SUSCEPTIBILITY OF RED FLOUR BEETLE, *Tribolium castaneum* (Herbst) (COLEOPTERA: TENEBRIONIDAE) TO INSECTICIDES

By

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(2017-11-128)

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THESIS

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DEPARTMENT OF AGRICULTURAL ENTOMOLOGY

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VELLANIKKARA, THRISSUR – 680656

KERALA, INDIA

2019

DECLARATION

I hereby declare that this thesis entitled **"Susceptibility of red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) to insecticides"** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award of any degree, diploma, fellowship or other similar title, of any other University or Society.

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CERTIFICATE

Certified that this thesis entitled **"Susceptibility of red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) to insecticides"** is a bonafide record of research work done independently by **Ms. Anusree R. P. (2017-11-128)** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

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Anusree R P

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Introduction

1. INTRODUCTION

The world population is growing by approximately 83 million people every year. The current world population is 7.7 billion and it is projected to reach 9.8 billion by 2050 and 11.2 billion by 2100. An increasing demand on the production of cereals and other food grains occurs due to this inevitable population growth. Since the arable lands are diminishing, reducing yield loss is a way to increase the availability of food grains to the burgeoning humanity. Postharvest losses constitute a major form of yield loss and accounts for one-third of the food produced every year (Gustavsson *et al*. 2011). Cereal crops suffer about 19 per cent loss by weight and (Lipinski *et al*. 2013) and 53 per cent loss on the basis of calorific content.

About 50 – 60 per cent of the grains produced in our country are stored in traditional structures (Grover and Singh, 2013), the rest in Food Corporation of India (FCI) and Central Warehousing Corporation (CWC) godowns. Insect pests cause 30 – 40 per cent loss to the stored grains (Abass *et al*. 2014; Boxall, 2002; Tapondjou *et al*. 2002). Insect damage cause both quantitative and qualitative loss to the stored grains including loss of viability of seeds.

The red flour beetle, *Tribolium castaneum* (Coleoptera: Tenebrionidae) is an important worldwide stored grain pest, causing postharvest losses of > 20 per cent in developing countries and up to 9 per cent in developed countries (Phillips and Throne, 2010). Larvae and adults of red flour beetle are external feeders or secondary pests and thus attack damaged grains or farinaceous materials. They readily adapt to stored grain environment due to its high fecundity rate and relative longevity (Boyer *et al.* 2012).

Even though several practices are available for stored grain pest management, use of insecticides is the quickest, effective and economic method to reduce the insect pest populations to acceptable levels (White and Leesch, 1995; Harein and Davis, 1992; Perez-Mendoza, 1999). However, selection pressure by insecticides had led to the development of resistance to insecticides (Boyer *et al*. 2012).

Among the storage pests, *T. castaneum* has demonstrated propensity to develop resistance to all classes of insecticides and fumigants used for managing it (Richards *et al*. 2008). *Tribolium castaneum* ranks 17th among the 20 most insecticide resistant arthropods in the world (Whalon *et al.* 2015) and it has already developed resistance against phosphine, methyl bromide, organophosphates, pyrethroids and insect growth regulators, which are commonly used for its management (Champ and Dyte, 1976; Collins, 1998; Dhaliwal and Chawla, 1995; El-Lakwah *et al.* 1996; Horowitz *et al.* 1998; Pacheco *et al.* 1994; Werner, 1997; Zettler and Arthur, 1997; Pimental *et al.* 2007).

Malathion, deltamethrin and dichlorovos are the insecticides recommended for the management of stored grain pests in FCI godowns. However, development of insecticide resistance has made the management of these pests a difficult task. In 1971 itself 37.76-fold resistance to malathion was reported in *T. castaneum* in India by Bhatia *et al.* (1971). Subsequently, resistance to malathion (Rajak *et al*. 1973; Champ and Dyte, 1976; Pasalu and Bhatia, 1983; Joia and Chawla, 1988; Dhaliwal and Chawla, 1995; Srivastava *et al*. 2001) and dichlorvos (Saxena and Sinha, 1989; Saxena *et al*. 1992) was reported from different parts of our country.

Detoxifying enzymes play a major role in conferring resistance in insects to insecticides. Enzymes that are important for detoxification of insecticides in insects include esterases/carboxylesterases, glutathione-S-transferases and cytochrome P450 monooxygenases (Shakoori *et al.* 2000; Enayati *et al.* 2005; Liu *et al.* 2015). Esterases catalyse the hydrolysis of thio- and phospho- ester bonds in insecticides (Myers *et al.* 1988; Wheelock *et al.* 2008) and have been implicated in the detoxification of major insecticide groups such as organophosphates, carbamates and pyrethroids (Wheelock *et al*. 2005). Glutathione-S- transferases detoxify the insecticides by conjugating them with glutathione (Armstrong, 1997). Glutathione-S-transferase was found to be responsible for resistance to organochlorines, organophosphates and synthetic pyrethroids (Enayati *et al.* 2005). Cytochrome P450s play a major role in detoxification/activation of several insecticides and

overexpression of cytochrome P450 was detected in insects resistant to almost all groups of insecticides (Feyereisen, 2005).

A major management tactic to overcome insecticide resistance is to rotate pesticides with different modes of action and this strategy will work if there is no cross-resistance between the pesticides used in the rotation (Tabashnik, 1989; Sparks and Nauen, 2015). This involves the utilization of pesticide classes with different modes of action to minimize the selection for resistance (Sparks and Nauen, 2015).

Use of pesticides for the management of pest population can lead to yet another undesirable fallout in the form of presence of pesticide residues in food. Safety of the food consumed has become a major concern and the end-users (consumers) demand assurance about the safe use of the pesticides in food production. Contamination of food grains with pesticides can lead to the rejection of the same in trade. Hence, it is critical to develop and validate sensitive methods for the detection and quantification of pesticide residues even at very low concentrations (Tsochatzis *et al*. 2010).

Hence, the current study had been conducted with the following objectives.

1. Evaluation of susceptibility of *Tribolium castaneum* population to selected insecticides
2. Studies on the biochemical basis of insecticide resistance in *Tribolium castaneum*
3. Evaluation of new insecticide molecules against different populations of *Tribolium castaneum*
4. Estimation of residues of selected insecticides in rice grains

Review of literature

2. REVIEW OF LITERATURE

Red flour beetle *Tribolium castaneum* (Herbst.) (Coleoptera: Tenebrionidae) is an important worldwide pest of stored products. Both larvae and adults are secondary pests of grains, dried fruits, flour, *etc*. in storage. The damage caused by them to various food commodities is estimated to be 15 – 20 per cent of total food grain production (Khattak and Shafique, 1986).

The literature pertinent to the research programme has been critically reviewed and provided under the following headlines

a. Status of insecticide resistance in *Tribolium castaneum* with respect to malathion, deltamethrin and dichlorovos
b. Role of detoxification enzymes in imparting resistance to malathion, deltamethrin and dichlorovos in stored product pest
c. Susceptibility of stored product pests to bifenthrin, chlorfenapyr, spinosad and flubenidamide
d. Pesticide residue analysis in stored food grains

2.1 STATUS OF INSECTICIDE RESISTANCE IN *Tribolium castaneum*

Among the various measures for protection of stored grains, chemical control with insecticides is the most commonly adopted control measure. But, resistance to insecticides is a critical factor contributing to the failure of chemical control of stored grain pests. In India, organophosphorous pesticides malathion and dichlorovos and the synthetic pyrethroid, deltamethrin are recommended for the management of stored product insects. Wide spread use of these insecticides in Food Corporation of India (FCI) godowns had led to the development of resistance to these insecticides.

2.1.1. Resistance to Malathion in Red Flour Beetle, *Tribolium castaneum*

Seven strains of *Tribolium castaneum* collected from Nigeria exhibited resistance to malathion (Parkin, 1962). Resistance factor varied from 10-60 in various strains of *T. castaneum*.

Lemon (1966) studied relative susceptibilities of *T. castaneum* and *Tribolium confusum* to 16 organophosphorus insecticides. Insecticides were applied topically with 0.016, 0.0475, 0.145 and 0.43 μg of toxicant per insect. Six insecticides, fenthion, fenitrothion, imidan, dicapthon, dimethoate and chlorthion were better than malathion. Diazinon showed more effectiveness than malathion against *T. castaneum* but not against *T. confusum*.

Speirs *et al*. (1967) conducted a study on ten strains of red flour beetle, *T. castaneum* collected during 1963 to 1965 from storage godowns in Georgia and Florida, United States, where malathion had been used for a long period. One of the strains required 11.3 times as much malathion as did the susceptible standard laboratory strain. Insects without any exposure to malathion in storage godowns were nearly as susceptible as the laboratory strain.

