized proventriculus is present. It is marked externally by 8 rows of thick denticles. The presence of a well developed proventriculus in the gut is a characteristic feature of the adult. At the posterior end of foregut stomodeal valve is present that marks the boundary between fore gut and mid gut.

Mid gut

Mid gut (MG) is the longest part of the alimentary tract. One of the characteristic features of the mid gut is the presence of numerous regenerative crypts. Histologically, the MG includes 3 regions. they are the anteriorwith

thick regenerative cells (RC) and moderate digestive cells (DC**),** the middle with thickly populated RCs and well developed DCs and the posterior with thick RCs and less DCs (Prasad and Singh, 2013).

Hind gut

It is the second largest part of the alimentary canal. Hind gut includes pylorus (PY), ileum (IL), colon (CL) and rectum (RT). Pyloric valve is present in between mid gut and hind gut. The malpighian tubules arise from pylorus.

2.2. REVIEW OF LITERATURE

The process of digestion in insects shows great variations due to the variation in consumption of food materials. The diet is correlated with the gut length. Slansky (1982) noticed that the successful adaptation of an insect to a particular food as its source of nutrients, essential for growth, development, reproduction and population maintenance, requires a unique combination of behavioral, physiological and biochemical processes. According to Pradhan (1939) the insects which consume higher protein containing diet have shorter gut in general. Thus the gut length is generally correlated with diet.

In general coleopterans have a short tubular foregut. The foregut of insect begins at the base of the mandible and maxillary and extends to the oesophagus and proventriculus (Tsai and Perrier, 1996; Lee *et al*., 1998). The function of the oesophagus of the insect is to pass food downwards to the midgut while the crop serves as a storage deposit for ingested food (Wigglesworth, 1965). The oesophageal glands secrete mucous which lubricates food and makes swallowing easy. At the joining of foregut and midgut, proventriculus may be present (Ekis and Gupta, 1971). The proventriculus is muscularly built in insects to withstand the pressure associated with the mechanical grinding of food in the chamber. Chapman

(1985) reported that proventriculus was absent in fluid feeders. In coleopterans it functions as grinding organ or as valve. The intima covering the proventriculus is highly developed, densely covered with usually coarse spicules and responsible for ingesting, transporting, storing, grinding, and filtering particles of food (Sinha, 1958; De Sousa *et al.,* 2013). The noncellular intima is homologous to the external body cuticle. This layer is secreted by the epidermal cells (Sinha, 1958).

The crop is usually absent or very slightly developed in beetle larvae and polyphagous adults, but it is usually present in adult Adephaga (Crowson, 1981). The crop may also serve as a site for preliminary or more complete digestion. Chun-Nu *et al.* (2000) reported the arrangement of the oesophagus of oriental fruit fly which was similar to that of palm weevil. The larva stores all its food in its big crop. Yoloye (1988) and Adedire (2002) reported identical structures in the alimentary tract of cockroach, *Periplaneta americana* and kolanut weevil, *Sophrorhinus insperatus* respectively. Functionally, the foregut is of considerable importance in storage and digestion, but it does not play a significant role in absorption. Structurally, the alimentary canal appears as a simple layer of epithelium, resting on a basement membrane, with a discontinuous layer of longitudinal and transverse muscles outside (Dow, 1987). Liu and Hua (2009); Wang *et al*. (2012); Zhong *et al*. (2015), investigated that the Orthoptera and Mecoptera which feed on solid food usually possess thick alimentary canals lined with the cuticular intima in foregut and hindgut. In contrast, Hemiptera which have piercing-sucking mouth parts, and feed on fluids, lack the cuticular layer (Chapman, 1998; Kerkut and Gilbert, 2013; Zhong *et al.,* 2015).

Mid gut is the longest division of the alimentary tract. The midgut of insects secretes most of the digestive enzymes and is the principal site of digestion (Gilmour, 1961; Dadd, 1970; Wigglesworth, 1972). The midgut was

the region for both digestion of food and absorption of nutrients. According to Wigglesworth (1965) and Billen and Buschinger (2000) the epithetlium was responsible for both the production of many digestive enzymes and the uptake and transfer of nutrients to the haemolymph. Schneider and Rudinsky (1969) and Lopez Guerrero (2002), noticed that the coleoptera midgut is long and coiled in adults like *Trypodendron lineatum* and *Cephalodesmis armiger*. According to Chapman (1998) in some species of Meloidae, the midgut is morphologically differentiated into broad anterior and narrow posterior regions. In some species of Cleridae, it is divided in to anterior, middle and posterior regions based on differences in size and shape and also midgut is covered by small papillae, which are the outward projections of crypts. The papillae contain regenerative cells. The insect midgut includes three types of epithelial cells: columnar, goblet, and regenerative cells showing variation in functions in various insects (Lewis, 1926; Waterhouse, 1952; Wigglesworth, 1965; Sarwade and Bhawane, 2013).

The columnar cells are concerned with secretion of enzymes and absorption and active transport of potassium and calcium ions is the function of goblet cells. Renewal and replacement of injured cells are concerned with regenerative cells and endocrine cells are endocrine in function.

Shinoda (1930) described the histology of midgut in insect shows three types of epithelial cells as described which includes columnar, goblet and regenerative cells showing variation in functions in different insects. (Lewis, 1926; Waterhouse, 1952; Wigglesworth, 1965). Chapman (1985) and Santos *et al*. (1984) studied and reported the presence of goblet cells in the Lepidopteron and Coleopteran larvae and described their fine structure and functions. Chapman (1972) reported the presence of goblet cells which has also been reported in the midgut epithelium of Ephemeroptera, Plecoptera and Trichoptera.

Hindgut is the last part of digestive system which includes ileum followed by colon and rectum and ends exteriorly with anus. The hindgut is lined by thin layer of permeable cuticle (Maddrell and Gandiner, 1980). Browne (1934, 1935); Jones (1940); Swingle (1950); Gupta (1965); Berberet and Helms (1972); Kumar and Adjei (1975) investigated the histomorphological analysis of alimentary canal in coleopteran insect species.

The present study aims at investigating the histomorphological changes in the proventriculus and mid gut of alimentary canal of adult *Odoiporus longicollis*, banana weevil. The histomorphological studies were carried out using light microscopy and ultra-structural studies by scanning electron microscopy.

2.3. MATERIALS AND METHODS

The mid gut and proventriculus of alimentary canal are taken for the histomorphological study. The adult insects were treated with 80% (0.7µg/µl), 50% (0.4µg/µl) and 10% (0.1µg/µl) of the lethal dose (LD_{50}) of carbosulfan insecticide topically for 20- 25 days. The control groups were treated with acetone topically. Before undergoing the practical process, the adults have been starved for 2-3 days so as to enable the insects to clear the food stuffs from their alimentary canal. After treatment of sub lethal concentrations of carbosulfan, insects were cut laterally, tergum was removed and alimentary canal was taken out.

2.3.1. Tissue Preparation for Light Microscopy

Alimentary canal of adult banana weevils were taken out and fixed in Bouin's fluid for 24 hr. The tissues were washed in different gradients of alcohol (70%-100%) and processed for preparing wax blocks using L mould. The techniques adopted here is according to the method described by Adedire (2002). Following standard histological procedures 4 - 5 µm thick paraffin

sections were prepared. After deparaffinisation and rehydration staining were carried out in Haematoxylin and Eosin. After dehydration tissues were mounted in DPX and examined under light microscope and microphotographs were taken using digital camera.

2.3.2. Tissue Preparation for Scanning Electron Microscopy

For tissue preparation, the gut of the treated and control insects were fixed in 2.5% gluteraldehyde in 0.1M phosphate buffer (pH 7) for 1 hr. The tissues were then washed in a graded acetone series (70%, 80%, 90% and 100%). In each gradient the tissues were kept for 1 hr each and dried in oven. The SEM images were taken using Hitachi SU6600 Variable Pressure Field Emission Scanning Electron Microscope (FESEM), available at NIT, Calicut.

2.4. RESULTS

2.4.1. Light microscopic studies

The different concentrations of lethal dose of carbosulfan (10%, 50% and 80% of LD_{50}) were treated with adult banana pseudostem weevil, *Odoiporus longicollis* and tissues were prepared and observed under light microscope for the major histomorphological changes occurred in the mid gut and proventriculus of the alimentary canal.

2.4.1.1. General histomorphology of mid gut

Mid gut of adult banana weevils is the longest part of the gut with the presence of regenerative crypts (RG) as the characteristic feature. The number of regenerative crypts is more in anterior region than in posterior mid gut. The RGs arises from the gut lumen (Gl) (Plate 2.1.). Generally the mid gut tissue contains peritrophic membrane, epithelium, basement membrane, and muscular layer (Plate 2.2.). In most of the cases peritrophic membranes are

broken due to the digestive action of epithelial cells below. There are different types of epithelial cells in the mid gut tissue like columnar epithelium (CC), Digestive cells (DC) and regenerative cells (RC). Columnar cells form the major volume of epithelial cells compared to others. They are long column like cells with prominent oval shaped nuclei at the centre of the cell (Plate 2.3.). They are concerned with the digestive enzyme production. Regenerative cells are seen at the base of the regenerative crypts for the formation of renewal of damaged cells. RGs have a swollen end at distal position bearing numerous cells called nidi, are located (Plate 2.4.).

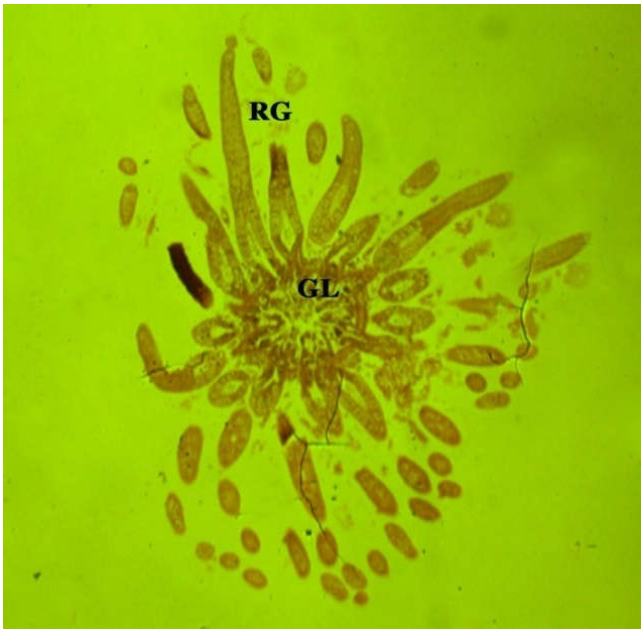


Plate 2.1: Cross section of the mid gut tissue of the adult banana pseudostem weevil (control) showing numerous regenerative crypts (RG) around the gut lumen (Gl) (4x)

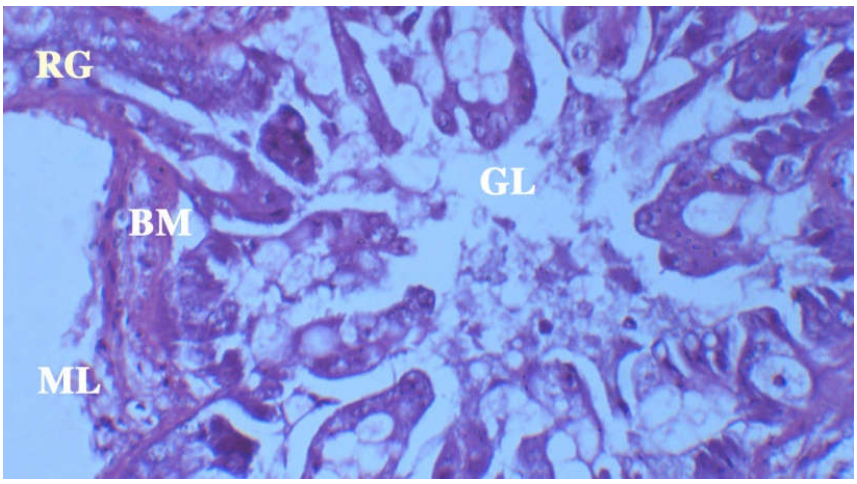


Plate 2.2: Cross section of the mid gut tissue of the control insect showing muscular layer (ML), basement membrane (BM), regenerative crypts (RG), and gut lumen (GL) (40x)

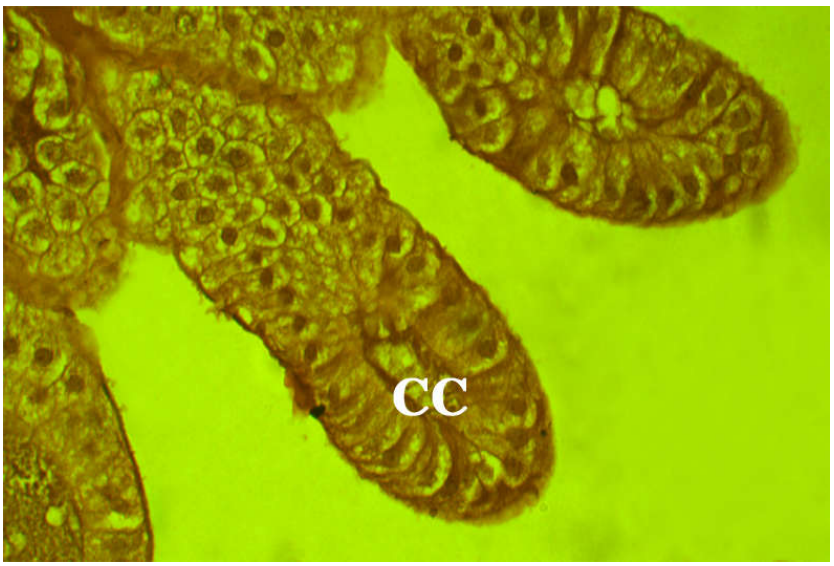
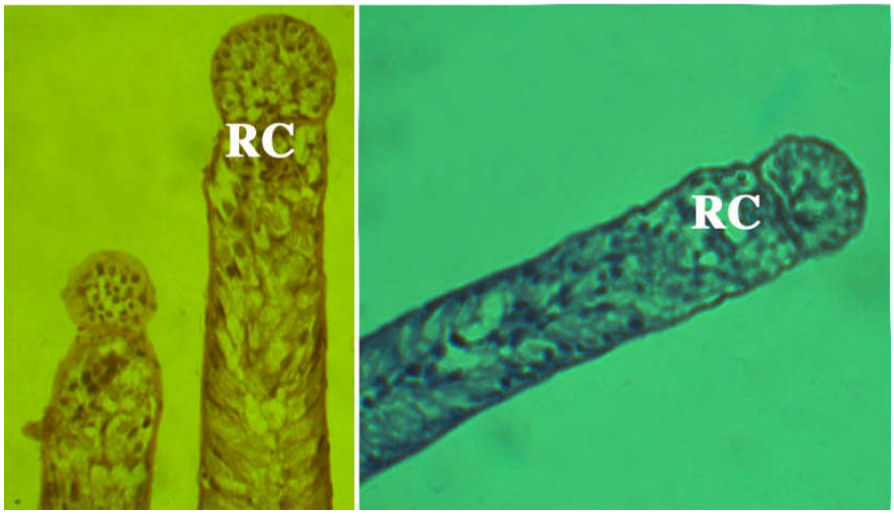


Plate 2.3: Cross section of the mid gut tissue showing regenerative crypts (RG) with columnar cells (CC) attached to the basement membrane (40x).



A B

Plate 2.4 A & B: Cross section of regenerative crypts (RG) of adult banana weevil showing nidi of regenerative cells (RC) (10x)

2.4.1.2. Effect of carbosulfan insecticide on adult mid gut tissue of banana weevil

Different concentrations of lethal dose (10%, 50% and 80%) were applied topically on the adult insects and toxicity was analysed. The results show that at 10% carbosulfan lethal dose application, the outer circular and inner longitudinal muscles got separated from basement membrane (Plate 2.5). The regenerative crypts show highly overlapped columnar cells. Columnar cells got detached from the basement membrane and migrated into the lumen of the crypts. Columnar cells got elongated and compressed (Plate 2.6). At the posterior mid gut region highly elongated columnar cells present. Excess vacuoles are present. The size of the nuclei becomes larger (Plate 2.7.). On treatment with 50% of LD_{50}, vacuoles are formed in excess number in the epithelial layer. Columnar cells are viewed as highly overlapped and congested (Plate 2.8 & 2.9). Tissues treated with 80% of LD_{50} of Carbosulfan lethal dose showed an enlarged nucleus and completely separated basement membrane (Plate 2.10 & 2.11).