Toppozada *et al.* (1969) exposed two local strains of *Sitophilus oryzae* and *T. castaneum* to insecticides treated papers or wheat to study the toxicity of insecticides. They found that lindane, malathion, dichlorovos and diazinon were more toxic than carbaryl, DDT and pyrethrins. *Tribolium castaneum* strains showed tolerance to lindane, whereas, *S. oryzae* was susceptible to lindane and malathion. *Sitophilus oryzae* was more susceptible to all tested insecticides than *T. castaneum*.

Dyte and Blackman (1970) conducted a study on malathion resistance in strains of *T. castaneum* from eleven countries using a discriminating dose bioassay. Malathion synergised with triphenyl phosphate was used to distinguish the malathion specific resistance from other types. Fifteen out of 17 strains of *T. castaneum* were resistant to malathion. Non-specific malathion resistance was detected in strains from Gambia, Kenya, Malawi, and Senegal and malathion-

specific resistance was detected in strains from England, The Gambia, Japan, Kenya, Malawi, Malaysia, Nigeria, Senegal and The Seychelles.

Champ and Campbell-Brown (1970) recorded malathion resistance in *T. castaneum* associated with peanuts and cereals in storage in eastern Australia and found a maximum resistance factor of 18.3. Those resistant strain from peanuts also showed tolerance to dichlorovos, fenitrothion, gardona, phoxim, cynox, abate, diazinon, dursban, hoe, carbaryl, arprocarb, promecarb, DDT, and lindane. They also recorded significant increase in tolerance to pyrethrins and synergised pyrethrins.

Fourty-eight samples of *T. castaneum* were collected from storage godowns and grain transport in New South Wales, Australia. Of this fourty-eight samples eight samples were resistant to malathion (Greening, 1970).

The first storage pest to develop resistance to malathion in India was *T. castaneum*, collected from Food Corporation of India godowns at Naraina, New Delhi (Bhatia *et al*. 1971). Naraina population of *T. castaneum* was 37.76-fold resistant to malathion, when compared to the susceptible strain maintained in the laboratory.

Pieterse *et al*. (1972) examined the susceptibility of seventeen strains of red flour beetle *T. castaneum* collected from warehouses and stores throughout Malawi to lindane and malathion. All strains were resistant to lindane, whereas, eleven strains were resistant to malathion. Cross-resistance to bromophos was exhibited by malathion resistant strains. Resistance in the eleven malathion resistant strains was of the non- specific type since it was not significantly affected by the synergist triphenyl phosphate.

Tribolium castaneum populations collected from Hapur and Meerut, Uttar Pradesh were having 16-21-fold resistance to malathion (Rajak *et al*. 1973).

Resistance to malathion was examined in six strains of red flour beetle from stored peanuts in Georgia and Alabama. Range of resistance ratio was 9.6 to 108.7 at LD_{50} and 19.2 to 89.7 at LD_{95}. Slopes of the dosage mortality regression lines

revealed that three strains were less heterogeneous and three strains were more heterogeneous for resistance than the susceptible laboratory strain (Zettler, 1974).

Wide spread resistance to insecticides in *T. castaneum* was reported by Champ and Dyte (1976) after a global survey. The survey found *T. castaneum* populations from Maharashtra, Rajasthan, Uttar Pradesh and Delhi as resistant to malathion.

Field populations *T. castaneum* in North Carolina were examined in the laboratory to determine the extent of resistance to malathion. Forty-four-fold resistance to malathion was exhibited by field populations in comparison to a laboratory maintained population. Since triphenyl phosphate (TPP) suppressed resistance in the population, it was categorised as malathion specific type resistance. Cross-resistance was not exhibited by malathion resistant strains towards four other organophosphorus insecticides such as pirimiphos-methyl, Ciba-Geigy CGA-20168, bromophos and fenetrothion (Bansode and Campbell, 1979).

Susceptibility to malathion, dichlorovos and pirimiphos- methyl were studied in field strains of Indian meal moth, *Plodia interpunctella*, the almond moth, *Ephestia cautella*, and the red flour beetle *T. castaneum,* collected from peanut and oilseed storage facilities throughout the South-eastern United States by Zettler (1982). All strains except one showed extremely high resistance to malathion. None of the strains showed resistance to dichlorovos and no significant cross resistance to pirimiphos-methyl was detected.

Different strains of *T. castaneum* from grain bins in 14 grain producing states of United States were screened for resistance to malathion by discriminating dose technique (Haliscak and Beeman, 1983). Measurable tolerance to malathion was showed by 31 out of 36 strains of *T. castaneum.*

Seventy-three-fold resistance to malathion was found in a strain of *T. castaneum* from Georgia and it was highly cross resistant to phenthoate. Resistance to malathion was inherited as a simple autosomal semi dominant trait. The strain did not show any cross resistance to structurally dissimilar carboxylate esters,

organophosphates, carbamates, chlorinated hydrocarbons, or pyrethroids (Beeman, 1983).

Responses of field strains of *T. castaneum, Rhyzopertha dominica, Oryzaephilus surinamensis, S. oryzae* and *Sitophilus zeamais* from New Caledonia to lindane, malathion and fenitrothion was studied by Brun and Attia (1983). Five out of seven samples of *T. castaneum* showed resistance to lindane and malathion. All the resistant strains were susceptible to malathion synergised with TPP, which indicated malathion specific resistance in *T. castaneum*. Strains of *R. dominica* were resistant to malathion and fenitrothion, but susceptible to lindane. Strains of *O. surinamensis* were resistant to all the three insecticides, whereas, strains of *Sitophilus* spp. were susceptible to them.

Field collected malathion resistant strains of *T. castaneum* was used by Pasalu and Bhatia (1983) to study the inheritance of resistance to malathion by making genetic crosses between the resistant and a susceptible strain. The study revealed that resistance in this strain was controlled by an autosomally inherited single major gene which is incompletely dominant.

A comprehensive survey from 1974-1982 in the grain storage facilities of New South Wales, Australia was carried out by Attia (1983). The survey revealed the incidence of multiple-organophosphorous (OP) resistant strains of *T. castaneum, T. confusum, R. dominica, O. surinamensis, P. interpunctella, E. kuehniella* and *E. cautella*. Except *O. surinamensis*, all the strains of other pests showed high levels of malathion resistance.

South Carolina strains of *T. castaneum* was used for dose discriminating laboratory tests for detection of malathion resistance by Horton (1984). Sixteen strains, out of 29, were resistant to malathion. Same experiment was conducted using *T. confusum* and only one strain showed malathion resistance. Resistant strains of *T. castaneum* showed malathion specific resistance since they were 100 per cent susceptible to the malathion – TPP treatments. All these strains were susceptible to pirimiphos-methyl within three hours after exposure.

Multiple and cross resistance characteristics of phosphine resistant *T. castaneum* was studied by Attia (1984). Phosphine resistant *T. castaneum* was resistant to malathion (77.9-fold) as well as lindane (5.2-fold).

Resistance to malathion and lindane and effectiveness of pirimiphos-methyl, fenitrothion, etrimfos, permethrin and deltamethrin were studied in populations of *T. castaneum, T. confusum, S. zeamais, Callosobruchus maculatus* and *Zabrotes subfasciatus* from Uganda. All the populations were resistant to malathion or lindane and some were resistant to both. Most effective among those insecticides was deltamethrin and least effective was permethrin (Evans, 1985).

Six field collected strains of *T. castaneum* showed 3.6 to 111-fold resistance to malathion (Beeman and Nanis, 1986). Navarro *et al.* (1986) reported that, resistance factor calculated from the LC_{50} values of different insect species against malathion were very high. Among the different species tested, *T. castaneum* had the highest resistant factor of 538.0, which was followed by *O. surinamensis* (x8.0), *S. oryzae* (x1.2) and *Rhyzopertha dominica* (x0.9).

A *T. castaneum* strain, resistant to malathion, was selected after 15 generations by White and Bell (1988). The resistance was malathion specific, autosomal, semidominant, monofactorial and TPP suppressible.

Halliday *et al*. (1988) examined the susceptibility of red flour beetles collected from peanut storage warehouses and processing facilities throughout Georgia and Alabama against three commonly used insecticides and two insecticides, whose registration is pending, by discriminating dose procedure. Malathion resistance was detection in 13 out of 15 strains with a survival rate of more than 90 per cent. Half of the strains were resistant to dichlorovos. All strains were susceptible to chlorpyrifos-methyl. One strain was resistant to pirimiphos-methyl which also showed high resistance to dichlorovos, revealing the cross resistance between dichlorovos and pirimiphos-methyl.

Organophosphate resistance was examined in four strains of red flour beetle, *T. castaneum,* and six strains of saw toothed grain beetles, *O. surinamensis* infesting

barley in Minnesota by using malathion, pirimiphos-methyl and chlorpyriphos-methyl. All strains of *T. castaneum* showed resistance to malathion but not by *O. surinamensis*. No cross resistance to pirimiphos-methyl or chlorpyriphos-methyl was exhibited by *T. castaneum.* Four strains of *O. surinamensis* were slightly tolerant to chlorpyriphos-methyl whereas all strains were susceptible to pirimiphos-methyl (Subramanyam *et al.* 1989).