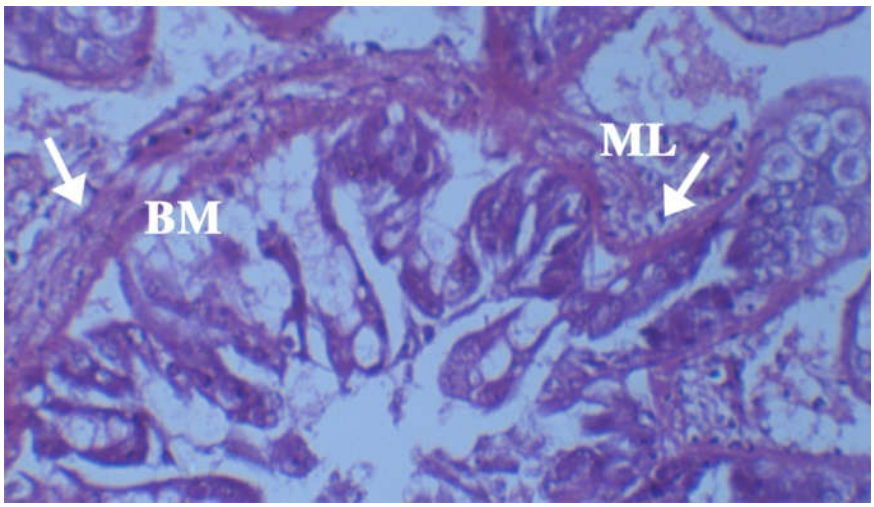
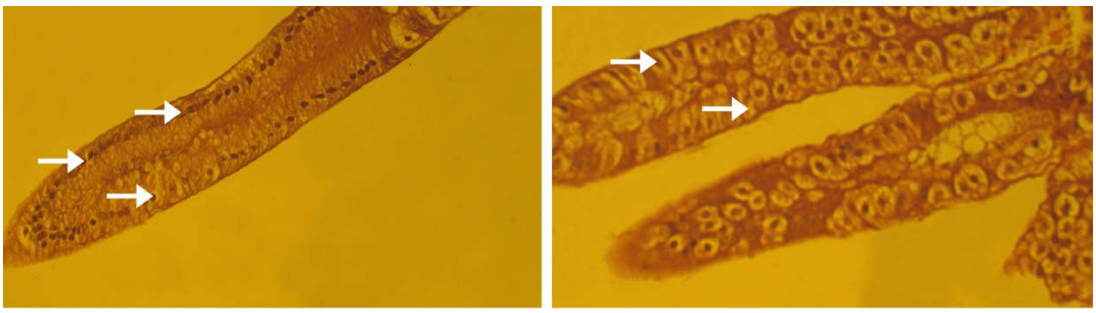
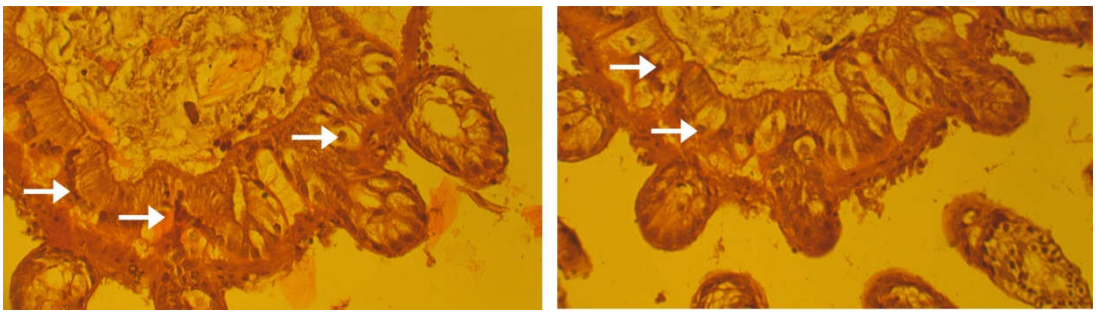


Plate 2.5: Cross section of the mid gut tissue of the adult banana weevil treated with 10% of LD_{50} showing the excessive thinning of the muscular layer (ML) (40X)



A **B**

Plate 2.6 A & B: Cross section of regenerative crypts (RG) of mid gut of adult banana weevil treated with 10% of lethal dose of carbosufan showing (A) the overlapped and congested cells of the columnar cells (CC) and (B) the migration of cells into the lumen of crypts (arrows) (10X).



A B

Plate 2.7 A & B: Cross section of posterior mid gut of regenerative crypts (RG) of adult banana weevil insects treated with 10% of lethal dose of carbosulfan showing (A)Overlapping of columnar cells (B) the enlargement of nucleus (arrows) (10X).

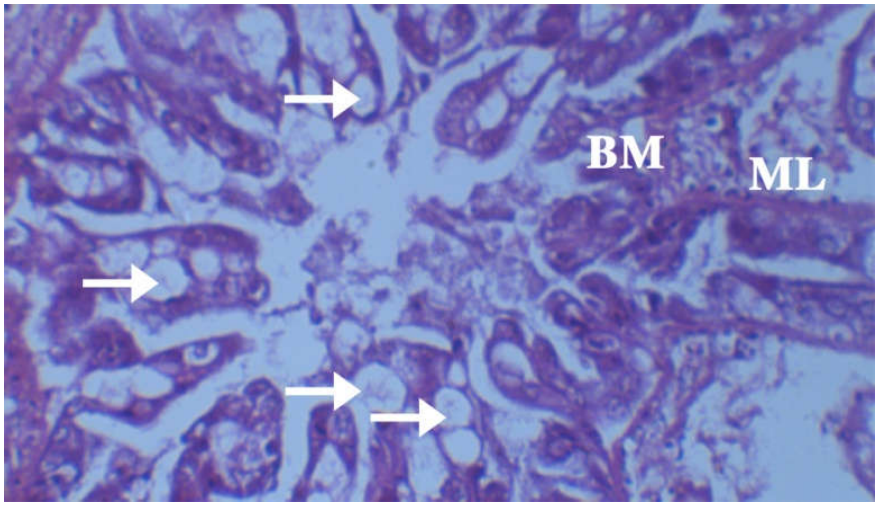
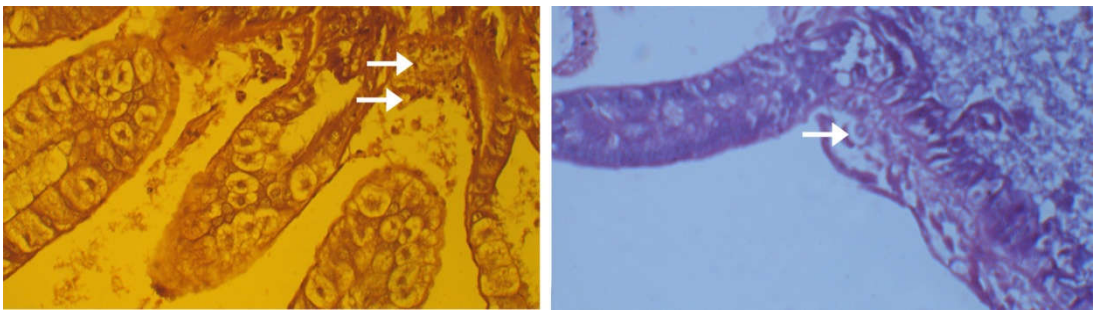


Plate 2.8: Cross section of the mid gut tissue of the adult banana weevil treated with 50% of LD_{50} showing excessive vacuolization (arrows) and separation of the outer muscular layer (ML) and basement membrane (BM) (40X).



A B

Plate 2.9 A & B: Cross section of mid gut RGs of adult banana weevil insects treated with 50% of lethal dose of carbosulfan(A.) showing ruptured Muscular layer (ML) (arrows) and detachment of columnar cells from the basement membrane (BM),(B.) showing ruptured muscular layer (ML), basement membrane (BM) and overlapped columnar cells (CC) (arrows) (10X)

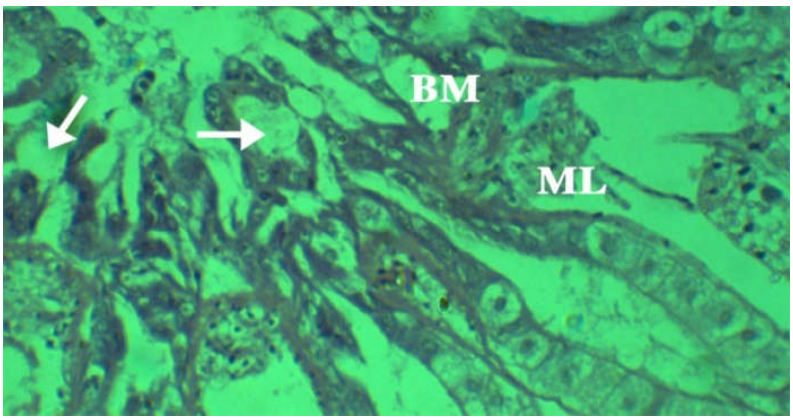


Plate 2.10: Cross section of the mid gut tissue of the adult banana weevil treated with 80% of LD_{50} showing the ruptured muscular layer (ML) and basement membrane (BM). Excessive vacuolization in the epithelium layer can be seen (arrows) (40X).

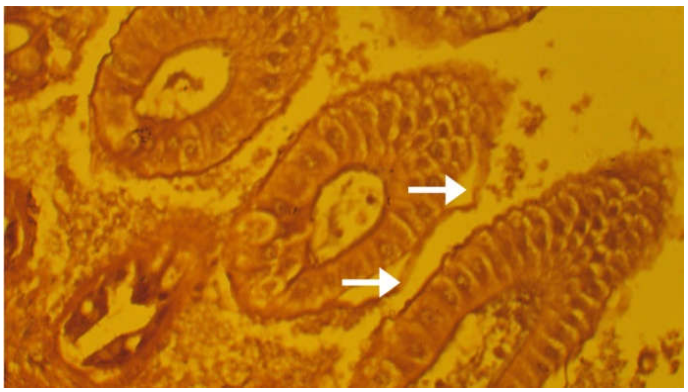


Plate 2.11: Cross section of mid gut of regenerative crypts (RG) of adult banana weevil insects treated with 80% of lethal dose of carbosulfan showing detached basement membrane (BM) from the columnar cells (CC) (arrows) (40X).

2.4.1.3. General histomorphology of proventriculus of adult banana pseudostem weevil

Proventriculus is the terminal region of fore gut. The crop opens posteriorly into proventriculus. The function of proventriculus is the mastigation of food. Histological observation reveals that proventriculus contains intima, epithelium and muscular layer. The characteristic feature of the proventriculus is the 8 rows of thick chitinous plates called denticles. Denticles are tooth like and used for grinding of food. Proventriculus tapers posteriorly and ends in mid gut. Stomodeal valve opens proventriculus into mid gut. A thick circular muscle layer covers the external surface of the proventriculus. A row of cuboidal epithelial cells lies on the circular muscles. Longitudinal muscles are located on the bases of the invaginations. Behind the epithelium there is a tough layer of chitinous intima which is thrown into 8 prominent ridges. The chitinous spicules arise from the intima joins to form sclerotized plates. At the posterior region the intima become thin and forms a narrow lumen tapering into stomodeal valve which ends in mid gut. This limits the entry of food into the mid gut (Plate 2.12 & 2.13).

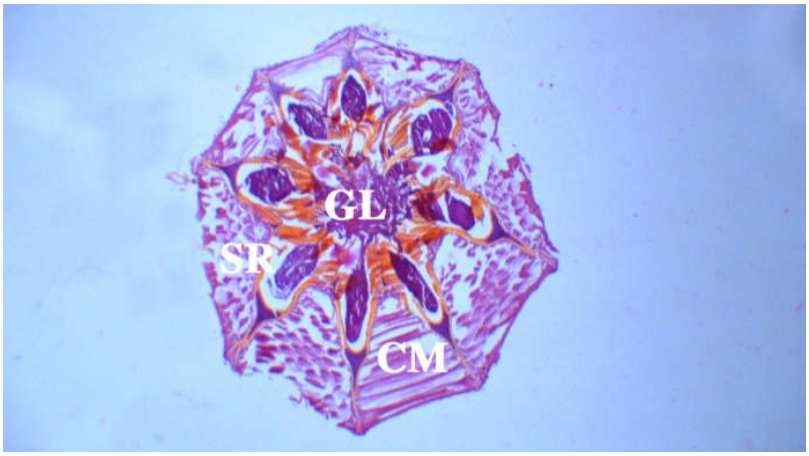
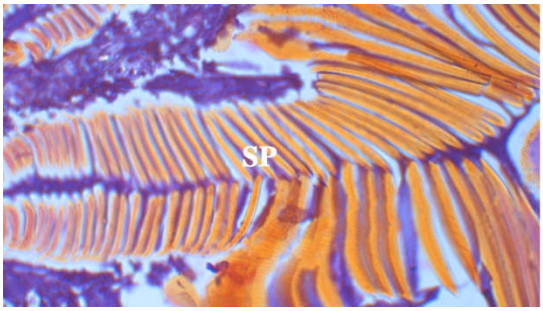
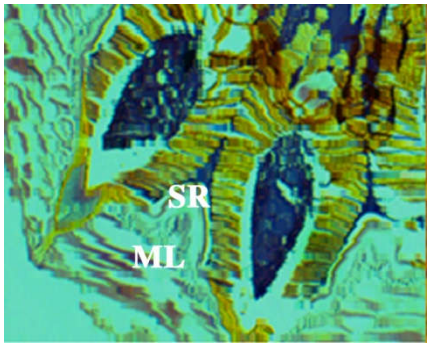


Plate 2.12: Cross section of the adult banana weevil proventriculus of gut of the control insect showing 8 chitinous denticles (SR), muscular layer (ML), and intima (IN) (4x).



A **B**



C

Plate 2.13 A,B,C: Cross section of the adult banana weevil proventriculus of the control insect showing sclerotized ridges(SR) bearing chitinous spicules (SP),epithelium (EP), intima (IN) and muscular layer (ML) (40X).

2.4.1.4. Effect of carbosulfan insecticide on proventriculus of gut in adult Banana weevil

Different doses of carbosulfan lethal dose on the foregut region were applied and observed its effect on the histomorphology of adult banana weevil proventriculus. On application of 10% carbosulfan lethal dose the arrangement of chitinous denticles found to be irregular. The teeth like bristles appear damaged at certain regions. The intima lining the muscle layers also thinned and separated from the chitinous plates. The chitinous intima is not continuous at certain regions. The epithelial layer found to be separated from the underlying intima. Muscular layer also appeared as disintegrated (Plate 2.14). At the 50% of lethal dose application disintegration of circular muscular layer appeared in the proventriculus. The cuboidal epithelial cell layer completely separated from intima. The cells of the epithelium found to be damaged and nucleus in pyknotic condition. The irregular and damaged arrangement of spines can also be seen in the proventriculus (Plate 2.15). On the 80% lethal dose application the damages on the chitinous plates were more compared to others. The epithelial layer completely got sloughed off from the intima. The intima does not form a continuous layer and separated from the underlying spines. The damages caused by the insecticide on the circular muscle layer are clearly visible (Plate 2.16).

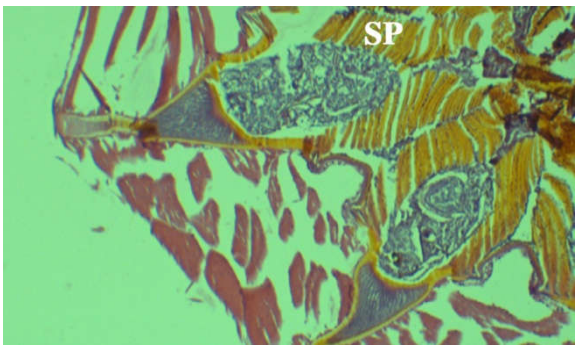
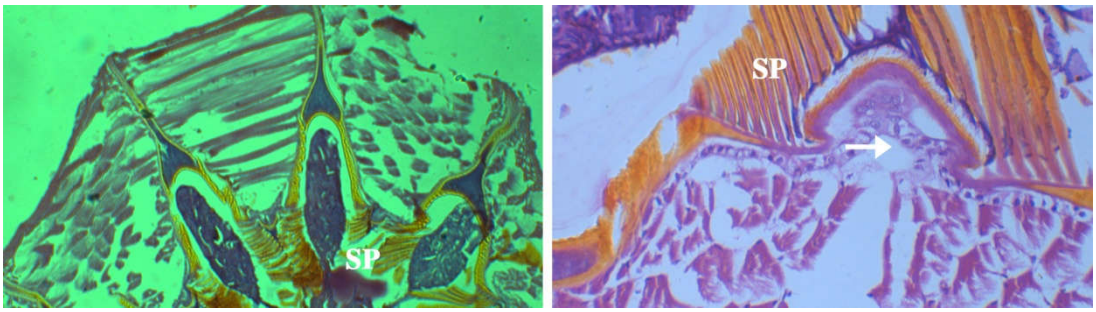


Plate 2.14: Cross section of proventriculus of adult banana weevil on application of 10% of lethal dose of carbosulfan showing damaged chitinous spicules (SP), intima (IN) and epithelium(EP) (10x).