Herron (1990) collected stored grain pests such as *T. castaneum, T. confusum, S. oryzae, Sitophilus granarius, O. surinamensis* and *R. dominica* from farms of southern and northern New South Wales and bioassayed, to test the resistance against malathion, fenitrothion, bioresmethrin, carbaryl, pirimiphos-methyl, chlorpyriphos-methyl and the fumigant phosphine. Fifty per cent populations of *O. surinamensis* were resistant to fenitrothion. Seventy per cent of *O. surinamensis* population and one population of *T. castaneum* were found to be resistant to pirimiphos-methyl. Resistance to chlorpyriphos-methyl was obtained in 39 per cent *O. surinamensis* populations. All species, except *S. granarius* showed resistance to malathion. All species exhibited low level of phosphine resistance.

Resistance to malathion was studied in eight strains of red flour beetle, *T. castaneum* collected from wheat stored in Oklahoma farms (Zettler and Cuperus, 1990). All strains showed resistance against malathion when compared with susceptible strain from Savannah laboratory. More intensive selection pressure with malathion in Oklahoma resulted in higher frequencies of malathion resistance in *T. castaneum*.

Twenty populations of *S. oryzae*, ten of *S. zeamais*, twenty of *R. dominica* and twenty-five of *T. castaneum* collected from storage facilities located in different regions of Brazil were tested for resistance to three insecticides *viz.,* malathion, pirimiphos-methyl and fenetrothion. Seven populations of *S. oryzae*, six of *R. dominica* and ten of *S. zeamais* were susceptible to all the three insecticides. Thirteen populations of *S. oryzae,* fourteen of *R. dominica* and all the twenty-five populations of *T. castaneum* were resistant to malathion. One population of *S. oryzae* and eight of *T. castaneum* exhibited cross resistance to pirimiphos-methyl

and fenitrothion. Two populations of *S. oryzae*, one of *R. dominica* and one of *T. castaneum* indicated cross resistance only to pirimiphos-methyl and two of *R. dominica* and two of *T. castaneum* only to fenitrothion (Pacheco *et al*. 1990).

All the *T. castaneum* strains collected from various locations of Kansas in 1987 were strongly resistant to malathion (Beeman and Wright, 1990). But the resistant strains were susceptible to chlorpyriphos-methyl and pirimiphos-methyl.

Exposing nine field strains and a laboratory susceptible strain of *T. confusum* to malathion residues on galvanized steel and plywood panels resulted in the control of a laboratory susceptible strain for 5 weeks. Three susceptible field strains were controlled for 3 weeks and the six resistant field strains were controlled for a period of 1 week only (Arthur and Zettler, 1991a).

When malathion resistant field strains and a malathion susceptible strain of *T. castaneum* was exposed to malathion treated galvanized steel and plywood surfaces, three field strains were highly resistant to surface residues of malathion (Arthur and Zettler, 1991b)

Field strains of red flour beetle were collected from flour mills of United States. Out of 28 strains collected, 93 per cent were resistant to malathion. At least 80 per cent of malathion resistance frequencies was detected in half of the strains and only two strains were susceptible to malathion (Zettler, 1991).

During 1991-92 fourty-three populations of *T. castaneum* was sampled from various types of storage premises representing different agro-climatic regions of Punjab. Among the collected populations, 56 per cent was resistant to malathion (Dhaliwal and Chawla, 1995).

Resistance to malathion was studied by Zettler and Arthur (1997) by collecting adults of 14 field strains of red flour beetle, *T. castaneum* from flour mills and found that all strains were resistant to malathion. The most resistant strain showed an LD_{50} of 104000 ppm.

Srivastava *et al*. (2001) carried out a survey throughout the country and malathion resistance was studied in thirteen populations of *T. castaneum* collected from National Seed Programme centres. Of the thirteen populations studied, eleven strains were resistant to malathion and the resistance level varied from 0.725 to 24.53 in the resistant populations of *T. castaneum*.

A study was conducted by Rahman *et al*. (2007) to examine the susceptibility of field strains of red flour beetle, *T. castaneum* collected from eight storage depots and one silo in Bangladesh to insecticides such as malathion, dichlorovos, fenitrothion, pirimiphos-methyl and phosphine. Results showed that all strains were resistant to all the insecticides tested. Among them, resistance was highest against malathion (18-fold) followed by dichlorovos, phosphine, pirimiphos-methyl and fenitrothion.

Assie *et al.* (2007) investigated the effect of population density on malathion specific resistance in *Tribolium castaneum*. A ten- fold increase in malathion resistance was achieved by low–density line after completing 33 generations whereas high–density line required only 17 generations. Malathion susceptible strain did not show any significant change in LC_{50} during selection.

Andric *et al*. (2010) examined the susceptibility of different populations of red flour beetle *T. castaneum* from Serbia to various contact insecticides and found that malathion was the least toxic among the insecticides evaluated.

2.1.2 Resistance to Dichlorovos in Red Flour Beetle, *Tribolium castaneum*

Ninety-five per cent mortality of confused flour beetle *T. confusum* was obtained with dichlorovos concentrations of 4.0, 2.7 and 1.9 µg/ l after 4, 8 and 12 hours of exposure, respectively (Harein *et al*. 1970).

Zettler and Jones (1977) reported that pirimiphos- methyl and dichlorovos as more toxic to malation resistant strains of *T. castaneum*. LD_{50} of dichlorovos in

malathion susceptible stain of *T. castaneum* was 0.051 mg/g of insect body, whereas it was 0.048 mg/ g of insect body in resistant strains.

Sixteen populations of *T. castaneum* collected from peanut storage and processing facilities located in Alabama and Georgia were tested for its resistance to dichlorovos (Halliday *et al.* 1988). Half of the strains collected showed resistance to dichlorovos and survival of *T. castaneum* was more in Dothan strain.

Dichlorovos resistance was studied by Saxena and Sinha (1989) in 13 samples of *T. castaneum* collected from different FCI godowns. Resistance to dichlorovos was exhibited by nine strains with resistance ratio ranging from 3.56 to 10.51.

Dichlorovos resistant strain of *T. castaneum* was developed through laboratory selection by Sinha and Chauhan (2004). The increase in resistance factor, based on LC_{50} values were x2.43, x3.51, x4.65, x6.31, x6.48, x7.82, x9.67, x10.58, x10.82 and x10.87 in each of ten successive generations.

Nine strains of *T. castaneum* was collected from different locations of Bangladesh and evaluated for their resistance to commonly used insecticides such as malathion, dichlorovos, fenitrothion, pirimiphos-methyl and phosphine. All the strains showed resistance to dichlorovos and the resistance factor ranged from 1.78 to 3.45 (Rahman *et al*. 2007).

Adults of the confused flour beetle, *T confusum* and pupae of the red flour beetle, *T. castaneum* were exposed to dichlorovos at a rate of 0.35 g/m^3 in Kansas State University pilot flour mill in open, obstructed and concealed mill locations. Mortality of *T. castaneum* pupae was 97 – 100 per cent and knock down mortality of *T. confusum* adults was 99 – 100 per cent in open and obstructed mill locations. However, it was 85 – 94 per cent in concealed condition for both adults and pupae (Subramanyam *et al*. 2014).

2.1.3 Resistance to Deltamethrin in Red Flour Beetle, *Tribolium castaneum*

Collins (1990) examined resistance of two strains of *T castaneum* to pyrethroids by using various insecticides. Both the strains showed 19 and 950-fold resistance to deltamethrin. Suppression of the resistance was achieved by piperonyl butoxide, which indicated that the major resistance mechanism was microsomal oxidation.

Daglish *et al*. (1992) reported that deltamethrin was highly effective against *T castaneum* and 96.60 per cent mortality was obtained after 14 days of exposure on peanuts treated with 5 mg/ kg of deltamethrin. However, malathion could not cause mortality of *T. castaneum* even at a dose of 50 mg/kg.

A strain of *T. castaneum* resistant to deltamethrin was obtained after six generations of selection in laboratory. Selection was initiated at 6 ppm and reached 200 ppm after six generations (Padhee *et al*. 2002).

Singh *et al.* (2003) studied the rate of resistance development to deltamethrin by laboratory selection in *T. castaneum*. The levels of resistance obtained after I, II, III, IV, V and VI generations were 2.651, 3.873, 8.969, 16.292, 48.782 and 72.272 - folds, respectively.