A **B**

Plate 2.15 A & B: Cross section of proventriculus of adult banana weevil on application of 50% of lethal dose of carbosulfan showing (A) damaged chitinous plates with spicules (SP), intima (IN) and circular muscles (CM), (B) pyknotic epithelium (EP) (10x).

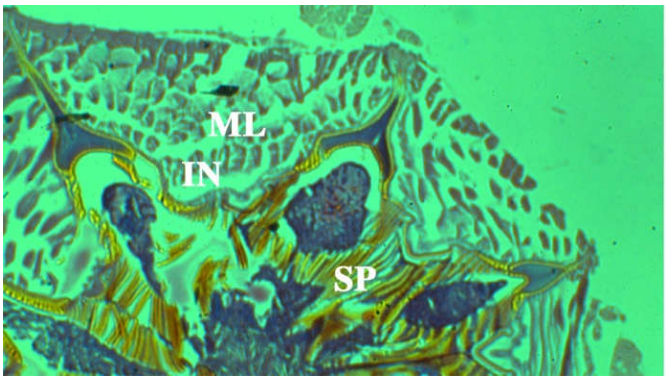
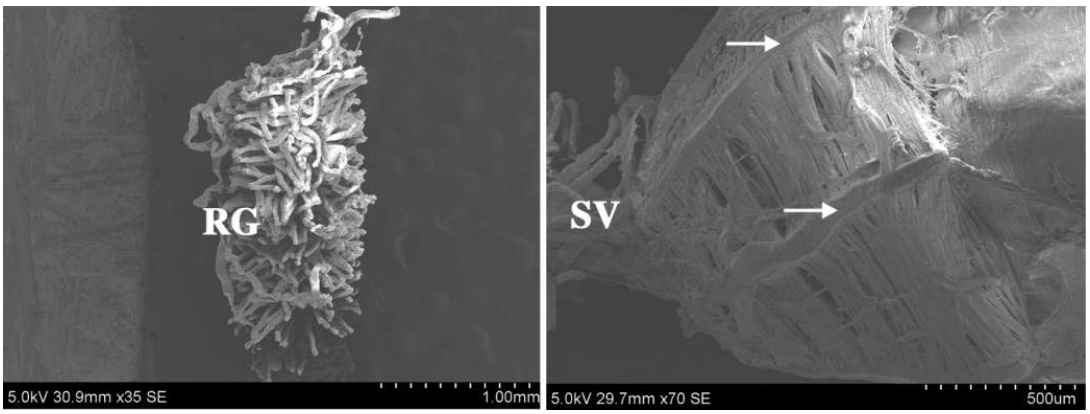


Plate 2.16: Cross section of proventriculus of adult banana weevil on application of 80% of lethal dose of carbosulfan showing damaged chitinous spicules (SP), intima (IN)and epithelium(EP) and circular muscle fibers (ML) (10x).

2.4.1.5. Ultra structural studies

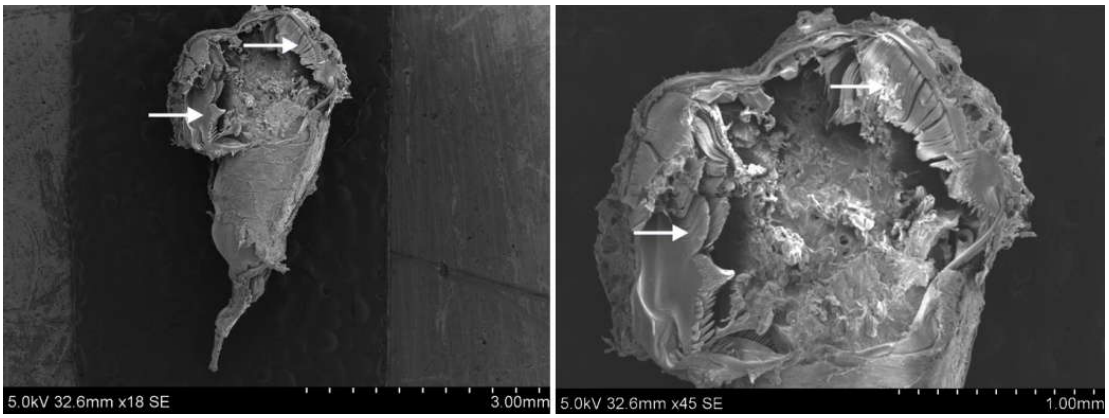
Ultra structural studies also support the effect of carbosulfan insecticide on the proventriculus and mid gut of the adult banana weevil. In general the epithelium of the mid gut has numerous regenerative crypts (Plate 2.17 A). Regenerative crypts are usually swollen at the distal end, taper medially and join with the mid gut. Midgut is usually broader at the middle region and narrows posteriorly. In foregut region after oesophagus and crop, externally a thickened chitinous structure called proventriculus is present (Plate 2. 17 B). On the surface of the proventriculus 8 thick folds of chitinous brown ridges is found which help in food mastigation. Externally in between the 8 ridges muscular layer is present. The ridges taper posteriorly ending in stomodeal valve which is the connection between fore gut and mid gut. The cross section studies reveal that chitinous plates are of three different types. The anterior thick and small spicules, median bristles which is more in number and posterior bristles closer to stomodeal valve which is larger than the median bristles (Plate 2. 18 A & B).

On application of the different concentrations of insecticide, the mid gut tissue appeared damaged in external appearance (Plate 2.19). The numbers of regenerative crypts are less in number compared to the control tissue. The regenerative crypts are damaged in all the three insecticide application. The distribution of regenerative crypts is uneven. The crypts appear clogged at the posterior region. 80% lethal dose application showed high damage in the regenerative crypts (RG). In the foregut region proventriculus appeared with disintegrated circular muscle fibers which are present externally. The thick brown ridges are not prominent in the treated insects (plate 2. 20.).



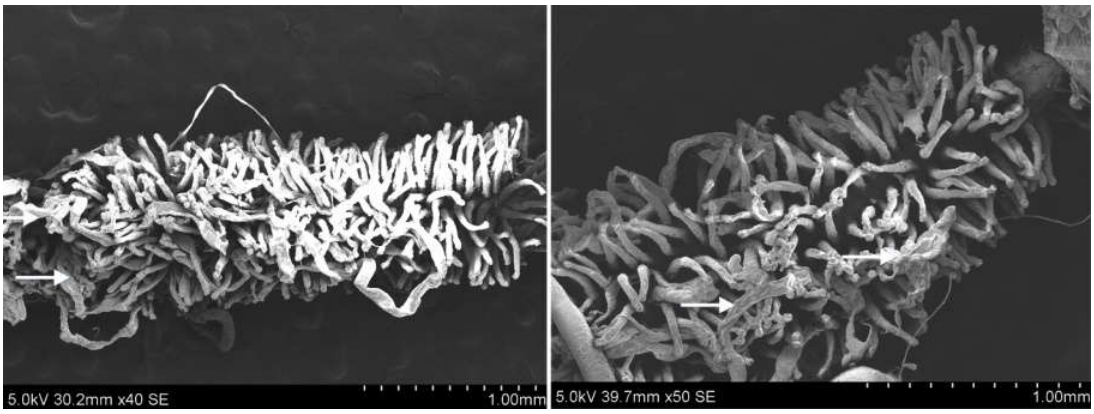
A B

Plate 2.17 A, B: SEM micrographs of (A) mid gut and (B) proventriculus of adult banana weevil showing sclerotized ridges (SR), and stomodeal valve (SV).

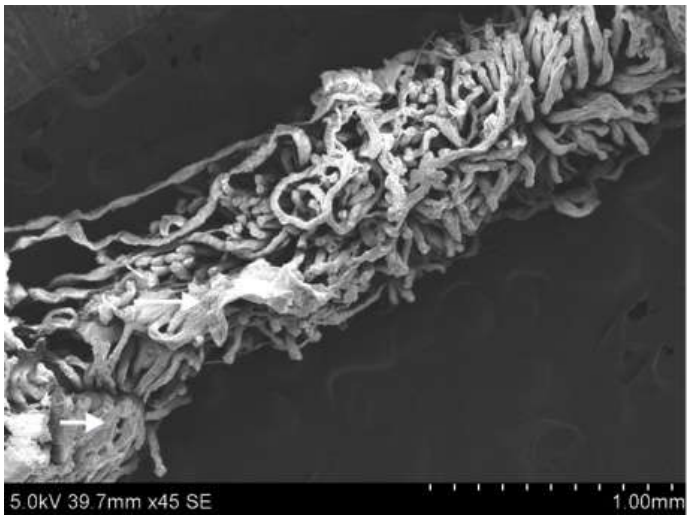


A B

Plate 2.18 A, B: SEM micrographs of longitudinal section of foregut showing anterior, median and posterior chitinous plates (arrows).

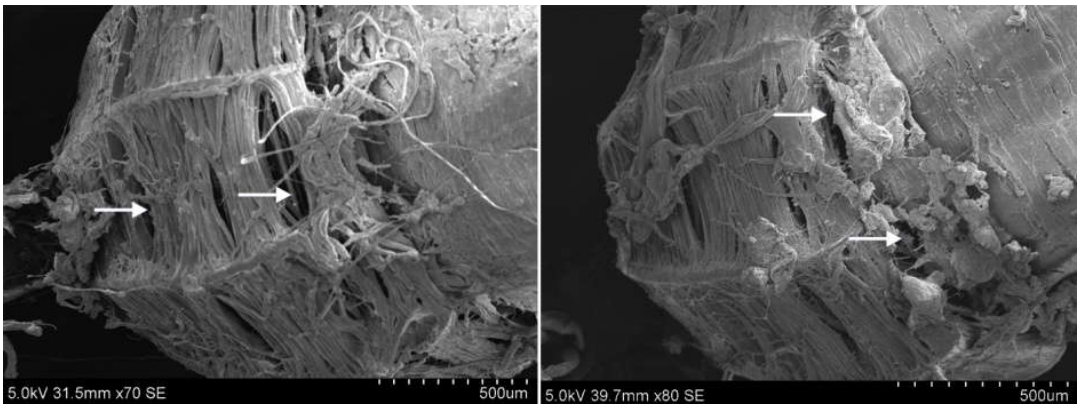


A B

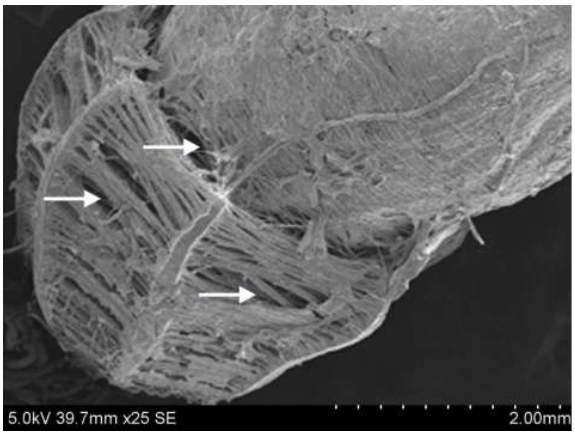


C

Plate 2.19 A, B, C: SEM micrographs of mid gut of adult banana weevil treated with (A) 10% lethal dose of carbosulfan, (B) 50% lethal dose of carbosulfan (C) 80% of lethal dose of carbosulfan showing the damaged regenerative crypts (RG).



A B



C

Plate 2.20 A, B, C: SEM micrographs of fore gut of adult banana weevil treated with (A) 10% lethal dose of carbosulfan, (B) 50% lethal dose of carbosulfan (C) 80% of lethal dose of carbosulfan showing the damaged proventriculus (PV) and muscular layer (ML) (arrows).

2.5. DISCUSSION

The alimentary canal of adult banana pseudostem weevil, *Odoiporus longicollis* is a long, muscular and tubular structure. Food mastigation, enzyme production and nutrient absorption takes place in different regions of alimentary canal. Alimenatary canal is divided into fore gut, mid gut and hind gut. Fore gut of adult weevil bears oesophagus, crop, proventriculus and stomodeal valve. Proventriculus is the region in which mastigation of food takes place. There are eight chitinous plates or ridges are present in the proventriculus.

On application of different sub lethal concentrations of carbosulfan on adult banana weevil, caused the histomorphological changes in the proventriculus of adult weevil. When 10% of LD_{50} was applied the chitinous denticles found damaged and irregular (Plate 2.14). On application of 50% of LD_{50} the muscular layer found ruptured. The nucleus of the epithelium that lines the intima become condensed and pycnotic (Plate 2.15). The chitinous spicules were damaged; the intima and epithelial layer were separated on application of the 80% lethal dose application (Plate 2.16).

Mid gut is the region in which digestive enzyme secretion takes place and is an important defense site against foreign pathogens. The mid gut cells are involved in secreting digestive juices. In banana weevil, numerous regenerative crypts can be seen externally covering the mid gut. They help in increasing the area of absorption. The numbers of regenerative crypts are different in various regions of mid gut.

On application of the 10% lethal dose of carbosulfan in adult banana weevil, damaged the external muscular layer (ML). Muscular layer is divided into outer circular muscles and inner longitudinal muscles. The columnar cells also found to be overlapped and migrated into the lumen (Plate. 2.5-2.7). 50%

lethal dose application of carbosulfan ruptured the muscular layer and basement membrane. Excessive vacuolation can be seen in the damaged tissue (Pates 2.8-2.9). The basement membrane is detached from the under lying columnar cells and ruptured muscular layer is also a characteristic feature the damaged tissue (Plates 2.10, 2.11).

The sub lethal concentrations caused tissue damages in mid gut and proventriculus of alimentary canal. The light microscopic studies and ultra-structural studies support the disintegration of tissues. The proventriculus of adult weevil has intima, epithelium and muscular layer. Zunuga *et al.* (1994) noticed similar results in other coleopteran insects such as *Dendroctonus parallelocollis, D. rhizophagus, D. valens*, *D. adjunctus* and that the same characteristic appeared in other orders such as *D. cingulatus* (Muralitharan, 1983) and *Chrysochoris purpureus* (Baskaran, 1970). The proventriculus contains 8 prominent ridges externally. The similar structure was noted in the proventriculus of *Hypothenemus hampei* (Coleoptera, Curculionidae, and Scolytinae) containing 8 tooth like structures (Rubio *et al.*, 2008). The complexity of the proventriculus is related to the consistency of the alimentary diet (Balogun, 1969; Cruz-Landim, 2009). The proventriculus of banana weevil is similar to that of other weevils. The studies on weevils by Eaton (1942), Judd (1952), Kissinger (1963), Balogun (1969), Baker *et al*. (1984), Sa´nchez *et al*. (2000), Rubio *et al*. (2008), and Bu and Chen (2009) reveals that eight chitinous plates were found in the proventriculus of fore gut. Four dentitions were observed at the lumen wall at the anterior proventriculus region, whereas six dentitions were found in the posterior region of the alimentary canal of the worker termite *Neotermes bosei* (leksono, 2006). Davey and Treherne (1963) showed six chitinous teeth in the proventriculus of P*eriplaneta americana* (Blattodea). Dennell (1942), Eaton (1942), Sa´nchez *et al*. (2000), and Rubio *et al.* (2008) reported that the proventriculus has an important role in digestion for limiting the transport of

large and non digestible particles to the mid gut. The musculature associated with the proventriculus help them to create high pressure for mastigation. (Chapman, 1985). The structural and functional characterization of the proventriculus of banana weevil is necessary for the better understanding of its digestion physiology.

According to Dow (1987), the mid gut is considered the most important region of the digestive system responsible for digestion and absorption. The carbosulfan insecticide treatment resulted in excessive vacuolization, elongation of columnar cells, and overlapped columnar cells (Plate 2.5-2.9). Similar histopathological changes were reported in *P. americana* (Sharma and Chattoraji, 1964; Cantwell *et al.*, 1966; Sutherland *et al.*, 1969; Bearwlad *et al.*, 1969; Ahi, 1985), *Spodoptera litura* treated with endosulfan, diazinon and dichlorvos (Lal *et al*., 1970), and in *Plebiogryllus guttiventris* treated with fenitrothion (Balakrishnan *et al*., 1987). Srivastava (1962), Rizvi and Khan (1973), Shukla *et al.* (1979), Sabesan and Ramalingam (1978) and Zutshi and Saxana (1989) reported the drastic effect of insecticide on the epithelium of insects. Present study in the mid gut resulted in the separation of basal membrane and disintegration of muscular layer (Plate 2.9 & 2.11). Previous works on *Spodoptera exi*gua larvae treated with diflubenzuron, malathion and Cypermethrin by Younes *et al.* (2002), on camel nasal bot fly, *Cephalopina titillator* larvae treated with two insect growth regulators, pyriproxyfen and chlorfluazuron by Bassiony and Nady (2005), on *Synthesiomyia nudiseta* larvae treated with the volatile oils of *Cupressus macrocarpa* and *Alpinia officinarum* by Khalaf *et al.* (2009) and on *R. ferrugineus* larvae treated with zinc sulfate by Al-Dhafar and Sharaby (2012) showed similar findings. In addition to the columnar cells, regenerative cells are also seen in the mid gut epithelium. Regenerative cells functions to replace and regeneratre the damaged cells.Regenerative cells

form groups called nidi (Chapman, 1985; Cruz-Landim, 1999, 2009; Wanderley-Teixiera *et al*., 2006; Rost-Roszkowska, 2008).

Mid gut epithelium of adult banana weevil is externally covered by circular and longitudinal muscle layers. On application of insecticide carbosulfan the regenerative crypts of mid gut is observed to be disintegrated and it also affected the circular and longitudinal muscle fibres (Plate 2.19). Similar observations were recorded by Shukla *et al.* (1979) and Zutshi and Saxena (1989) on the midgut of *Aulacophora foveicollism* and *Gryllodes sigillatus*, by the effect of pyrethrum. Muckherji and Hardas (1954) reported that the muscle layer in the 3rd instar hopper of *Schistocera gregaria* F. was degenerated due to parathion poisoning. Similar findings were noticed by Lal *et al.* (1970) in caterpillar of *Spodoptera litura* F. when treated with diazinon. Soliman and Soliman (1958) reported that parathion, when fed to 5th instar larvae of *Prodenla litura* F. completely destroyed the mid gut epithelium.

Ultra structural studies also support the effect of carbosulfan on the mid gut tissues and proventriculus of the alimentary canal of the adult banana stem weevil (Plates 2.19, 2.20). Carbosulfan insecticide caused cytotoxic effect on the tissues and changed the histomorphology of cells. The effect of the carbosulfan may change the physiology of the gut of the Banana weevil.

CHAPTER 3

EFFECT OF XENOBIOTICS ON WING SHAPE VARIATION OF ADULT BANANA PSEUDOSTEM WEEVIL, *Odoiporus longicollis*, OLIV. (COLEOPTERA: CURCULIONIDAE) USING LANDMARK BASED GEOMETRIC MORPHOMETRIC ANALYSIS

3.1. INTRODUCTION

Historically, taxonomic classification and understanding of biological diversity have been based mainly on morphological descriptions (Adams, 2004). In the early twentieth century, comparative biology entered a transition from the description field and quantitative science, where morphological analysis had a similar revolution of quantification (Bookstein, 1997). This made possible the combination of multivariate statistical methods and new ways to visualize a structure (Adams and Funk, 1997; Dryden and Mardia, 1998). Shape analysis is one of the statistical methods for understanding morphological variability. According to Dryden and Mardia (1998), shape is the geometric feature of an object except for its size, position and orientation. Due to the advancement in statistics, geometry and biology analysis of shape has become more quantitatively described, which lead to the development of geometric morphometrics; the fast and reliable way of studying biological forms (Tabugo *et al*., 2012).

Geometric morphometric is a method to study the form in two or three dimensional spaces. Here shape and morphological structures are based on landmarks and the morphology is represented by coordinates of a set of landmark points (Bookstein, 1991). According to Merckx and Van Dyck (2006); Monaghan (2008); Otaki *et al*. (2010), phenotype of any organism can change under certain environmental conditions to increase its fitness which allow them to adjust its phenotype to the local conditions. Beldade and Brakefield (2002); Prieto and Dahners (2009) have examined the effects of environmental factors on the shapes of organisms. Morphometry is important in biology for the quantitative description of organisms. Geometric morphometric is a more sophisticated method for the description of shape based on the landmarks. Land mark based geometric morphometric are used

mostly for the study of sexual dimorphism in organisms where homologous points in biological structures are studied. It is one of the important areas of evolutionary biology. Sexual dimorphism is an important source for phenotypic variation in organisms. Zelditch *et al*. (2004) and Klingenberg (2008) described landmarks as points that can be located precisely on each specimen under study with a clear correspondence in a one-to-one manner from specimen to specimen. According to Bookstein (1997) outlines and curves can be analyzed by semi land marks which are the points that fall at defined intervals along a curve between two landmarks. For studying the semi land marks Procrustes superimposition, Fourier analysis and Eigenshape are used. According to Macleod and Rose (1993), Macleod (1999), in eigenshape, the coordinate points of an outline or curve are converted to a phi function, which is a list of the angles from one point to the next one in the series. To describe the positions of outline coordinates fourier methods use sine and cosine harmonic functions. According to Goodall (1991); Rohlf *et al*. (1996); Dryden and Mardia (1998) in Procrustes superimposition the shape information is extracted and the other components of variation in size, position and orientation can be removed.

Coleoptera (known as beetles) are the largest insect order, containing 380000 named living species classified into more than 160 families (Mckenna *et al*., 2015). The body of coleopterans is covered by tough exoskeleton which protects its membranous body parts. The fore wing is modified into hardened elytra which are usually not used for flight. It serves as a protective cover over hind wing (Frantsevich, 2011). Hind wing is membranous and folded under the elytra (Beutel and Haas, 2000; Haas, 2006). Hind wings are folded longitudinally and transversely under elytra. According to Haas *et al*. (2000), the hind wing must have a certain size to be aerodynamically functional, which makes them distinctly larger than the thickened fore wings. It is easy to hide and escape from predators with the folded wings (Haas, 2006). Studies

on the wing folding mechanisms of beetles by Beutel and Haas (2000) explains that longitudinal folding was already present in the earliest stem-lineage representatives of the Lower Permian, whereas transverse folding evolved in the Middle Permian with the formation of a closed sub elytral space. The hind wings are unfolded during flight (Muhammad *et al*., 2010; Truong *et al*., 2014).

Studies of morphological variances of insect wings have been done using geometric morphometric analysis by Klingenberg and Zaklan (2000), Villemant *et al*. (2007) and Bai *et al*. (2011). Using the geometric morphometric, analysis of insect wings have applied in taxonomy by Bubliy *et al*. (2008); Lyra *et al*. (2010); Lorenz *et al*. (2012); Su *et al*. (2015). Bubliy *et al*. (2008) and Jaramillo (2015) did the studies on dipterans, hymenopterans by Rattanawannee *et al*. (2010) and coleoperans and odonata were studied by Su *et al*. (2015) and Demayo *et al*. (2011).

The present study aims to determine the effect of xenobiotics on adult banana pseudostem weevil, *Odoiporus longicollis* using landmark based geometric morphometric analysis.

3.2. REVIEW OF LITERATURE

Geometric morphometric (GM) can provide valuable information on phenotypic variability and population structure. It is a low cost tool for the shape analysis (Dujardin, 2011). According to Pavlinov (2001), the complex shape of an organism cannot easily be summarized by using linear measurements as in traditional morphometrics. In general, it is not possible to generate graphical representations of shapes from the linear distances (Adams *et al*., 2004). So, researchers explored alternative methods of analyzing morphological shapes. One of the methods is known as landmark based

geometric morphometrics. The research field of this method involves studies on the structures that Cartesian coordinates can be taken (Pavlinov, 2001).

D'Arcy Thompson at the beginning of the century proposed the idea of landmark based geometry (Lynch, 2004). Rohlf (1990) mention that the emergence of computer technology facilitates the record of morphometric information. Bookstein (1991) developed the method of relative warps and shape coordinates for the analysis of morphometric variation based on landmark data providing additional inputs that help taxonomists in an excellent way. At the present time, geometric morphometric studies have gained significant support especially among anatomists (Lockwood *et al*., 2002) and taxonomists (Rohlf, 1993; Alibert *et al*., 2001; Gumiel *et al*., 2003) by the use of various morphological characters.

Many works have been done using geometric morphometric analysis for the species level discrimination of insects. Several geometric morphometric studies have been carried out in Iran for the geographic variations among the populations of *Chilo suppressalis* (Walker), *Cydia pomonella* L., *Ectomyelois ceratoniae* (Zeller), *Helicoverpa armigera* (H¸bner) by Zahiri (2003); Alipanah *et al*. (2004); Khaghaninia *et al*. (2008), Mozaffarian *et al*. (2005, 2007) and Khiaban *et al*. (2010). According to Brancucci (1980) in *Cantharidae*, the venation of hind wings was suggested to be of diagnostic value in the subfamily level based on the comparative morphology. Aytekin *et al*. (2007) applied the geometric morphometry for the identification of stingless bees by the analysis of the forewings and also used to resolve taxonomic problems in bumble bees. Francoy *et al*. (2008) used the technique for the identification of honey bee sub species and to examine changes in the morphometric profile of some Africanized honey bee populations. Rehn (2003) studied the wing venation pattern of Odonata. Using

landmarks based analysis Zahiri *et al.* (2006) recorded the wing sexual dimorphism in one species of rice stem borer, *Chilo suppressalis.*

Jirakanjanakit *et al*. (2008), Dujardin *et al*. (2009), Henry *et al.* (2010), Devicari *et al.* (2011) and Vidal *et al*. (2011) have characterized the wings of culicids. The importance of morphometric method in blow flies taxonomic identification was reported by Vásquez and Liria (2012) and Nuñez and Liria (2016). Geometric morphometric methods are now commonly used in studies of Klingenberg *et al*. (2010); Adams (2011); Martınez Abadıas *et al.* (2012) for the evolutionary quantitative genetics. Adams (2004); Langerhans *et al*. (2004); Adams *et al*. (2007) used this method to reveal phenotypical changes associated with species interactions. Geometric morphometric methods are applied for the study of fluctuating and directional asymmetry by Klingenberg *et al*. (2002) and Schaefer *et al*. (2006). It is also used to identify convergent and parallel evolution (Stayton, 2006; Adams, 2010; Adams & Nistri, 2010; Piras *et al*., 2010). This method has wide applications like discovering phylogenetic and macroevolutionary trends (Sidlauskas, 2008; Klingenberg and Gidaszewski, 2010; Monteiro & Nogueira, 2011) and to reveal ontogenetic patterns in human evolution (Bookstein *et al*., 2003; Mitteroecker *et al*., 2004; Mitteroecker and Bookstein, 2008), among other applications. Benitezetal (2012) studied the morphology of tenebrionid beetles and found that individuals of the same species can vary their geometric body shape responding to different climatic conditions. Morphometric methods (conventional and geometric) have been used to assist in identifying many insect species (Daly, 1985; Baylac *et al*., 2003). In the fruit fly genus Anastrepha, conventional morphometry has been used to characterize populations of the *A. fraterculus* complex by Hernandez-Ortiz *et al*. (2004,

2012); Selivon *et al*. (2005). Ruttner (1988) used standard morphometric in honey bee studies by measuring different wing angles, indices and distances.

Wings show many advantages over other organs in insects. The wings can be easily compared because of their two dimensional structure. Each taxon has a specific morphology of wing. It was Comstock (1893) who first popularized the use of insect wing venation for traditional classification (Kunkel, 2004). Landmarks can be easily formed by the intersection of veins. According to (Pavlinov, 2001) wings are solid or rigidly articulated structures; they have become very useful tools for geometric morphometric studies. Louis (1970) and Ito (1985) did the study of wing shape variation using inter landmark distances. This method is replaced by modern geometric morphometric method (Rohlf and Marcus, 1993). In geometric morphometry the size and shape of wings are analysed for heritability and environmental sensitivity (Klinberg, 2010). Generally, wing morphometric analyses can provide good insights into various categories, such as identifying populations within a species, as shown by the analysis of geographic variation in populations of *Drosophila lummei* Hackman (Haas and Tolley, 1998), *D. serrata* Malloch (Hoffman and Shirrifs, 2002), *Scythris obscurella* (Scopoli) (Roggero and Dentreves, 2005), *Calopteryx splendens* (Harris) (Sadeghi *et al*., 2009).

3.3. MATERIALS AND METHODS

3.3.1. Sampling sites.

The adult banana stem weevil, *Odoiporus longicollis* were collected and analyzed for the present study. Two sites were selected to study the xenobiotic effects on the adult *Odoiporus longicollis* as following. The sample insects were collected from Vellalssery in Kozhikode district. The

sample collection were made during January to June 2017. A total of 94 insects collected from the (Site 1) banana plantations treated with chemical pesticides like carbosulfan, fipronil, chlorpyrifos etc. Of the 94 insects collected, 46 were males and 48 were females. Another group of 110 adult banana weevils were collected from (Site 2) banana plantations in the Chathamangalam Panchayath in Kozhikode district where organic fertilizers were used. Of these 60 were males and 50 were females. The adult banana weevil exhibits sexual dimorphism. Male and female were identified based on their rostral surface. The rostral surface was rough in males and smooth in females. After identification, the adult insects were killed and hind wings were carefully removed using forceps. Insect wings were separated and photographed with a scale bar included to record the size of each specimen.The statistical differentiation was performed by one way ANOVA and size variation by centroid size.

3.3.2. Landmarks

For the morphological analysis, 18 landmarks for hind wings of banana weevil were identified. The locations of landmarks were chosen based on the intersection of veins or vein base, vein end or apex. The “anterior”, “posterior”, “proximal”, and “distal” veins or plates were used to describe the detailed position of landmarks. Landmarks are defined as points of correspondence on each object that matches between and within populations (Dryden & Mardia, 1998). Wings were dissected from anesthetized specimens and documented by using CANON EOS 7 D camera.

Landmarks for geometric morphometric analysis were digitized using tps.dig software. Analysis and interpretation was done using MorphoJ software using principal component analysis, canonical variate analysis.

MorphoJ (Version 1.06d) was used for landmark data analyses. MorphoJ is a software package enabling geometric morphometric analysis for two-dimentional and three-dimensional landmark data and designed for the analysis of actual biological data (Klingenberg, 2011). Prior to further analyses, the landmark data of wings were imported into MorphoJ, and a complete Procrustes fit was conducted by orthogonal projection to correct size and orientation. The digitized images were superimposed by generalized procrustes superimposition. The position description of landmarks and the average shape of hind wing of weevil were identified.

3.3.3. Principal Component Analysis

PCA is the most widely used method for exploratory multivariate analysis (Klingenberg and McIntyre, 1998; Klingenberg and Zaklan, 2000). For perceiving wing shape variation between sexes PCA analysis was performed, which was visualized by using deformation grid and discriminant analysis. In this study, MorphoJ generates covariance matrices from landmark data sets of 204 specimens after Procrustes superimposition. Based on the covariance matrices, PCA was used to analyse and display the patterns of covariation of positions of landmarks throughout the wing. Principal components (PCs) are visualised directly as patterns of simultaneous displacements of landmarks in relation to one another.

3.3.4. Canonical Variate Analysis (CVA)

CVA is a method used to find the shape features that best distinguish among multiple groups of specimens (Gumiel *et al*., 2003; Villemant *et al*., 2007). CVA based on the 204 specimens was used to explore the wing variance of male and female weevils collected from site1 and site 2.

3.4. RESULTS

3.4.1. Landmark Position

Adult banana pseudostem weevil, *Odoiporus longicollis was* identified with about 18 landmarks for the hind wing (Plate 3.1). After identification of male and female insects using its rostral features (Plate 3.2, 3.3), Landmarks were digitized using tps.dig software. MorphoJ software is used for the analysis and interpretation of landmark analysis. For calculating the error percentage landmarks were redigitalized twice. Table 3.1 and 3.2 decribes the landmark positon and average shape of hind wing of banana weevil.