Populations of *T. castaneum* and *T. confusum* collected from grain and food storage facilities of 18 different locations of Italy were subjected to insecticide resistance study by Rossi *et al.* (2010). Seven populations of red flour beetle and eleven populations of confused flour beetle were used in bioassays with six contact insecticides. Confused flour beetle from Molise, assayed with deltamethrin gave highest resistance ratio. Susceptibility of *T. castaneum* populations to malathion, diazinon and pyrethrin was high compared to that of *T. confusum* populations. Resistance ratio obtained in bioassay indicated that insecticide resistance was not a wide spread problem in Italian strains of *T. castaneum* and *T. confusum*.

Vojoudi *et al*. (2012) evaluated the efficacy of chlorpyrifos, abamectin and deltamethrin against red flour beetle *T. castaneum* adults on different surface substrates such as glass, ceramic tile, plastic and paper discs. The results revealed that chlorpyrifos was the most toxic and deltamethrin was the least toxic chemical against *T. castaneum*. All the tested insecticides were more effective on glass surface followed by ceramic tile, plastic and paper discs.

Development of resistance in *T. castaneum* towards deltamethrin was achieved by Singh and Prakash (2013) after six generations of selection from a laboratory susceptible strain through topical application method. The selection started at the dose of 0.0004 per cent, which was increased during successive generations and reached up to 0.026 per cent in the sixth generation. The resistance ratio of selected strain was found to be 370.5-fold in sixth generation, as compared to the susceptible strain.

Velki *et al*. (2014) studied toxicity and repellency of dimethoate, pirimiphos-methyl and deltamethrin on adult red flour beetle, *T. castaneum*. Based on LC_{50} values, toxicity was the highest for pirimiphos-methyl, followed by dimethoate and the least for deltamethrin.

Sehgal and Subramanyam (2014) evaluated the efficacy of suspension concentrate formulation of deltamethrin on concrete surfaces and hard red winter wheat against 12 field strains of red flour beetle, *T. castaneum*; six strains of saw toothed grain beetle, *O. surinamensis*; and four strains of lesser grain borer, *R. dominica*. Deltamethrin failed to provide complete mortality of adults of *T. castaneum* and *O. surinamensis* field strains on concrete, but was effective against *R. dominica* strains. Fourteenth day mortality of six *T. castaneum* field strains, three *O. surinamensis* strains and one *R. dominica* strain were significantly lower than that of the corresponding laboratory strains on wheat treated with 0.5 mg (AI) / kg of deltamethrin.

Efficacy of deltamethrin was studied in adults of lesser grain borer, *R. dominica*; granary weevil, *S. granarius*; red flour beetle, *T. castaneum*; rice weevil,

S. oryzae; maize weevil, *S. zeamais* and warehouse beetle, *Trogoderma granarium* by exposing insects to insecticide treated brown rice for one, four, eight and 24 hours. Progeny production was assessed by rearing these insects on treated brown rice mixed with varying amounts of untreated rice. Immediate and delayed mortality of exposed adults did not cross seven per cent at any exposure interval. However, progeny production of *T. castaneum* was lower in the mixture of untreated rice with treated rice (Kavallieratos *et al*. 2015).

Susceptibility of four different field populations of red flour beetle, *T. castaneum* collected from Faisalabad, Jhang, Sahiwal and Sargodha were evaluated by bioassay using deltamethrin, permethrin and spinosad. Strains from Faisalabad, Jhang and Sahiwal exhibited higher tolerance ratio of 26.5-fold, 21–fold and 18.6–fold, respectively, in comparison with Sargodha strain against deltamethrin. Faisalabad strain showed 9.5-fold tolerance ratio against permethrin, whereas all strains were susceptible to spinosad (Riaz *et al*. 2018).

2.2 ROLE OF DETOXIFICATION ENZYMES IN IMPARTING RESISTANCE TO MALATHION, DELTAMETHRIN AND DICHLOROVOS IN STORAGE PESTS

Enzymes are involved in the detoxification of insecticides, enabling insects to evolve resistance to insecticides. Esterases, monooxygenases and glutathione-S-transferases are the major enzyme groups imparting insecticide resistance in insects.

2.2.1 Esterase Mediated Resistance

Most of the commonly used insecticides such as synthetic pyrethroids, carbamates and organophosphates are esters. Esterases hydrolyse these ester bonds leading to decrease in toxicity of insecticides. Esterases that hydrolyse carboxylic acid esters are called as carboxylesterases and they are the primary group of esterases in insects.

Dyte and Rowlands (1968) studied the resistance mechanism in a malathion resistant strain of *T. castaneum*. They inferred that the resistance mechanism was

due to enhanced detoxification by carboxylesterases. Similar to this study, Greening (1970) also found out that malathion resistance in *T. castaneum* was due to carboxylesterase.

The study conducted by Beeman *et al.* (1982) showed that, carboxylesterase activity was higher in six resistant strains of *Poldia interpuctella* from North – Central United States than susceptible laboratory strain. Major hydrolysis product was α – monoacid in all resistant strains which indicated the presence of carboxyl esterase - type malathion – specific resistance in Indian meal moth.

Beeman and Schmidt (1982) reported that malathion specific resistance in Indian meal moth could be suppressed by nontoxic carboxylestearse inhibitors. The carboxylesterase activity in a malathion resistant strain, *Kano*, was double than that of the susceptible *bb* strain (Wool *et al*. 1982).

Collins *et al*. (1992) reported that increased level of esterase activity was responsible for resistance to chlorpyriphos-methyl in *O. surinamensis.*

Elevated levels of esterase caused malathion and fenitrothion resistance in a multi–organophosphorus resistant strain of saw-toothed grain beetle, *O. surinamensis*. Ten-fold higher esterase activity was exhibited by resistant strain than susceptible strain. Along with that, V_{max} of the enzyme from resistant strain was 7-fold higher than that from susceptible laboratory strain. Evidence of elevated glutathione-S- transferase and cytochrome P450 were lacking in the resistant strain (Conyers *et al*. 1998).

Fifty-fold resistance to malathion was detected in field collected Mexican population of *R. dominica* compared to a susceptible laboratory population. Biochemical studies proved that esterase activity was 1.3-fold higher in the Mexican population than the laboratory population. Cytochrome P450 and glutathione-S- transferase were not responsible for the increased resistance in Mexican population (Guedes and Zhu, 1998).

According to Shakoori *et al*. (2000), carboxylesterase activity was highest among different esterases in all strains of *R. dominica* collected from different parts

of Pakistan. The carboxylesterase activity of Lahore (352%), Sialkot (198%) and Chichawatni (214%) were significantly higher than susceptible Karachi strain.

A malathion resistant *T. castaneum* strain (MN61) had high activity of esterase isozyme, EST2. Addition of TPP to malathion synergised the effect of pesticide, which indicated that resistance to malathion was mediated by carboxylesterase (Wool and Front, 2001).

Higher carboxylesterase activity was found in malathion and chlorpyriphos-methyl resistant strains of saw-toothed beetle, *O. surinamensis* compared to that of susceptible strain (Lee and Lees, 2001).

Haubruge *et al*. (2002) reported that, 44-fold increase in malathion carboxylesterase activity (MCE) relative to a susceptible strain resulted in specific resistance to malathion in a strain of *T. castaneum*. Purified carboxylesterase from susceptible and resistant strains had a similar molecular weight of 62000 Da.

Four field collected populations of *S. zeamais* had reduced susceptibility to cypermethrin. Bioassays with synergists like piperonyl butoxide, diethyl maleate and TPP indicated that cypermethrin resistance was mediated by esterases (Ribeiro *et al*. 2003).

2.2.2 Glutathione-S- transferases

Glutathione-S-transferases are the enzymes found in aerobic organisms which play a key role in detoxification of both endogenous and xenobiotic compounds such as insecticides. They are also involved in intracellular transport, biosynthesis of hormones and protection against oxidative stress (Enayati *et al.* 2005)

According to Reidy *et al*. (1990) resistance in *T. castaneum* against cyfluthrin, fenitrothion and malathion was associated with increased levels of glutathione-S- transferase activity. Glutathione-S-transferase activity was approximately four to six-fold higher in resistant strains than susceptible strains.

Fragoso *et al.* (2003) reported that there was more than 2–fold increase in glutathione-S- transferase activity in pyrethroid resistant strain of maize weevil, *S. zeamais*, than the susceptible strain. Those strains were resistant to cypermethrin, deltamethrin and permethrin.

Studies conducted by Dou *et al.* (2006) revealed that, specific activity of GST was significantly higher in dichlorovos and phosphine resistant strains of *Liposcelis bostrychophila*, compared to their susceptible counterparts. Dichlorovos resistant strain showed higher specific activity of GST than phosphine resistant one.

Elevated GST activity was exhibited by pyrethroid – resistant strain of *S. oryzae* compared to susceptible strain. K_m and V_{max} values of GST in resistant strain were two-fold higher than the susceptible population (Fragoso *et al*. 2007).