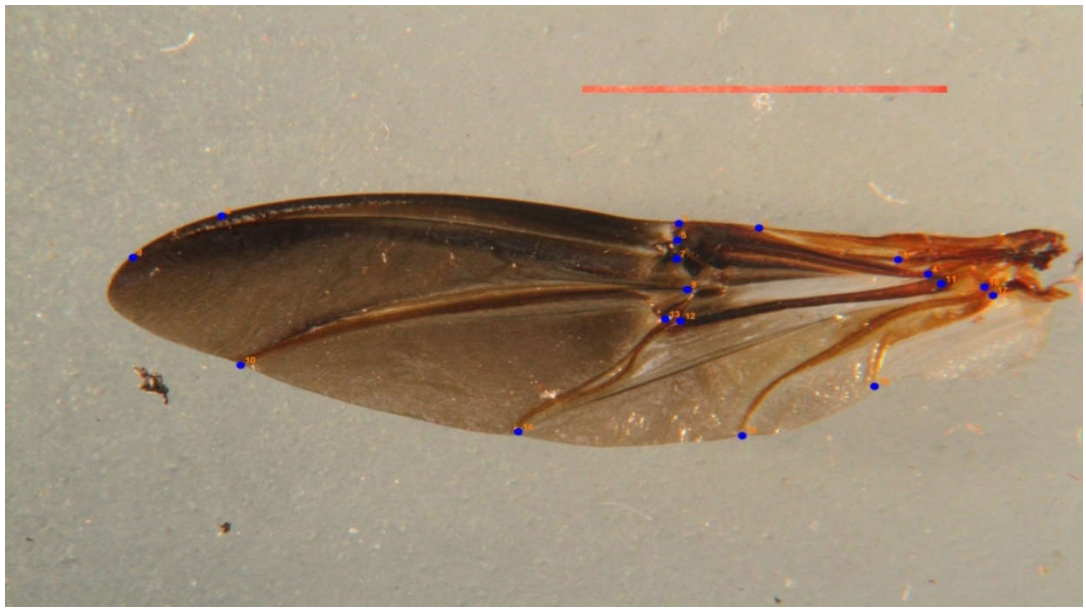


Plate 3.1: Digitalized image of hind wing of *Odoiporus longicollis* with landmark points

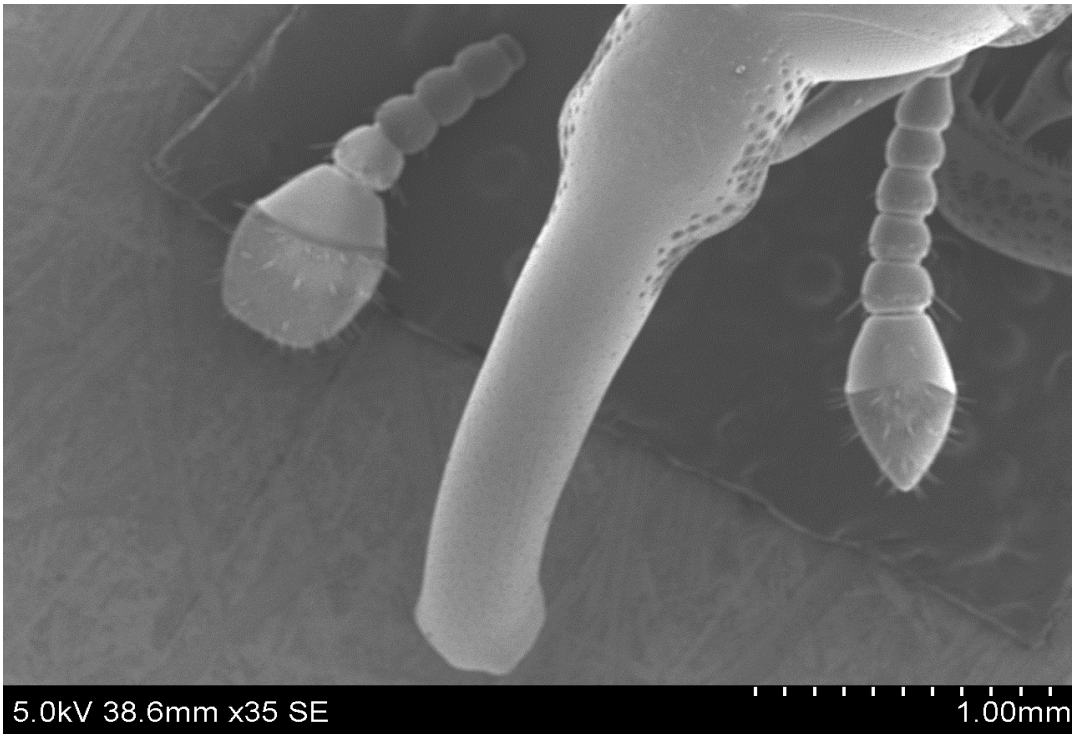


Plate 3.2: SEM micrograph of adult banana weevil showing smooth rostrum in female

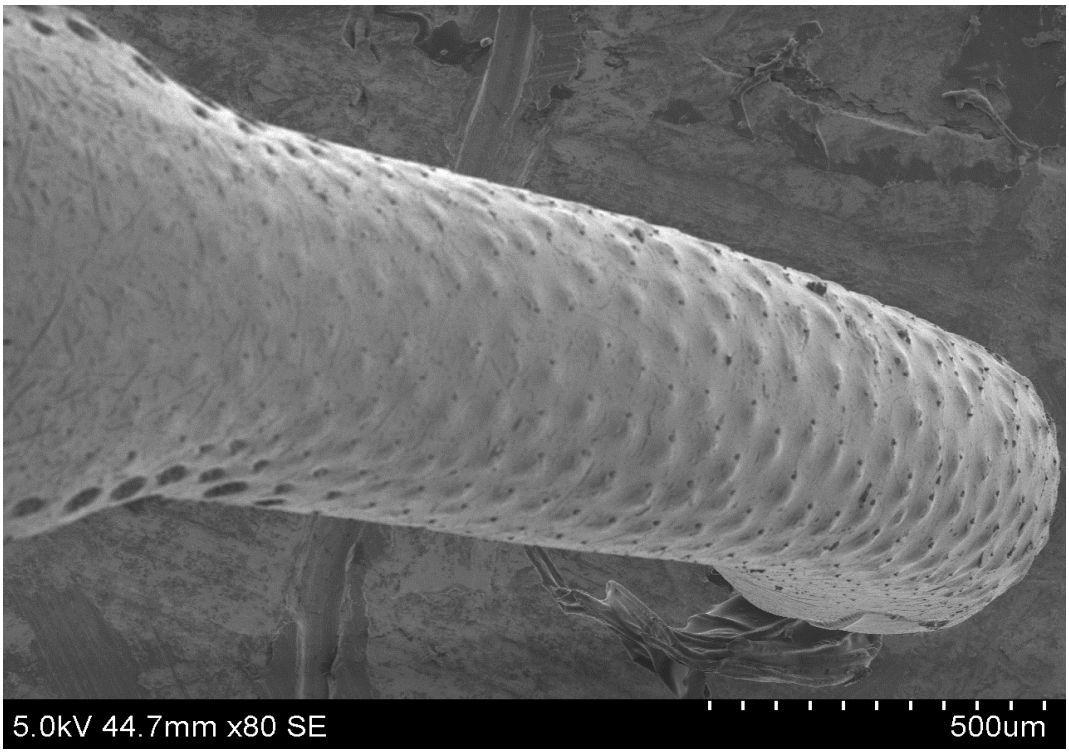


Plate 3.3: SEM micrograph of adult banana weevil showing rough rostrum in male

Table 3.1: Landmark position description

LANDMARK	DESCRIPTIVE LOCATION	DESCRIPTION
1	SC(B)	BASAL END OF THE SUB COSTA
2	SC(D)	DISTAL END OF THE SUB COSTA
3	HI	ANTERIOR HINGE
4	R(B)	BASAL END OF ANTERIAL RADIAN
5	RA 3 (B)	BASAL END OF THE ANTERIAL RADIUS□
6	RA□(D)	DISTAL END OF ANTERIAL RADIUS □
7	RA□+RP□(B)	BASAL END OF ANTERIAL RADIUS□+ POSTERIOR RADIUS □
8	RA□+RP□(D)	DISTAL END OF ANTERIAL RADIUS□+ POSTERIOR RADIUS □
9	RP□(B)	BASAL END OF RADIUS POSTRIOR□
10	RP□(D)	DISTAL END OF RADIUS POSTRIOR□
11	MP□+ 2 (B)	BASAL END OF POSTERIO MEDIAN□+ 2
12	MP□+ 2 (D)	DISTAL END OF POSTERIO MEDIAN□+ 2
13	RP□+ 4 (B)	BASAL END OF RADIUS POSTERIOR□+ 4
14	RP□+ 4 (D)	DISTAL END OF RADIUS POSTERIOR□+ 4
15	CuA(B)	BASAL END OF CUBITO ANAL
16	CuA(D)	DISTAL END OF CUBITO ANAL
17	AA(B)	BASAL END OF ANTERIOR ANAL
18	AA(D)	DISTAL END OF ANTERIOR ANAL

Table 3.2: Mean configuration of the 18 landmarks plotted of the hind wing of banana weevil

Average shape:		
Lmk.	Axis 1 (x)	Axis 2 (y)
1	0.212183	0.040238
2	0.066874	0.067889
3	-0.01495	0.073999
4	0.221102	0.026861
5	-0.01013	0.053726
6	-0.40523	0.084442
7	0.001336	0.035049
8	-0.50031	0.030826
9	0.01428	0.013527
10	-0.39773	-0.06543
11	0.236769	0.015323
12	-0.00354	-0.02094
13	-0.01789	-0.01254
14	-0.15329	-0.12886
15	0.264069	0.010233
16	0.047532	-0.13545
17	0.275997	0.004872
18	0.162914	-0.09377

3.4.2. PCA and CVA analysis

On the basis of analysis of hind wing of banana weevil, *Odoiporus longicollis* the data obtained showed significant variation in size and shape of hind wings of two sexes. The Procrustes analysis were performed on the acquired landmarks data of wings to determine the average shape within the subgroup of adult male and female beetles and also among and between both contaminated and uncontaminated insects (Table 3.6 &3.7). The landmarks were then superimposed to optimize the distances from a common centroid shape. The result of the study provides information about the average shape and variation in wing morphology between male and female adult beetles *Odoiporus longicollis* and data were graphically represented. Using the canonical variate analysis (CVA) and principal component analysis (PCA), the symmetry between the hind wings of male and female adult insects were analyzed in uncontaminated and pesticide affected group which is then graphically represented. Also, the wing shape variations caused by the pesticides on *Odoiporus longicollis* was also represented in shape decomposition grid for contaminated and uncontaminated group.

Table 3.3: Principal Component Analysis (PCA) of hind wing of banana pseudostem weevil.

	Eigenvalues	% Variance	Cumulative %
1	0.00114	36.882	36.882
2	0.00056	18.122	55.004
3	0.000221	7.159	62.163
4	0.000188	6.089	68.252
5	0.000114	3.684	71.937
6	9.71E-05	3.141	75.077
7	8.79E-05	2.844	77.921
8	7.83E-05	2.534	80.455
9	6.53E-05	2.113	82.569
10	6.04E-05	1.955	84.524
11	5.45E-05	1.762	86.286
12	4.85E-05	1.568	87.854
13	4.66E-05	1.506	89.36
14	4.05E-05	1.309	90.669
15	3.62E-05	1.172	91.841
16	3.08E-05	0.996	92.837
17	2.67E-05	0.865	93.702
18	2.52E-05	0.817	94.519
19	2.17E-05	0.701	95.219
20	2E-05	0.648	95.867
21	1.86E-05	0.603	96.47
22	1.59E-05	0.514	96.983
23	0.000014	0.453	97.436
24	1.32E-05	0.428	97.864
25	1.23E-05	0.397	98.261
26	1.15E-05	0.371	98.632
27	9.78E-06	0.316	98.948
28	9.44E-06	0.305	99.253
29	6.98E-06	0.226	99.479
30	5.82E-06	0.188	99.667
31	5.64E-06	0.182	99.849
32	4.65E-06	0.151	100

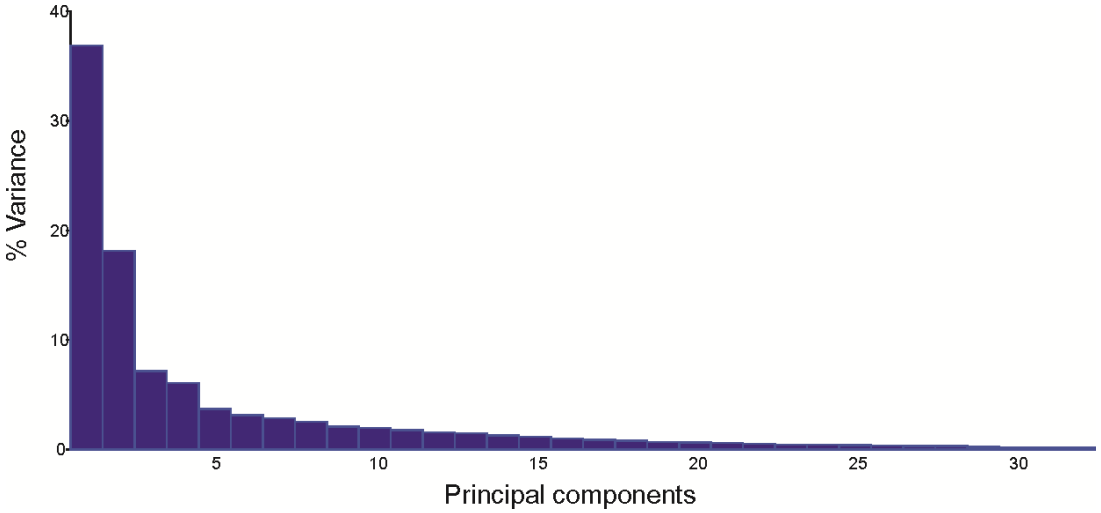


Fig 3.1: Percentages of variance in principal components (PCA) of hind wings of banana weevil, *Odoiporus longicollis*

In principal component analysis, the first principal component has the highest variation. The variations decrease with each component. The cumulative percentage of variation of first three principal components was 62.163%. The variations percentages of PC1, PC2, and PC3 were 36.882, 18.122, and 7.159 respectively. The results show the asymmetry between control and contaminated groups. The significant P-value ($p<0.0001$) obtained in left and right hind wing analysis shows the variation and asymmetry between the contaminated and uncontaminated groups. The two groups are ordered along with the two principal components and the plot obtained shows that the conataminated and uncontaminated groups are well separated. It describes the distinguished nature of the two sub sets (Table 3.3 & Fig 3.1-3.7).

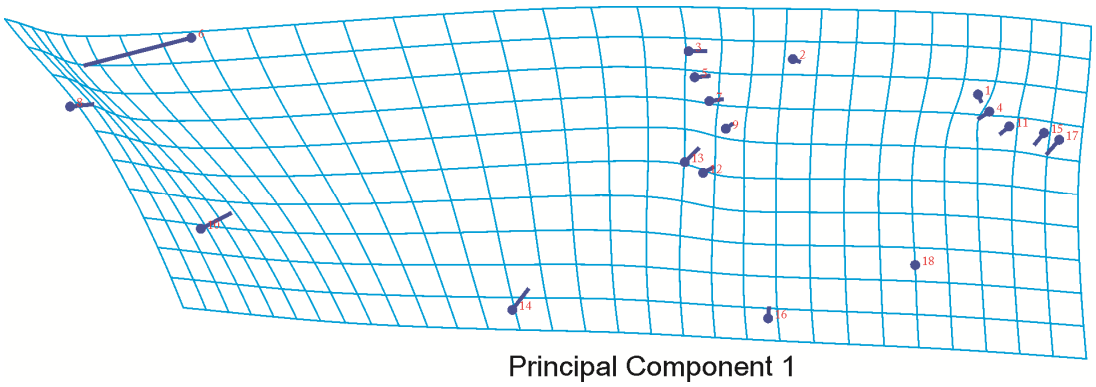


Fig 3.2: Thin plate deformation grid showing the shape variation of hind wings of adult beetle *Odoiporus longicollis* by PC1 (36.882%).

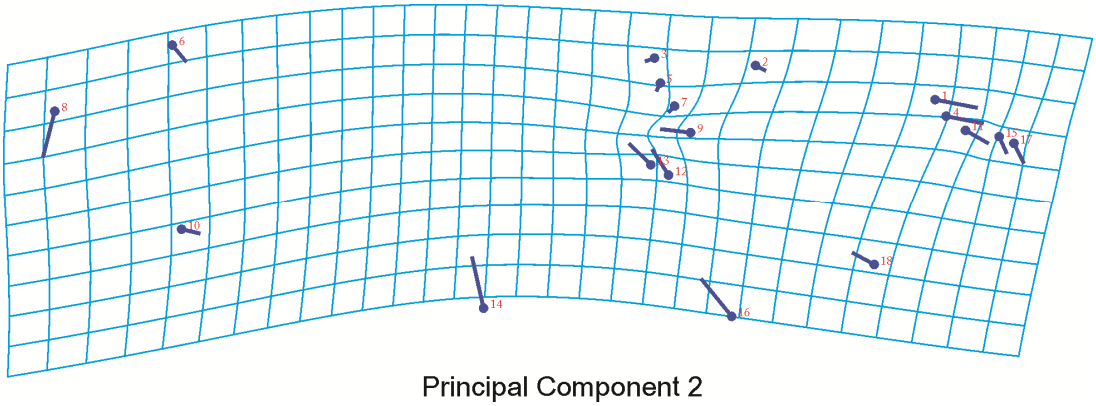


Fig 3.3: Thin plate deformation grid representing the shape variation of hind wings of adult beetle *Odoiporus longicollis* by PC 2 (18.122%).