Dou *et al*. (2009) conducted a similar study in *L. bostrychophila* and found that specific activities of GSTs purified from dichlorovos and phosphine resistant strains were higher than the susceptible strain. Significantly higher affinity to the substrate GSH was shown by GSTs of both resistant strains.

2.2.3 Cytochrome P450 monooxygenases

Cytochrome P450 monooxygenases are important enzymes involved in detoxification and/or activation of xenobiotics such as pesticides. In insects, they play a major role in detoxification of insecticides leading to insecticide resistance (Liu *et al.* 2015).

Cohen (1982) studied the activity of cytochrome P450 in insecticide resistant and susceptible strains of *T. castaneum*. It was found that levels of cytochrome P450 were 4-fold and 3-fold higher in larvae and adults of resistant strains in comparison with susceptible strain.

A similar study was conducted by Rose and Wallbank (1986) in susceptible and fenitrothion resistant strains of saw-toothed beetle *O. surinamensis*. Cytochrome P450 levels were 3 and 10-fold higher in resistant larvae and adults, respectively, compared with the susceptible population.

Collins *et al.* (1992) reported that four fenitrothion resistant strains of *O. surinamensis* exhibited seven to 8.5 times higher levels of cytochrome P450 than the reference strain. Seven to eleven times enhanced activity of cytochrome P450 was observed in organophosphate resistant strain of *O. surinamensis.* Strain with the highest resistance to fenitrothion showed the highest enzymatic activity (Kotze and Wallbank, 1996).

Cytochrome P450 mediated detoxification was identified as the major mechanism of deltamethrin resistance in QTC279 strain of *T. castaneum* (Zhu *et. al*., 2010). *CYP6BQ9* gene was identified as the gene responsible for deltamethrin resistance. The gene *CYP6BQ9* is predominantly expressed in the central nervous system, which contains the voltage gated sodium channels, the site of action of deltamethrin.

2.3 SUSCEPTIBILITY OF STORED GRAIN PESTS TO BIFENTHRIN, CHLORFENAPYR, SPINOSAD AND FLUBENIDAMIDE

A mechanism to combat insecticide resistance in insects is to rotate insecticides with different mode of action. The present study evaluated the effectiveness of bifenthrin, chlorfenapyr, spinosad and flubenidamide to different strains of *T. castaneum.*

2.3.1 Susceptibility of Stored Product Pests to Bifenthrin

Bifenthrin is a synthetic pyrethroid insecticide acting on voltage gated sodium channels. It's a non-alpha cyano pyrethroid insecticide widely used for the management of agricultural pests.

Emulsifiable concentrate and wettable powder formulations of bifenthrin were more toxic to a susceptible strain of *T. castaneum,* when compared to malathion and dichlorovos, by film residue method of bioassay (Reddy, 2002). The LC_{50} values of malathion, dichlorovos and bifenthrin were, 0.00503, 0.01777 and

0.00112 per cent, respectively. Deltamethrin EC was more toxic than both EC and WP formulations of bifenthrin.

Daglish and Wallbank (2003) reported that, effective control of *R. dominica* could be obtained with bifenthrin (0.5mg/ kg) + piperonyl butoxide (7mg/ kg) in combination with either chlorpyriphos-methyl or fenitrothion. Complete, or almost complete control of a range of other beetle pests could be achieved by combining bifenthrin + piperonyl butoxide with either chlorpyriphos-methyl (10mg/ kg) or fenitrothion (12mg/ kg).

Combination of bifenthrin (0.5 mg/ kg) + piperonyl butoxide (7mg / kg) + chlorpyriphos-methyl (10 mg/ kg) was highly effective against beetle and psocid pests of sorghum up to seven months of storage. Complete mortality was obtained in seven out of 13 strains of beetles and psocids tested. Three out of four strains of *R. dominica*, one strain each of *C. ferrugineus* and *L. bostrychophila* and two strains of *L. decolor* were the susceptible ones (Daglish *et al*. 2003).

Toxicity of emulsifiable concentrate (EC) and wettable powder (WP) formulations of bifenthrin against susceptible and phosphine resistant strains of *T. castaneum* was evaluated by Pathrose *et al.* (2005) by film residue and direct spray methods of bioassay. Bifenthrin EC in both the strains and bifenthrin WP in susceptible strain were significantly more toxic to *T. castaneum* as film residue than as direct spray.

Bioefficacy of bifenthrin as a prophylactic spray against four major stored grain pests was studied by Reddy and Swamy (2006). LD_{50} values of bifenthrin were 3.389, 46.65, 4.509 and 5.573 when treated on concrete slabs and 4.629, 48.0, 5.056 and 6.042 when treated on jute strips for *S. oryzae*, *T. castaneum*, *T. granarium* and *R. dominica*, respectively.

Alleoni and Ferreira (2006) studied the potential of bifenthrin against *S. zeamais* and *S. oryzae* by exposing them to corn treated with 16 ml commercial product/ ton (0.4 ppm) of bifenthrin. It was found that bifenthrin was efficient to

control the adults of the two weevils up to 150 days after treatment and young forms of *S. zeamais* and *S. oryzae* up to 180 and 120 days after treatment, respectively.

Studies conducted by Kljajic *et al*. (2006) revealed that bifenthrin was highly effective against adults of three *Sitophilus* species. Calculated LD_{50} values after six, 24 and 48 hours of exposure on filter paper impregnated with insecticide were 4.83, 1.73 and 0.96 µg/ cm^2, respectively, in *S. granarius*. In case of *S. oryzae*, LD_{50} values were 10.76, 4.07 and 2.67 µg/cm^2 and for *S. zeamais* LD_{50} values were 9.26, 4.85 and 3.15 µg/ cm^2, after six, 24 and 48 hours of exposure, respectively.

2.3.2 Susceptibility of Stored Product Pests to Chlorfenapyr

Chlorfenapyr is a broad spectrum insecticide and acaricide, which has contact and stomach action. Chlorfenapyr belongs to pyrrole group of insecticide, which are uncouplers of oxidative phosphorylation, thereby affecting ATP synthesis.

Arthur (2008) studied the efficacy of chlorfenapyr against adults of *T. castaneum* and *T. confusum* exposed on concrete, vinyl tile, and plywood surfaces at a rate of 1.1 g AIm^{-2}. Insects were held without food for seven days after an exposure period of two and four hours. Even though beetles survived initial exposure, survivability decreased during the seven-day holding period. *T. confusum* was more susceptible than *T. castaneum* and none of the *T. confusum* exposed to concrete and tile survived after fourth and fifth day, respectively. Survival rate of beetles was more on concrete than tile or plywood.

It was reported that mortality of *T. castaneum* after six days of exposure on partially treated (14.4 per cent of total area) concrete arena with 1.1 g a.i./m^2 of chloefenapyr was 60.0 ± 10.6 per cent (Arthur and Fontenot, 2012).

Arthur (2013) reported complete mortality of *T. castaneum* and *T. confusum* after 24 hours of exposure on concrete treated with chlorfenapyr at the rate of 1.1 g a.i/ m^2. No progeny was produced in post treatment period of zero to eight weeks.

Chlorfenapyr, alpha cypermethrin, deltamethrin and pirimiphos-methyl were applied on different storage bags such as woven polypropylene, biaxially oriented polypropylene and kraft paper bags to control adults and larvae of *T. granarium*. While considering the immediate mortality after one, three and five days of exposure, chlorfenapyr and pirimiphos-methyl were the most effective insecticides against both life stages of *T. ganarium* with > 90 per cent mortality after fifth day of exposure (Kavallieratos and Boukouvala, 2018).

2.3.3 Susceptibility of Stored Product Pests to Spinosad

Aerobic fermentation of soil actinomycete *Saccharopolyspora spinosa* leads to the formation of natural products called as spinosyns. Spinosad is a mixture of spinosyn A and D.

Adults of *R. dominica, S. oryzae* and *T. castaneum* were used to study the contact toxicity of spinosad at an exposure period of 24 or 48 h on treated glass Petri dishes. Based on LD_{50} values, *R. dominica* adults were 462 and 192 times more susceptible to spinosad than *T. castaneum* and *S. oryzae*, respectively and *S. oryzae* adults were two times susceptible than *T. castaneum* (Toews and Subramanyam, 2003).

Toews *et al.* (2003) studied the knockdown and mortality of eight species of stored product beetles exposed to different surfaces treated with spinosad. Mortality of all species exposed to spinosad was 99 – 100 per cent, except for *Tribolium* spp.

Similar experiment was conducted against resistant beetle and psocid pests of stored grains in Australia by Nayak *et al*. (2005). Hundred per cent adult mortality and progeny reduction was obtained with *R. dominica* after 14 days of exposure at 1 mg (a.i.) / kg of spinosad. However, spinosad was less effective against *S. oryzae, T. castaneum* and *O. surinamensis*. The psocid, *Liposcelis entomophila* was completely susceptible to spinosad after 28 days of exposure at 1 mg (a.i.) / kg and 92 per cent progeny reduction was observed after 14 days of exposure. Spinosad exhibited moderate effectiveness against *Liposcelis bostrychophila, L. decolor and L. paeta*.

Subramanyam (2006) observed that, lesser grain borer, *R. dominica*, was highly susceptible to spinosad at 1 mg/kg of grain. Whereas, spinosad was not effective in killing adults of saw-toothed grain beetle, *O. surinamensis* and red flour beetle, *T. castaneum*. Susceptibility was exhibited by neonates of these two species to spinosad at 1 mg/ kg.

The study conducted by Huang *et al.* (2007) revealed that, complete mortality of *R. dominica, C. ferrugineus, S. oryzae* and *S. zeamais* could be obtained after 14 days of exposure on hard white winter wheat treated with 1 mg (AI)/ kg of spinosad. More than 94 per cent mortality was exhibited by *T. castaneum* and *T. confusum* in the same experiment.

Efficacy of spinosad as a grain protectant was evaluated by Subramanyam *et al*. (2007) in three Kansas farms. Insect population in newly harvested hard red winter wheat, that was applied with spinosad at 1 mg (a.i.)/kg of grain, was compared with that of untreated grains. Very low densities (≤ 3 live adults/kg of sample) of red flour beetle, *T. castaneum*; rusty grain beetle, *C. ferrugineus*; and saw-toothed grain beetle, *O. surinamensis* were found in the treated grains whereas there were no live adults of lesser grain borer, *R. dominica* in the treated grains.

The study conducted by Nikpay (2007) revealed that, adult mortalities of *R. dominica, S. oryzae* and *O. surinamensis* were ≥ 90 per cent after seven days of exposure on maize, wheat and sorghum treated with spinosad at the rate of 1 mg (a. i.)/kg. Whereas *T. castaneum* was less susceptible and adult mortality was < 13 per cent on spinosad treated sorghum and maize and 65 per cent on treated wheat

Huang and Subramanyam (2007) reported that > 98 per cent adult mortality was observed in *C. ferrugineus, R. dominica, O. surinamensis*, *S. oryzae* and *S. zeamais* after 12 days of exposure on spinosad treated corn at 1 and 2 mg a.i./kg. Egg–to–larval survival and egg–to–adult emergence of *Plodia interpunctella* were completely suppressed upon treatment with spinosad at ≥ 0.5 mg/ kg. However, survivability of *T. castaneum* adult was 16 per cent at 1 mg/ kg after 12 days

A similar study was conducted by Athanassiou *et al*. (2008a) and reported an adult mortality range of *S. oryzae* between 61 and 98 per cent after 14 days of exposure on wheat treated with 50 ppm spinosad dust formulation. Adult mortality of *R. dominica* after 14 days of exposure on grains treated with 50 ppm spinosad ranged between 91 and 100 per cent.

Effectiveness of spinosad was also studied against different populations of confused flour beetle, *T. confusum* obtained from Greece, Italy, Portugal, Denmark, Germany and France. Adults and larvae of *T. confusum* were exposed on wheat treated with dust formulations of spinosad at 0.06 and 0.19 ppm. Population from Greece exhibited the highest tolerance against spinosad whereas populations of Germany and Denmark showed the least tolerance. Mortalities of adults of the Greek, German and Danish populations were 2, 25 and 62 per cent, respectively after 7 days of exposure on wheat treated with 0.06 ppm spinosad. However, it was 6, 27 and 28 per cent in the case of larvae of the same strains at the same concentration of 0.06 ppm (Athanassiou *et al*. 2008b).

Complete adult mortality of *R. dominica* was obtained at doses of ≥ 0.125 ppm of spinosad dust after 14 days of exposure on the treated substrate. In contrast, > 95 % adult mortality was observed in *S. oryzae* after 14 days of exposure at 1.25 ppm only. Significantly high mortality of *S. oryzae* was observed on spinosad treated wheat than on treated barley and maize (Chintzoglou *et al*. 2008a).

Insecticidal effect of spinosad dust in combination with diatomaceous earth was studied against *S. oryzae* and *T. confusum* by Chintzoglou *et al*. (2008b). Mortality, ranging between 83 and 100 per cent, was obtained in *S. oryzae* exposed for 14 days on wheat treated with spinosad alone. Whereas, the presence of diatomaceous earth, combined with spinosad, could not significantly increase *S. oryzae* mortality compared with spinosad alone. The mortality of *T. confusum* exposed for 14 days on wheat treated with 1.25 ppm a. i. of spinosad was 14 per cent but increased to 33 per cent in the presence of diatomaceous earth.

A similar experiment was conducted by Vayias *et al*. (2009a) in European strain of *T. confusum* and reported that at each dose of spinosad, mortality of test insect increased as the rate of diatomaceous earth increased.

Bioassays were carried out to examine the insecticidal effect of spinosad against adults of *R. dominica, S. oryzae*, as well as adults and larvae of *T. confusum* at doses of 0.01, 0.1. 0.5 and 1 ppm on different grain commodities. Mortalities of *T. confusum* adults were 58, 52, 72 and 69 per cent for wheat, maize, rice and barley, respectively after 21 days of exposure at 0.1 ppm. Whereas, it was 56,53,86,83 per cent in case of *S. oryzae*. Adult mortality was 100 per cent in *R. dominica* with dose rates $\geq$ 0.1 ppm after seven days of exposure, with the exception of maize, for which mortality was 99.4 per cent (Vayias *et al.* 2009b).

Complete mortality of *T. castaneum* was obtained after 21 days of exposure on safflower treated with 300 ppm of spinosad (Khashaveh, 2009).

Adult mortality of *R. dominica* was 78 and 72 per cent after 40 hours of exposure on wheat and maize, respectively, treated with 1 ppm of spinosad. Progeny production was completely arrested when parental adults were exposed for >8 or >4 hours on wheat and maize, respectively, treated with spinosad of same dose (Athanassiou *et al*. 2010).

Vayias *et al.* (2010) studied the insecticidal action of the combined use of spinosad and deltamethrin against stored product pests. Mortalities of *S. oryzae* and *S. granarius* were 73 and 88 per cent after seven days of exposure on wheat treated with 0.5 ppm of spinosad. On the contrary, 0.125 ppm of deltamethrin was more effective against *T. confusum* than spinosad alone and combinations of the two insecticides.

Sadeghi *et al*. (2011) reported that maximum mortalities of *T. castaneum* and *O. surinamensis* were obtained after 72 h of exposure on treated glass Petri dishes with 500 ppm of spinosad. However, strains of *S. oryzae* were more susceptible to spinosad and complete mortality was observed after 72 h on Petri dishes treated with 400 ppm of spinosad. LC_{50} values of spinosad, estimated for adults of *T,*

castaneum, *S. oryzae* and *O. surinamensis* were, 1287, 279 and 53.17 ppm, respectively.

Efficacy of spinosad was studied by Athanassiou *et al*. (2011) against six stored product insects and found that adult mortality of *R. dominica* was 100 per cent on wheat treated with 0.1 and 0.5 ppm of spinosad. However, mortality of *S. oryzae* did not exceed 62 per cent. Complete mortality of *S. granarius* was obtained with 0.5 ppm of spinosad. Even though 97 per cent mortality of *C. ferrugineus* was obtained with 0.5 ppm of spinosad, *O. surinamensis* and *Liposcelis bostrichophila* were less susceptible to spinosad.

Potency of spinosad against the cowpea bruchid, *Callosobruchus maculatus* in storage was studied by Kumari (2011) and found that spinosad brought about 50 per cent mortality in 20.51 to 33.09 hours. Ninety per cent mortality was observed in 2.6 to 3.3 days and spinosad was most effective as an inhibitor of oviposition and egg hatchability.

Efficacy of spinosad dust against major storage insect pests was evaluated by Mutambuki *et al.* (2012). Low susceptibility was exhibited by *T. castaneum* than other insects. Less than 18 dead insects of *T. castaneum* were observed after 20 weeks of exposure on wheat treated with spinosad (1.44 ppm).

Hameed *et al*. (2012) evaluated the toxicological effects of spinosad (Tracer 240 SC), neem and Kanair on the red flour beetle, *T. castaneum* with five concentrations namely 0.5, 1.0, 1.5, 2.0 and 2.5 per cent at different exposure intervals of 24, 48, 72 and 168 hours by filter paper dip method. Among the tested insecticides spinosad was the best and produced a maximum mortality of 55 per cent at 2.5 per cent dose in 168 h exposure time and a minimum of 16.66 per cent with 0.5 per cent concentration at 24 h exposure time.