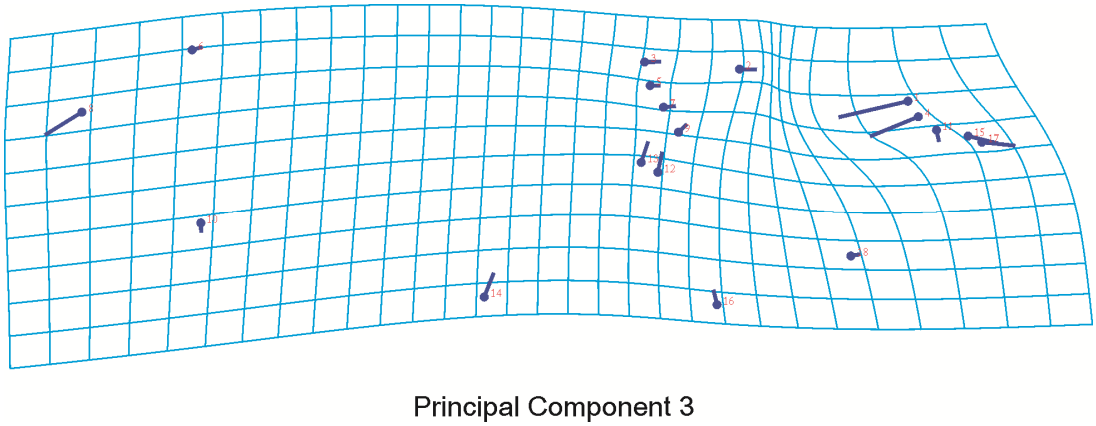


Fig 3.4: Thin plate deformation grid representing the shape variation of hind wings of adult beetle *Odoiporus longicollis* by PC 3 (7.15%).

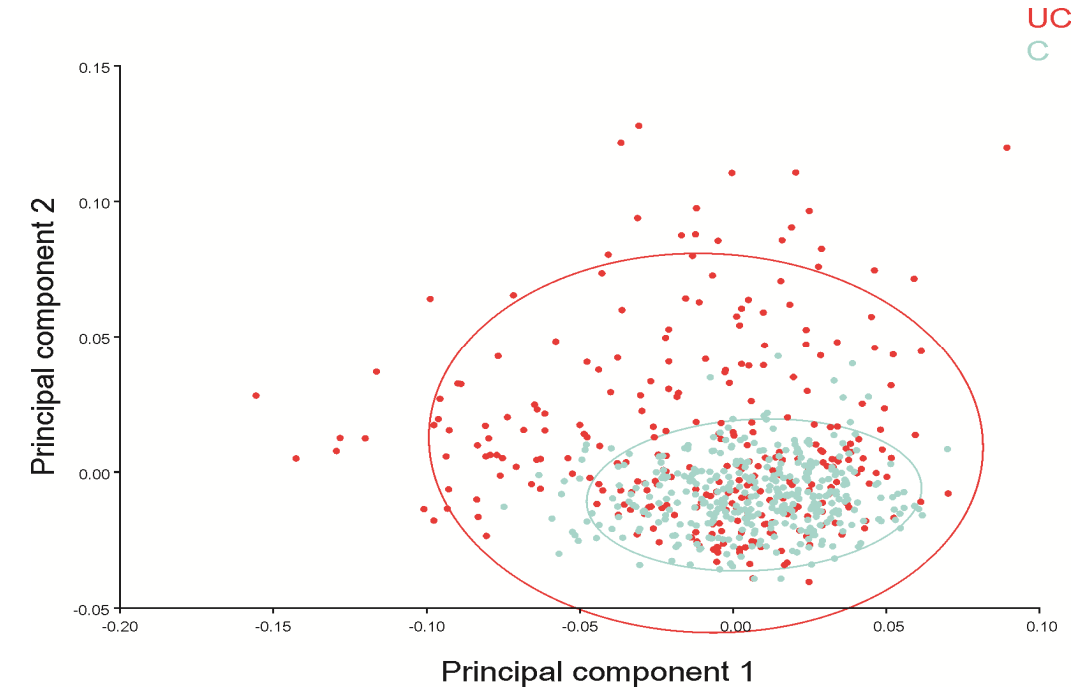


Fig 3.5: PCA plot of contaminated (Site 1) and uncontaminated groups (Site 2) - (PC1/PC2).

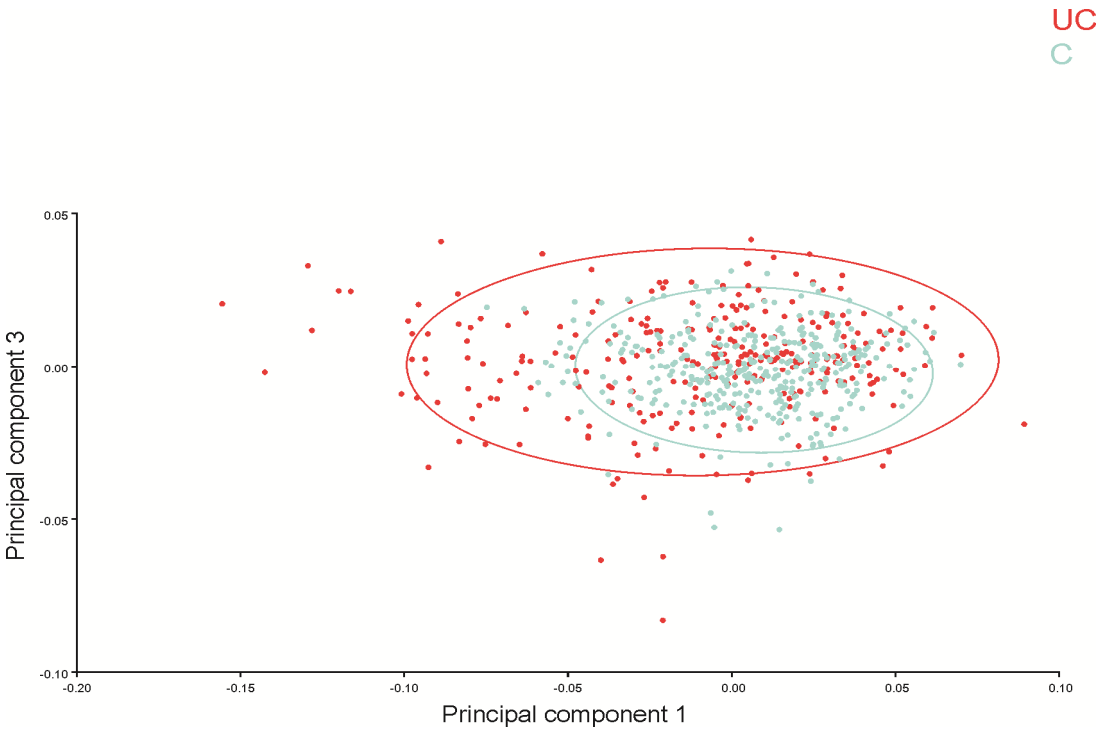


Fig 3.6: PCA plot of contaminated (Site 1) and uncontaminated insects (Site 2) representing the percentage of variance in principal components PC 1 & PC 3.

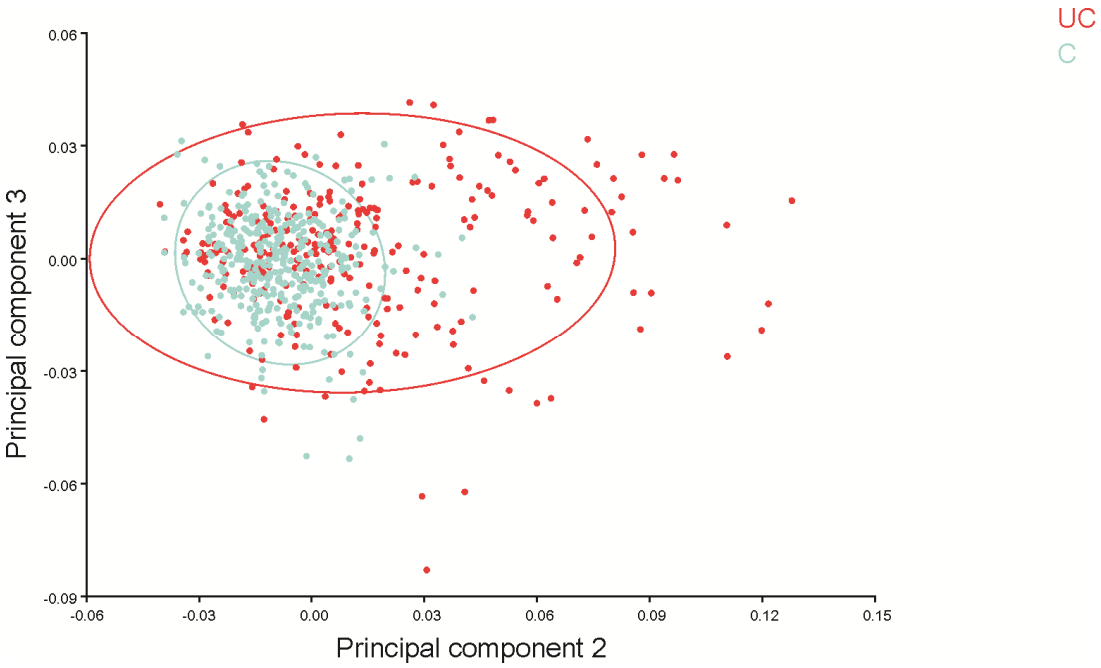


Fig 3.7: PCA plot of contaminated (Site 1) and uncontaminated insects (Site 2) representing the percentage of variance in principal components PC 2 & PC 3.

Male and female Hind wing Size and Shape Variations (uncontaminated) (Site 2)

Among the control group, the male and female showed significant ($p<.0001$) variations indicating the wing asymmetry. The differences in hind wing shape of male *O. longicollis* in control and experimental conditions were compared and visualized through graphs. Using the thin plate deformation grid the wing shape differences between control and contaminated individuals were shown. The male and female insects showed distortions in the wing which is represented in the graph. The land marks that showed maximum deviations are at the distal regions of the wing. They are distal end of radius posterior□+ ₄ (RP□+ ₄ (D)), distal end of anterial radius □ (RA□(D)),distal end of anterial radius□+ posterior radius □(RA□+RP□(D)) (Fig 3.8 & 3.9).

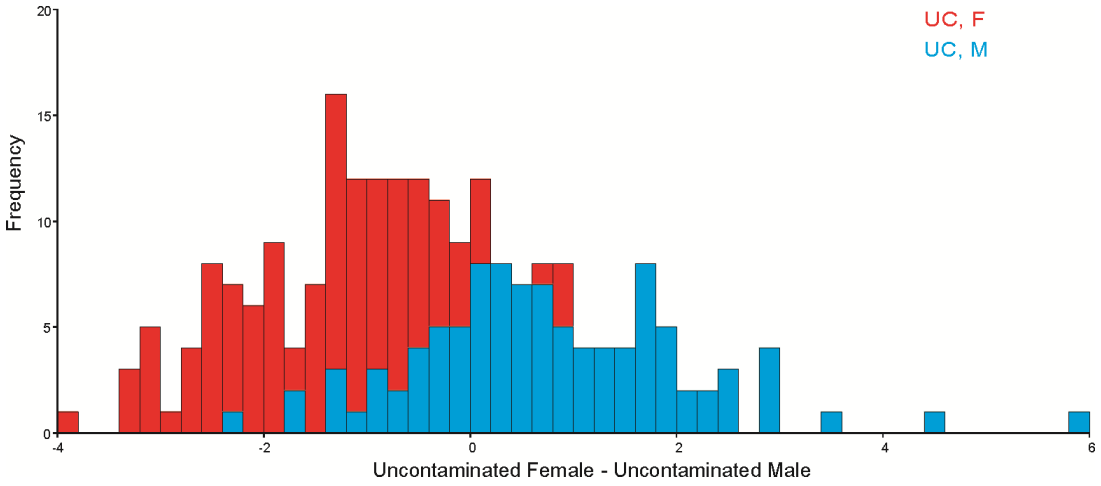


Fig 3.8: Discriminate analysis data showing variation in male and female hind wings insects of uncontaminated area (Site 2).

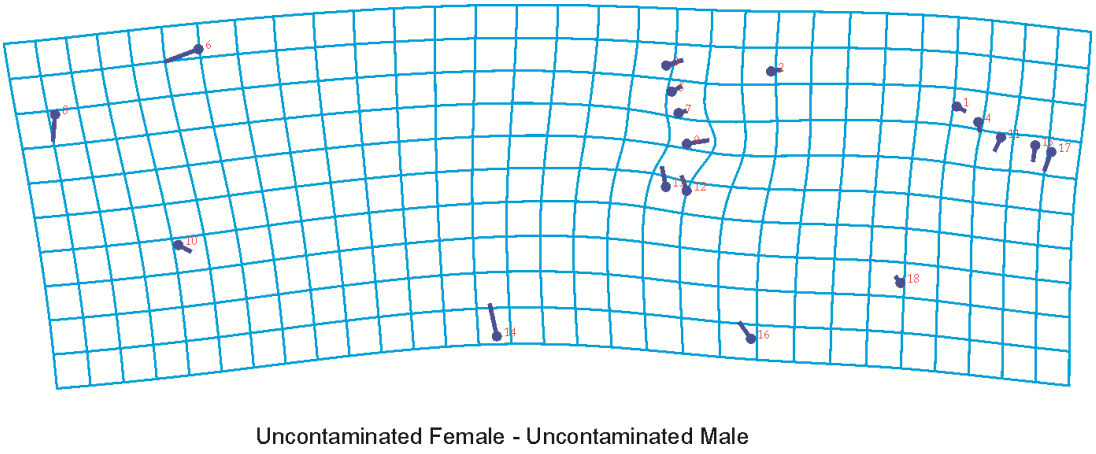


Fig 3.9: Thin plate deformation grid representing uncontaminated insects hind wings of both sexes.

Male and female Hind wing Size and Shape Variations (contaminated) (Site 1)

The insects collected from the pesticide contaminated area shows a high variation in size and shape of the hind wing. The P- value obtained ($p<.0001$) showed significant variation in size and shape. The land mark point six showed highest variation in position compared to others. Distal end of anterial radius□, RA□ (D) is the land mark at this position. Landmark number 18 (AA(D)) and 4(R(B)) showed no deviation in position. This

implies the position of these landmarks were almost similar in the case of forewings of contaminated male and female insects (Fig3.10&3.11).

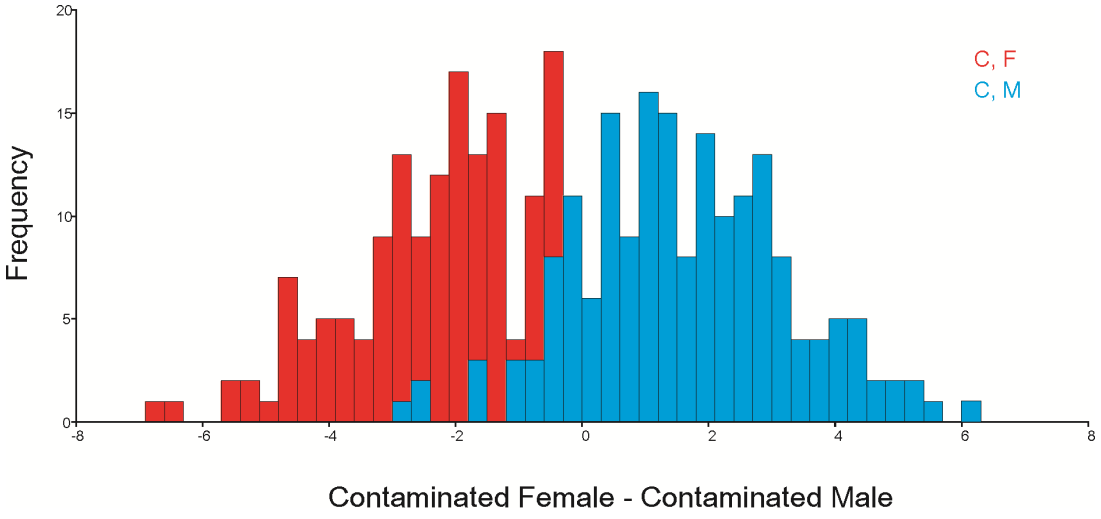


Fig 3.10: Discriminant analysis representing hind wings of male and female insects from contaminated area (Site 1)

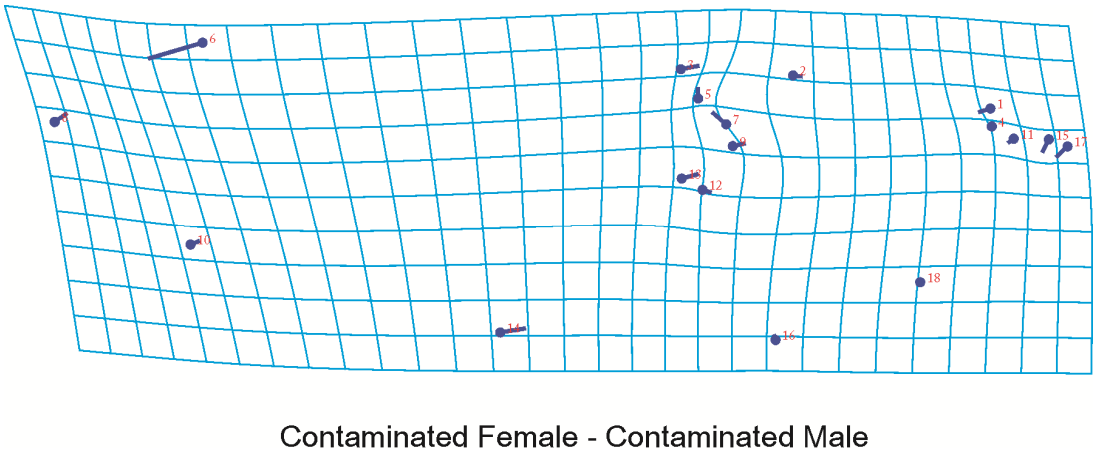


Fig 3.11: Thin plate deformation grid representing contaminated insects hind wings of both sexes

Site 1 female and Site 2 male hind wing size and shape variations

The discriminant analysis graph revealed that most of the landmark points in the forewing showed high deviation. The complete separation between male and female in the graph denotes the shape deterioration of individuals of male and female from site 2 and site 1 respectively. Here also there is a significant variation of hind wing size and shape ($p < .0001$). Distal end of the sub costa, anterior hinge, basal end of anterial radian, basal end of the anterial radius□, distal end of anterial radius □, basal end of anterial radius□+ posterior radius □, distal end of anterial radius□+ posterior radius □, basal end of radius posterior□, distal end of radius postrior□, basal end of posterio median□+ 2 , distal end of posterio median□+ 2 , basal end of radius posterior□+ 4 , distal end of radius posterior□+ 4 , basal end of cubito anal, distal end of cubito anal, basal end of anterior anal are the points of deviations. The thin plate deformation grid shows the wing shape differences between contaminated and uncontaminated insects (Fig 3.12 & 3.13).

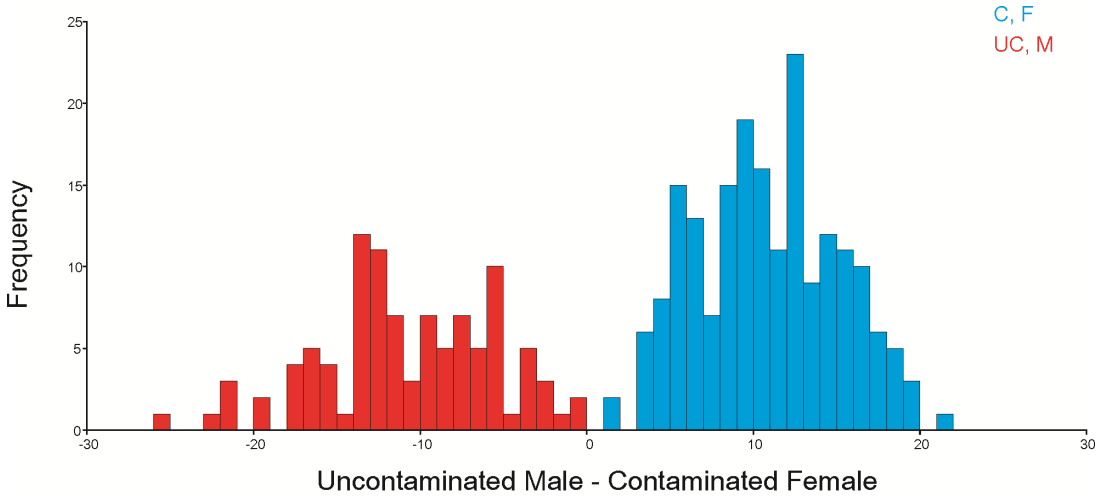


Fig 3.12: Discriminate analysis data showing variation in uncontaminated male (Site 2) and female from contaminated area (Site 1).

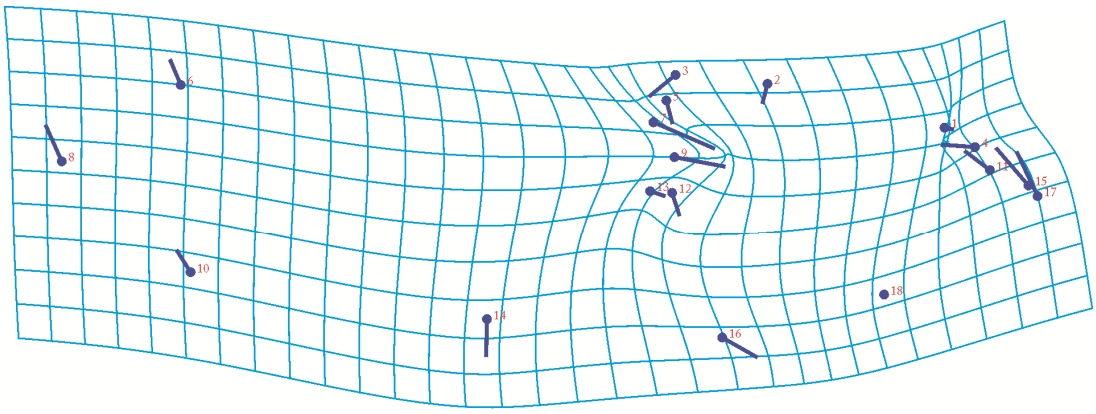


Fig 3.13: Thin plate deformation grid representing contaminated insects (Site 1) hind wings of female and uncontaminated male (Site 2).

Site 2 female and Site 1 male hind wing size and shape variations

The difference in the hind wing shape and size between control and contaminated insects were compared and visualized through discriminant analysis and thin plate deformation grid. Distal end of anterial radius □, basal end of radius posterior□, basal end of anterial radius□+ posterior radius □, basal end of anterior anal, basal end of cubito anal, basal end of posterio median□+ ₂ , basal end of anterial radian andbasal end of radius posterior□+ □ showed deviations. The highest variation is observed in distal end of anterial radius □. Significant P value obtained in forewing shape of female ($p<0.0001$) indicated asymmetry between undisturbed and disturbed individuals (Fig 3.14& 3.15).

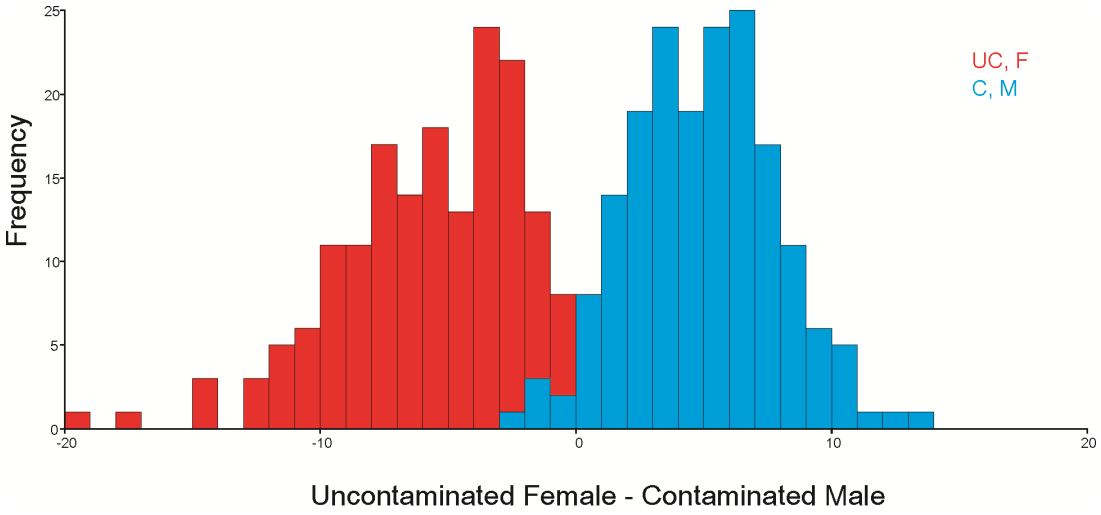


Fig 3.14: Discriminant analysis representing hind wings of uncontaminated female (Site 2) and male from insects from contaminated area (Site 1).

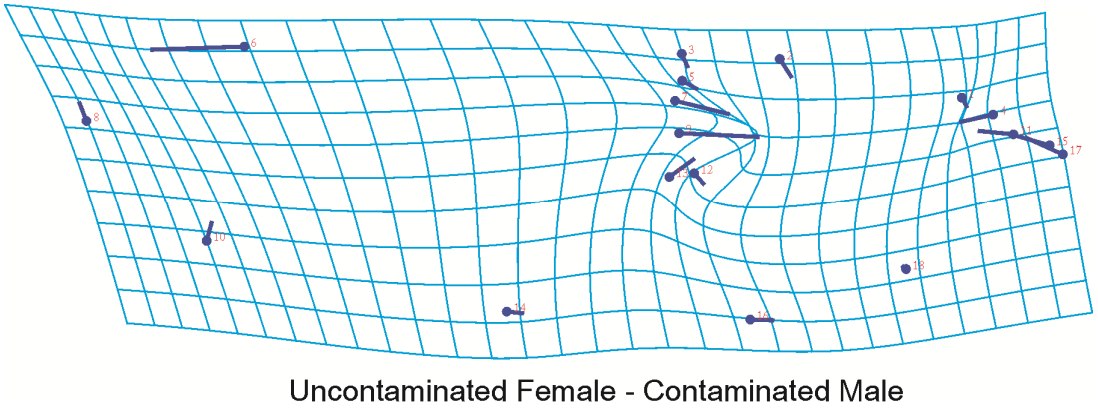


Fig 3.15: Thin plate deformation grid representing contaminated insects (Site 1) hind wings of male and uncontaminated female (Site 2)

Hind wing size and shape variations of females from Site 1 & 2.

The deviation in hind wing shape of the site 2 female from contaminated females (Site 1) is shown in the graph. The thin plate deformation grid of the hind wings of both site 1 & 2 females exhibited deviation in almost all landmarks points. The minimum deviation was observed in landmark number 18 (distal end of anterior anal) and maximum

deviations were seen in landmark number 7 [RA□+RP□(B)], 9 [RP□(B)], 15 [CuA(B)],17 [AA(B)], 6 [RA□(D)]. The highly significant P value (p<.0001) indicates the difference in the size and shape of the insect hind wings (Fig 3.16 & 3.17).

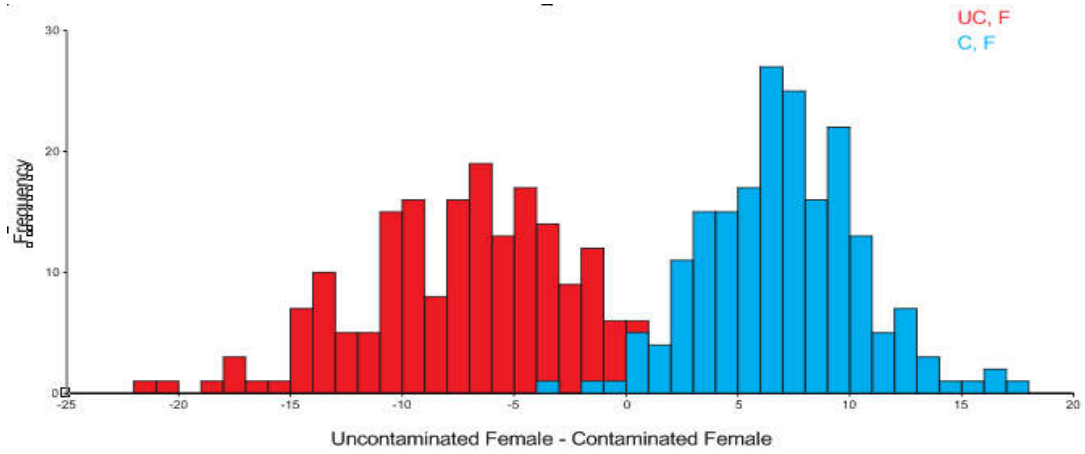


Fig 3.16: Discriminant analysis representing female insect hind wings of contaminated and uncontaminated area (Site 1& 2).

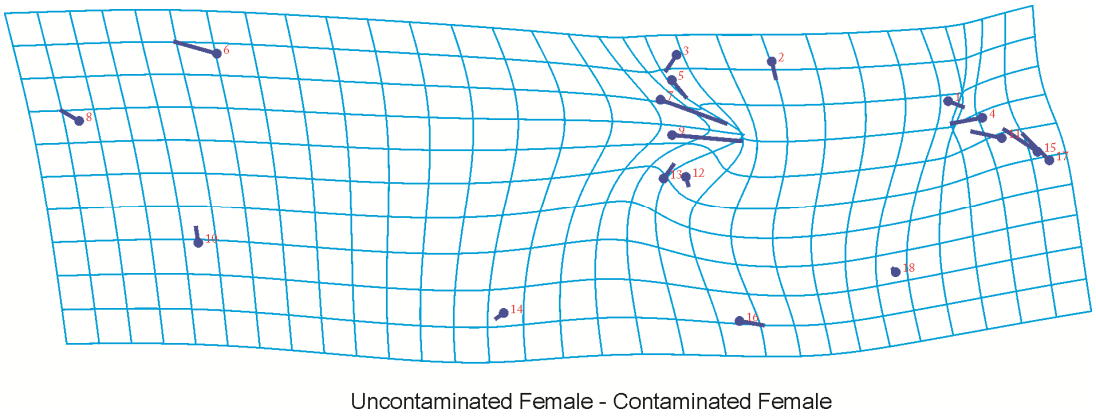


Fig 3.17: Thin plate deformation grid representing female insect hind wings of contaminated and uncontaminated insects (Site 1& 2).

Contaminated and uncontaminated male (Site 1& 2) hind wing size and shape variations

In this comparison almost all land marks shows the deviations except landmark 18 (distal end of anterior anal). All the land marks showed the highest deviation in its position. The shape variation was also observed between left and right hind wings of disturbed and undisturbed male, which revealed that they showed asymmetry in forewings. The P value obtained was <.0001, indicating the significant difference (Fig. 3.18 & 3.19).

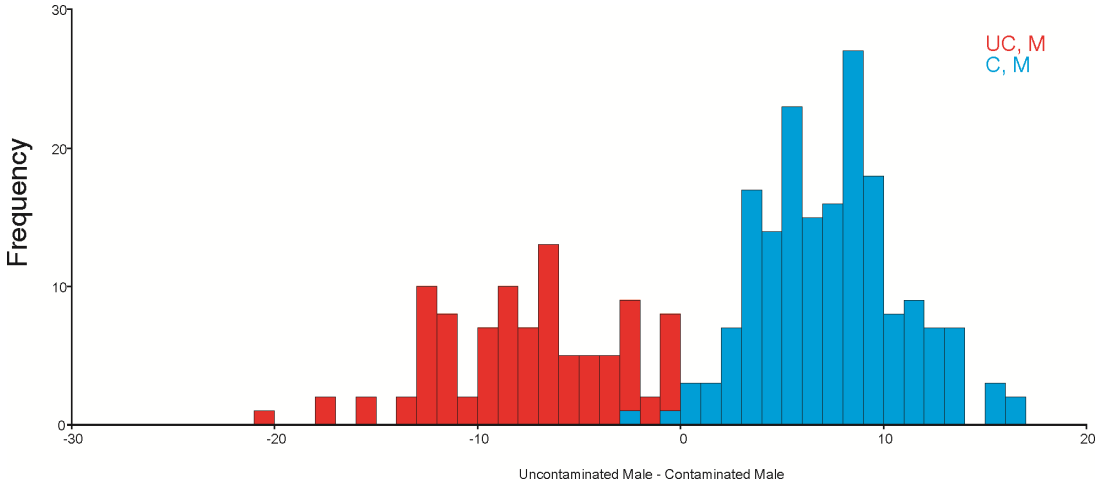


Fig 3.18: Discriminant analysis representing male insect hind wings of contaminated and uncontaminated area (Site 1 & 2).