The adult mortalities of *T. castaneum* after 7 and 14 days of exposure on spinosad treated (1 mg/ kg) wheat were 2 to 18 per cent and 4 to 58 per cent, respectively. No progeny production was observed in four out of 11 field strains of *T. castaneum* exposed to spinosad-treated wheat. All six field strains of *O.*

surinamensis were less susceptible to spinosad even after 14 days of exposure. Whereas 99 – 100 per cent mortality was obtained in strains of *R. dominica* after 7 and 14 days exposure on spinosad treated wheat (Sehgal *et al*. 2013).

Andric *et al*. (2013) reported that, adult mortality of laboratory strain of *T. castaenum* did not exceed 30 per cent after seven days exposure on wheat treated with 5 mg/ kg of spinosad. However, mortality was increased to 75 and 97 per cent after 14 and 21 days of exposure, respectively, on treated wheat. Mortality of malathion resistant strain of *T. castaneum* was only 87 per cent even after 21 days of exposure on spinosad treated wheat.

Athanassiou *et al*. (2016) reported that application of spinosad either with *Beauveria bassiana* or diatomaceous earth resulted in low mortality of *T. confusum* for any of the combinations used.

2.3.4 Susceptibility of Stored Product Pests to Flubendimaide

Flubendiamide is a phthalic diamide insecticide acting on ryanodine receptors. It is a stomach poison, which is highly effective against lepidopteran pests.

Bhogeesh *et al*. (2014) reported that infestation of groundnut pod borer, *Caryedon serratus* on stored groundnut could be controlled by treating the packaging material with flubendimaide 480 SC at 100 ppm. The insecticide treatment did not affect seed quality attributes like moisture content, germination and vigour index.

Study conducted by Raju and Jyothi (2016) revealed that pulse beetle (*Callosobruchus maculatus*) damage was significantly low (0.25 %) in stored cowpea seeds even after three months of storage after treating with flubendiamide 480 SC at 2 ppm.

2.4 ANALYSIS OF PESTICIDE RESIDUES IN STORED PRODUCTS

Thompson *et al*. (1970) determined residues of organochlorine insecticides in grain, pulses and nuts using gas chromatograph (GC) along with electron capture detector. Less than 0.1 ppm of BHC residue was detected in most of the samples. Trace amounts of DDE was observed in about 10 per cent of samples and DDT residue was very rare.

A procedure was developed by Crisp and Tarrant (1971) for the determination of residues of dichlorovos and malathion from wheat using gas-liquid chromatography with a phosphorus-sensitive detector. Recovery per centage of both the insecticides were between 87 and 99.

Desmarchelier *et al*. (1977) tested the accuracy and reproducibility of analytical methods for the residue estimation of five organophosphorus grain protectants namely, chlorpyriphos-methyl, malathion, fenitrothion, methacrifos and pirimiphos-methyl. Detection by gas–liquid chromatography equipped with specific phosphorus detector was a satisfactory procedure

Malathion, bromophos, iodofenphos and pirimiphos-methyl residues in milled fractions of wheat were determined by gas- liquid chromatography after six months of storage. Bran and middlings contained high levels of insecticide residues whereas, residue was very low in flour. Low levels of iodofenfos occurred in flour than malathion, bromophos or pirimiphos-methyl (Mensah *et al*. 1979).

Another multi-residue method was used by Bottomley and Baker (1984) for the determination of organochlorine, organophosphorus, synthetic pyrethroid and carbaryl residues in grains. Organochlorine residues were detected by gas- liquid chromatography using an electron capture detector and a flame-photometric detector was used for the detection of organophosphorus pesticide residues. Synthetic pyrethroids and carbaryl were determined by high-performance liquid chromatography using an ultraviolet spectrophotometric detector.

Residues of pyrethroid grain protectants namely bioresmethrin, phenothrin, fenvalerate, permethrin and deltamethrin together with the synergist piperonyl butoxide in paddy were determined by reverse-phase high-performance liquid chromatography at 225 nm. Limit of detection was 0.05µg/ ml (Haddad *et al*. 1989).

A multi-residue analysis method was developed by Walorczyk (2007) for 122 gas chromatography amenable pesticides in cereal grains and certain feedstuffs. Matrix clean- up was done by dispersive solid phase extraction method followed by residue detection using gas chromatography/triple quadrupole tandem mass spectrometry. The recovery range was between 73 and 129 per cent.

Residues of malathion, fenitrothion and their metabolites in barley were analysed using gas chromatography (GC) equipped with a nitrogen- phosphorus detector (NPD). Residue levels at the beginning of storage exceeded maximum residue limits (MRLs). Whereas 65–72 per cent degradation of malathion and isomalathion and 85 per cent degradation of malaoxon was observed during the storage period. Residue level of fenitrothion was 80 per cent for the short-term storage (Uygun *et al*. 2007).

Matrix clean-up in multiresidue analysis could be done by mixed- mode solid-phase extraction method. Residue analysis using gas chromatography with electron capture and mass spectrometric detectors were promising with recoveries ranging from 73–117 per cent for 25 pesticides (Balinova *et al*. 2007).

Development and validation of a method for the determination of eight pesticide residues in rice was done by Tsochatzis *et al*. (2010) by using high performance liquid chromatograph equipped with diode array detector. Limit of detection and limit of quantification ranged from 0.002 to 0.200 mg kg^{-1} and 0.006 to 0.600 mg kg^{-1}, respectively. An acceptable recovery of 74 – 127 per cent was obtained.

Another method for determination of pesticide residues in rice was proposed by Uddin *et al.* (2011) using capillary gas chromatography with a µ-ECD detector

and a HP-5MS capillary column. Recovery was in the range of 74 – 111 per cent and relative standard deviation (RSD) was in the range of 2.41 – 12.42 per cent.

Kolberg *et al.* (2011) developed a multiresidue analysis for the determination of 24 pesticides in wheat, wheat flour and bran with the help of gas chromatography coupled to mass spectrometry. Pesticides were extracted using QuEChERS method.

Samples of barley, oat, rye and wheat were tested for pesticide residues and 77.5 per cent of the samples were free from residues. Whereas, 13.75 per cent of samples contained residues. Most frequently detected residues were chlorpyriphos-methyl and pirimiphos-methyl (Lozowicka *et al*. 2014).

Materials and methods

MATERIALS AND METHODS

The present investigation on "Susceptibility of red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) to insecticides" was carried out at College of Horticulture, Kerala Agricultural University, Vellanikkara, Thrissur. Facilities at the Pesticide Residue Testing Laboratory and AINP on Agricultural Ornithology were utilized at different stages of study. The details of the materials used and methods followed are described in this chapter.

3.1 COLLECTION OF RED FLOUR BEETLE *Tribolium castaneum*

Different population of red flour beetle, *T. castaneum* were collected from five different godowns of Food Corporation of India (FCI) situated at different geographic locations of Kerala such as, Thikkodi (Kozhikkode district; 11.494295, 75.625879), Olavakkode (Palakkad district; 10.807528, 76.628972), Mulangunnathukavu (Thrissur district; 10.596744, 76.206517), Angamaly (Ernakulam district; 10.191658, 76.374888) and Valiyathura (Thiruvananthapuram district; 8.470714, 76.924354). Susceptible strain of *T. castaneum* was procured from Division of Entomology, Indian Agricultural Research Institute (IARI), New Delhi, which was maintained without exposure to any insecticide for more than 35 years.

3.2 REARING OF THE TEST INSECTS

Population of *T. castaneum* were reared in the laboratory at 30 ± 2°C and 80 ± 5 per cent relative humidity as per the method described by Bhatia and Pradhan (1968). About 15 – 20 adults obtained from different FCI godowns and IARI were introduced into separate plastic containers (17 cm x 11 cm) containing 250 g of sterilized wheat flour fortified with 5 per cent (wt./wt.) brewer's yeast. Ten such containers were initially prepared for each strain and kept in a culture room protected with ant well. After allowing five days for oviposition, the adults were sieved out and transferred to fresh rearing containers (Plate 1). This process was

Plate 1. Rearing of *Tribolium castaneum*

repeated in order to obtain a regular supply of adult insects of known age. Rearing containers were regularly examined and wheat flour was sieved every day following the first sighting of adult beetle to obtain one day old adults. Such adults were maintained in plastic containers containing fresh wheat flour for 15 days. Adults (17 ± 2 day old) obtained in this manner was used for bioassay.

3.3 EVALUATION OF SUSCEPTIBILITY OF *TRIBOLIUM CASTANEUM* TO INSECTICIDES

3.3.1 Susceptibility of *Tribolium castaneum* to Malathion, Dichlorovos and Deltamethrin

Susceptibility of six population of *T. castaneum* was evaluated against FCI recommended insecticides such as malathion, dichlorovos and deltamethrin by residual film method of bioassay. Technical grade insecticides (Sigma Aldrich) dissolved in acetone were used for bioassay. Stock solutions of 1000 ppm were prepared in volumetric flasks using acetone as solvent and were diluted serially to obtain required concentrations.