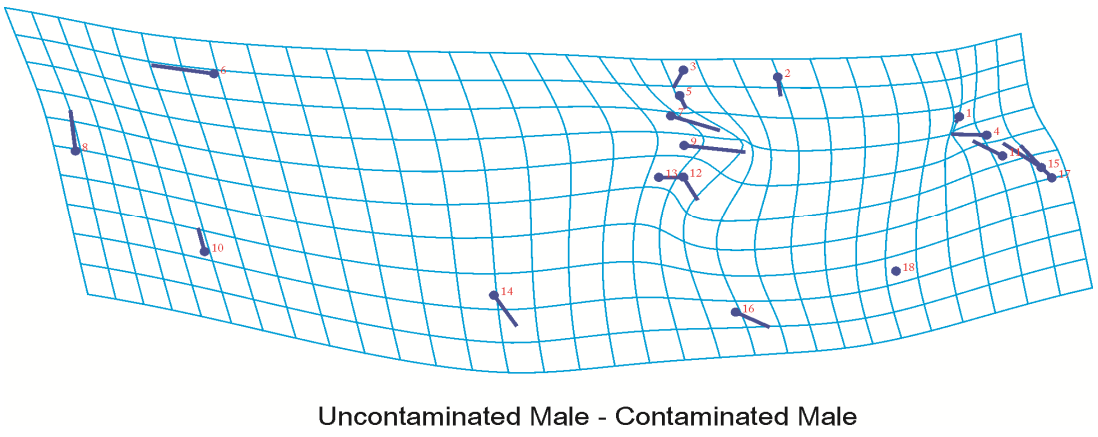


Fig 3.19: Thin plate deformation grid representing male insect hind wings of contaminated and uncontaminated area (Site 1& 2).

The canonical variate analysis showed significant change in wing shape. From about 690 observations, 664 are included in the data analysis (Table 3.4). Canonical variate analysis contributes 87.047% variation by CV1. The cumulative percentage of variation produced by CV1, CV2, and CV3 is 100 (Table 3.5). The thin plate deformation grid shows the variation due to CV1. The canonical variate analysis (CVA) was performed to determine the shape features between multiple groups of the individuals. The scatter plot of CVA represented shape variations between the control and treated male and female insect hind wings. The four groups are well separated and form four well distinguished groups (Fig 3. 20 & 3.21).

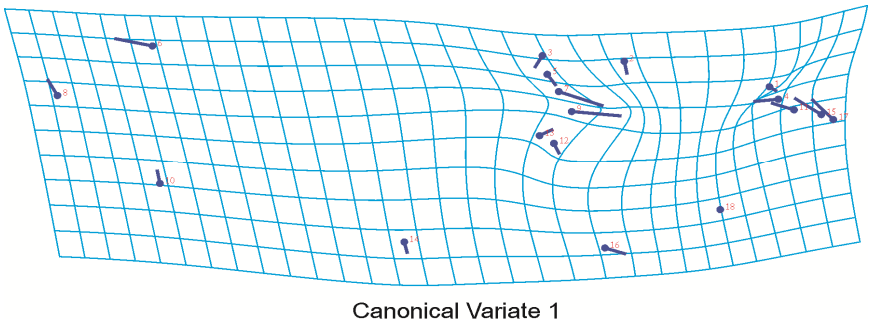


Fig 3.20: Canonical variate plot showing the variation by CV1

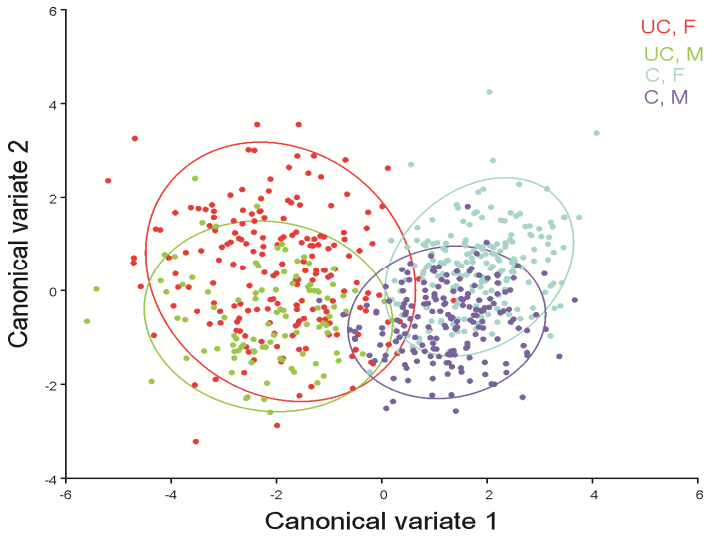


Fig 3.21: Canonical variate plot showing the distortion of hind wings of control and contaminated insects of both the sexes, based on the shape of left and right areas

Table 3.4: The dataset contains 690 observations of uncontaminated (UC) and contaminated (C) male (M), female (F) for analysis.

	Groups	Observations
1	UC, F	190
2	UC, M	100
3	C, F	193
4	C, M	181

Table 3.5: CVA Analysis of hind wings of banana pseudostem weevil.

	Eigenvalues	% Variance	Cumulative %
1	3.184033	87.047	87.047
2	0.291618	7.972	95.02
3	0.18217	4.98	100

The data have been subjected to statistical analysis using procustes ANOVA. Table 3.6 and 3.7 provides the data on statistical analysis of different groups of insects collected from two different collection areas (Site 1 & 2). In all the sample collection there exist a significant difference between the contaminated (Site 1) and uncontaminated (Site 2) areas. The p value in all the cases were less than 0.001 ($p<0.0001$). There is a significant variation between the samples groups collected from these areas.

Table 3.6: Data on the statistical analysis showing significant difference between contaminated and uncontaminated groups (Site 1 & 2).

Mahalanobis distances among groups:				**P-values from permutation tests**			
	UC, F	UC, M	C, F		UC, F	UC, M	C, F
UC,M	1.4908			UC, M	<.0001		
C,F	3.8303	4.1653		C, F	<.0001	<.0001	
C,M	3.3349	3.5624	1.4727	C, M	<.0001	<.0001	<.0001
Procrustes distances among groups:				P-values from permutation tests			
	UC, F	UC, M	C, F		UC, F	UC, M	C, F
UC,M	0.0196			UC,M	0.0004		
C,F	0.0371	0.0389		C,F	<.0001	<.0001	
C,M	0.0437	0.041	0.0191	C,M	<.0001	<.0001	<.0001

TABLE 3.7: Results of Procrustes analysis of variance (ANOVA) and decomposition of shape

Centroid size: Procustes ANOVA		**HIND WINGS CONTROL AND PESTICIDE EFFECTED**			
EFFECT	**SUM OF SQUARES**	**MEAN SQUARE**	**dF**	**F**	**P(param.)**
Individual	**50930039.755611**	**1083617.867141**	**47**	**1.18**	**0.2862**
Side	**171469678.781694**	**171469678.781694**	**1**	**186.75**	**< .0001**
Ind * Side	**43154622.414642**	**918183.455631**	**47**	**0.14**	**1.0000**
Error 1	**659849487840002**	**6665146.341818**	**99**	**3.96**	**< .0001**
residual	**720107583.824735**	**1682494.354731**	**428**		
SHAPE: Procustes ANOVA		**HIND WINGS CONTROL AND PESTICIDE EFFECTED**			
EFFECT	**SUM OF SQUARES**	**MEAN SQUARE**	**dF**	**F**	**P(param.)**
Individual	**0.24462334**	**0.0001626485**	**1504**	**1.02**	**0.3682**
Side	**0.21390323**	**0.0066844758**	**32**	**41.82**	**<.0001**
Ind * Side	**0.24041356**	**0.0001598494**	**1504**	**1.55**	**<.0001**
Error 1	**0.32765134**	**0.0001034253**	**3168**	**1.22**	**<.0001**
residual	**1.16214470**	**0.0000848529**	**13696**		

3.5. DISCUSSION

The current study examined the symmetry and comparison of wing patterns of *Odoiporus longicollis* collected from two selected sites such as pesticide treated Vellalassery area and pesticide untreated Chathamangalam area. In this study total 18 landmarks were identified and marked on hind wing. Procrustes superimposition which is the most important analysis of geometric morphometric was performed where, only the shape information is considered. Since, geometric morphometric is an emerging area in biology only few reports are available on the existing study on shape information of insects. Recent study on dimorphism by Espra *et al*. (2015) in the shape of the wings in *Lucilia sericata* using geometric morphometric methods showed that the variations observed could be genetic or could be mere reflections of the existence of high phenotypic plasticity due to environmental changes during growth and development of the larvae. According to Mendes *et al.* (2007) using only wing features it is now possible to identify species of various groups of insects with the help of computational morphometric identification systems.

From the study it is clear that the male and female hind wing of *Odoipoprus longicollis* exhibits wing variation. The P value for Procrustes distance of male and female hind wing among the site 2 group was 0.0004. The P value for mahalanobis distance of male and female hind wing among the control group was less than 0.0001. The P value for Procrustes distance of male and female hind wing among the site 1 group was also less than 0.0001. Based on the PCA and CVA study it is very clear that there exists a great variation in hind wing size and shape among and between the uncontaminated (Site 2) and contaminated groups (Site 1) (Table 3.6 &3.7).

In this study it is identified that most of the landmark variations occurred in distal region than proximal part. Similar studies were also

conducted by Jing Ren *et al*. (2017) shows that the apical part of the hind wings of leaf beetles has an important influence on hind wing shape variance by PCA. In beetles hind wings are used for flight and it is kept in transversely folded condition under the elytra when not in use. According to Haas *et al*. (2000) the hind wing as the flight organ, must have a certain size to be aerodynamically functional, which makes them distinctly larger than the thickened fore wings. This will prove that the areas of the beetle hind wing relevant to transverse folding importantly influence hind wing shape variations. Transverse wing folding is one of the advantages of coleopterans, dermapterans and some species of blattiodea (Forbes, 1926; Haas and Beutel, 2001; Haas, 2006; Beutel *et al.,* 2014). The CVA results (Figs 3.20 & 3.21) show that the different groups of male and female insects exhibit variation and asymmetry in hind wing, since Mahalanobis and Procrustes distances (Tables 3. 6) for each group are significantly different ($p<.0001$). It also suggests that the hind wing shape is useful for the discrimination of both male and female adult *Odoioporus longicolls* and also with the pesticide affected individuals.

The geometric morphometrics represent a reliable tool in the taxonomic research and also in further study on the evolution of the hind wing shape of coleopteran beetles. Geometric morphometric studies by Hoffmann *et al*. (2005) shows that the size and shape of the wing can be used as an indicator of stressful environmental conditions and to evaluate the developmental noise. The present study on the effect of xenobiotics on the *Odoiporus longicollis* was very significant and it explains that xenobiotics influence wing size and shape.

SUMMARY

SUMMARY

The banana pseudostem weevil *Odoiporus longicollis* Oliv. (Coleoptera: Curculionidae) is one of the important pests of bananas and plantains. Infestation with this pest is one of the major limiting factors which cause 10-90% yield loss by afflicting major share of the damage in the banana pseudostem. The pest has established itself in all the varieties of banana especially on Nendran in Kerala. The weevil attack interferes with the plant growth and reduces the plant vigour leading to plant death and reduction of bunch weight. For the successful and productive banana cultivation the number of pseudo stem weevil population must be reduced. The control of the banana pseudo stem weevil is necessary to maximize the banana production. The present study investigated the effect of sub lethal toxicity of carbosulfan on the adult banana pseudostem weevil, *Odoiporus longicollis.*

The insects were collected from Annassery, Vellalassery, Feroke Chathamangalam, Nadakkavu, Kuttiady and Thottilpalam areas in Kozhikode district, Kerala. Biochemical assays were conducted using the chemical insecticide, carbosulfan. Glutathione -s- transferase (GST), Monooxygenase or mixed function oxidase (MFO) and Acetylcholinesterase (AChE) activities in banana pseudo stem weevil were studied. The adult insects were treated with 10%, 50% and 80% of lethal dose (LD_{50}) for 24 hr, 48 hr and 72 hr. Gut, reproductive organs, fat body and also the whole insect were examined for the enzyme analysis.

The GST activity was increased after the treatment of the insect with sub lethal concentration. This result shows that GSTs of the banana weevil are involved in the detoxification of carbosulfan. GST enzyme activity was dose dependent and time dependent. Compared to the three tissues used in the study, fat body showed an elevated GST enzyme activity over the others. It

may be inferred that the increased activity of GST in fat body is the indication of development of a defensive internal mechanism in the animal to cope with the reactive toxicant. The GST activity was reduced over different time interval. This shows that there is a reduction in detoxifying activity in insects over time.

The MFO activity is significantly increased in all tested samples compared to the control. In most of the samples the activity was high at 48 hr. Increased MFO levels in the entire treated sample suggest its role in the detoxification process in adult banana pseudostem weevil. Thus the detoxifying enzymes have an important role in the development of resistance in adult banana pseudostem weevil which enables the insect to adapt to high stress conditions induced by the insecticides.

Acetylcholinesterase (AChE) is the target of both organophosphate and carbamate insecticides. They degrade the enzyme necessary for the nerve impulse transmission leading to the death of the insect. Compared to other tissues, fat body showed the highest AChE activity suggesting the involvement of metabolic resistance mechanism in this insect population. The analysis of enzyme activity shows an increased detoxifying enzyme levels compared to controls in most of the samples indicating development of a resistance mechanism.

Fat body, reproductive organs, and gut of the banana pseudo stem weevil were tested for the presence of enzymes degrading the commonly used systemic insecticide, carbosulfan. Enzyme extracts prepared from the tissues were incubated for 60 min at different concentrations indicated the presence of enzymes that degraded carbosulfan into several products having different retention time (HPLC). From the results it appears that the tissues consists of enzymes that can degrade the insecticide carbosulfan. It was found that fat

body is better sources of detoxifying enzymes than the other tissues. Using the standard libraries the nature of the products formed should be identified.

The sublethal dose of carbosulfan insecticide damages to the gut and proventriculus were studied by histomorphological examination of the adult insect gut. Alimentary canal of insects is important defense barrier against the chemical insecticide. Any changes in the tissue structure indicate the toxicity. Mid gut is the region where the digestive enzyme action is more and absorption takes place.To increase the absorptive area there are numerous regenerative crypts in the gut region. The structural and functional role of proventriculus is important to understand its digestion physiology. There are eight prominent ridges on the proventriculus externally. The light microscopic studies and ultra-structural studies support the disintegration of tissues in the gut of adult weevil by application of sublethal dose of insecticide. The carbosulfan insecticide treatment resulted in excessive vacuolization, elongation of columnar cells, pyknotic nuclei formation and overlapped columnar cells of the gut tissue. On application of insecticide carbosulfan the regenerative crypts of mid gut was observed disintegrated and it also effect the circular and longitudinal muscle fibres. Ultra microscopic studies reveal that the regenerative crypts are disintegrated. The number and structure of the regenerative crypts are deviated from the normal sample tissue. The cytotoxicity of carbosulfan has affected the gut tissues and changed the histomorphology of cells.

The effects of xenobiotic exposure on the wing pattern of the adult banana weevil were investigated using the geometric morphometric method. The specimens were collected from two different fields like Site 1 viz, Vellalassery, Kozhikode district (area where carbosulfan is predominantly used as pest control agent and inter alia fipronil, chlorpyrifos etc.) and Site 2 viz, Chathamangalam, Kozhikode district (area where pesticides are not

used). 94 and 110 specimens were collected and examined from Site 1 & Site 2 respectively during the period from January to June 2017. For the study of the wing shape analysis about 18 land marks of hind wings were identified. Principal component analysis and canonical variate analysis were conducted. The cumulative percentage of variation of first three principal components was 62.163%. The CVA results showed that the different groups of male and female insect exhibits variation and asymmetry in hind wing, as the Mahalanobis and Procrustes distances for each group are significantly different ($p<.0001$). Based on the PCA and CVA studies it has become very clear that there exists a great variation in hind wing size and shape among and between the groups of insects examined from site 1& 2. On the basis of the analysis of the data of the hind wings, it is found that there is a significant variation in the hind wings of both the sexes and also between the insects collected from the polluted and unpolluted area. The significant P-value ($p<0.0001$) obtained in left and right hind wing analyses shows the variation and asymmetry between the contaminated and uncontaminated groups. The hind wings in beetles are used for the flight and the wing folding mechanisms are important for the beetles. The hind wing size and shape variation among and between the male and female insects indicate the effect of xenobiotics on the banana pseudostem weevil.

The investigation reveals that the exposure of adult banana pseudo stem weevil, *Odoiporus longicollis* Oliv. to carbosulfan induced excessive production of detoxifying enzymes which is a clear sign of the internal defense mechanisms working against the pesticide. The study also investigated the histomorphological changes caused by the exposure of carbosulfan to the selected tissues of banana pseudostem weevil apart from the pattern of changes in the wing morphology in the cases where field specimens collected from two different sites, area having pesticide application with carbosulfan as predominant and area without application of any

pesticides. The study cautions, though the use of carbosulfan is effective in controlling the dangerous pest, the prolonged usage of pesticide can eventually lead to the development of resistance as evidenced from the increased presence of detoxifying enzyme which is characteristic of the development of resistance mechanism in insects.

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