A preliminary bioassay was conducted by using wide range of concentrations of each insecticide to arrive at the actual concentration required for bioassay for all the six population, separately. Bioassay involved a minimum of six concentrations for each population, including control, which were replicated thrice. One millilitre of prepared concentration of insecticide was pipetted out into a 9 mm Petri plate (in the case of control, acetone alone was used). Petri plate was rotated thoroughly to obtain a thin and uniform film of insecticide/solvent over the surface of Petri plate. After air drying, ten adult insects (17 ± 2 day old) were released in to each Petri plate. Insects were pre-starved for a period of two hours before bioassay. Observations on mortality was taken after 48 h. Moribund insects were counted as dead. Number of dead insects were counted and LC_{50} was calculated using probit analysis (Finney, 1971) for each insecticide and for each population using Polo PC software. Mortality was corrected, as per Abbott's formula whenever required (Abbott, 1925). Resistance ratio for each insecticide was calculated using the formula given below.

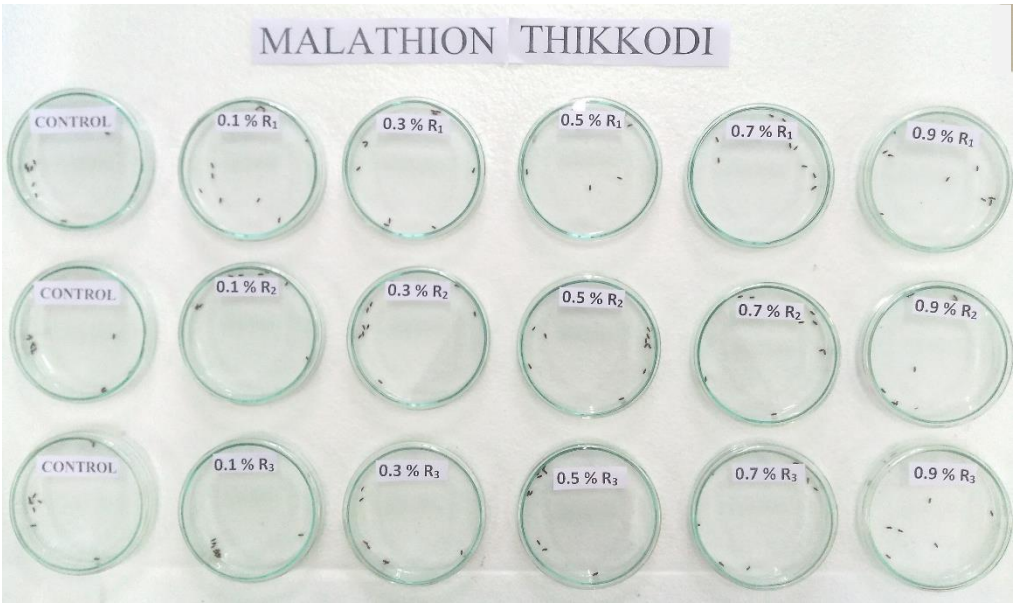

Plate 2. Residual film bioassay with malathon in Thikkodi population of *Tribolium castaneum*

$$\text{Resistance ratio} = \frac{LC_{50} \text{ of field collected strain}}{LC_{50} \text{ of susceptible strain}}$$

3.4 STUDIES ON BIOCHEMICAL BASIS OF INSECTICIDE RESISTANCE

3.4.1 Sample Preparation

Insect sample (16 mg) was homogenized in 700 µl of sodium phosphate buffer (pH 7.4) and centrifuged at 10000 rpm for 20 minutes at 4°C to remove coarse particles. The supernatant was utilized for protein and enzyme assay.

3.4.2 Estimation of Protein

Total protein present in six strains of *T. castaneum* was estimated as per the procedure given by Lowry *et al.* (1951).

3.4.2.1 Preparation of Standard Bovine Serum Albumin (BSA) Solution

A stock bovine serum albumin (BSA) (SRL, Mumbai) solution was prepared by dissolving 50 mg of BSA in 50 ml of double distilled water in a volumetric flask. Working standard was prepared by pipetting out 10 ml of stock solution and making up to 50 ml with double distilled water in a volumetric flask, so that, 1 ml of the solution contained 200 µg of protein. Different aliquots of 100, 200, 300, 400, 500, 600 and 700 µl were pipetted out in to different test tubes from the working standard and made up to 1 ml with double distilled water. A test tube with double distilled water alone served as blank. The reagents used are given below.

Reagent A: 2% sodium carbonate in 0.1 N sodium hydroxide.

Reagent B: 0.5 % copper sulphate solution in 1% sodium potassium tartarate solution.

Reagent C: Mixture of 50 ml of reagent A and 1 ml of reagent B, prepared just prior to the use.

Reagent D - Folin-Ciocalteu reagent (FCR): the commercial FCR was diluted in 1:1 ratio with double distilled water before use.

Five millilitres of reagent C was added to all the test tubes including blank. The contents in the test tube were mixed well and allowed to stand for 10 minutes. Afterwards, reagent D (0.5 ml) was added, mixed thoroughly and incubated at room temperature in dark for 30 minutes. Absorbance of the developed blue colour was read at 660 nm using spectrophotometer (Model: Agilent Cary 60 UV Vis). Standard graph was drawn by using the OD values and the corresponding concentrations of BSA (Fig. 1).

3.4.2.2 Total Protein Estimation in Tribolium castaneum

Fifty micro litres of supernatant/ enzyme extract prepared was taken and 2.5 ml of reagent C was added. After an incubation period of 10 min., 250 µl of reagent D was added. Reaction mixture was kept in dark at room temperature for 30 min. Absorbance readings were taken at 660 nm in spectrophotometer. Protein content was calculated from the standard graph and expressed in mg/ ml.

3.4.3 Estimation of Carboxylesterase

Esterase assay was carried out using the method described by van Asperen (1962).

3.4.3.1 Preparation of α-naphthol Standard

Stock solution of 10 mM α-naphthol (Merck) was prepared by dissolving 0.03605 g of α-naphthol in 25 ml methanol. Working standards of varying concentrations (100 µmol, 200 µmol, 300 µmol, 400 µmol, 500 µmol and 600 µmol) were prepared by pipetting out different aliquots (10 µl, 20 µl, 30 µl, 40 µl, 50 µl and 60 µl) from the stock solution and made up to 1 ml with methanol. Two millilitres of extraction buffer (sodium phosphate buffer, pH 7.4) was added to working standards. Phosphate buffer alone served as blank. The reaction mixture was incubated for 10 min. at 30°C with constant stirring. Dye solution (50µl)

containing 22.5 mg fast blue RR salt (SRL, Mumbai) in 2.25 ml double distilled water and 5% sodium dodecyl sulphate in double distilled water (2:5 v/v) was added to the reaction mixture. The mixture was again incubated for 5 min. at 37°C for colour development. The intensity of red colour was measured in spectrophotometer at 600 nm. Standard curve was prepared with OD values obtained and the corresponding concentrations (Fig 2).

3.4.3.2 Estimation of Carboxylesterase Activity in Tribolium castaneum

Fifty micro litres of enzyme extract was taken for enzyme assay. To this extract, 1 ml of 30 mM α-naphthyl acetate (SRL, Mumbai), as enzyme substrate, dissolved in acetone (0.028 g α-naphthyl acetate in 5 ml acetone), was added. The subsequent steps as described earlier for the preparation of standard curve was followed for the estimation of carboxylesterase in *T. castaneum.*

3.4.4 Estimation of Glutathione-S- transferase (GST)

Glutathione-S- transferase estimation was done by the method of Kao *et al*. (1989).

3.4.4.1 Estimation of Glutathione-S- transferase Activity in Tribolium castaneum

Fifty microliters of 50 mM 1-Chloro-2, 4-Dinitro Benzene (CDNB) (SRL, Mumbai) and 150 µl reduced glutathione (GSH) (SRL, Mumbai) were added to 2.75 ml of sodium phosphate buffer (pH 6.5). Fifty microliters of prepared enzyme extract was added to this mixture. The contents were gently shaken, incubated for 2 – 3 min and then transferred to a cuvette for reading in UV spectrophotometer. Reaction mixture without enzyme served as blank. Absorbance at 340 nm was recorded for 5 minutes at 30 sec intervals. The GST activity was calculated using the formula

$$\text{CDNB-GSH conjugate (µmol mg protein}^{-1}\text{ min}^{-1}) = \frac{\Delta \text{ Abs in 5 min x 3 x } 1000}{9.6 \text{ x 5 x mg of protein}}$$

Extinction coefficient for CDNB-GSH conjugate – 9.6 mM/cm.

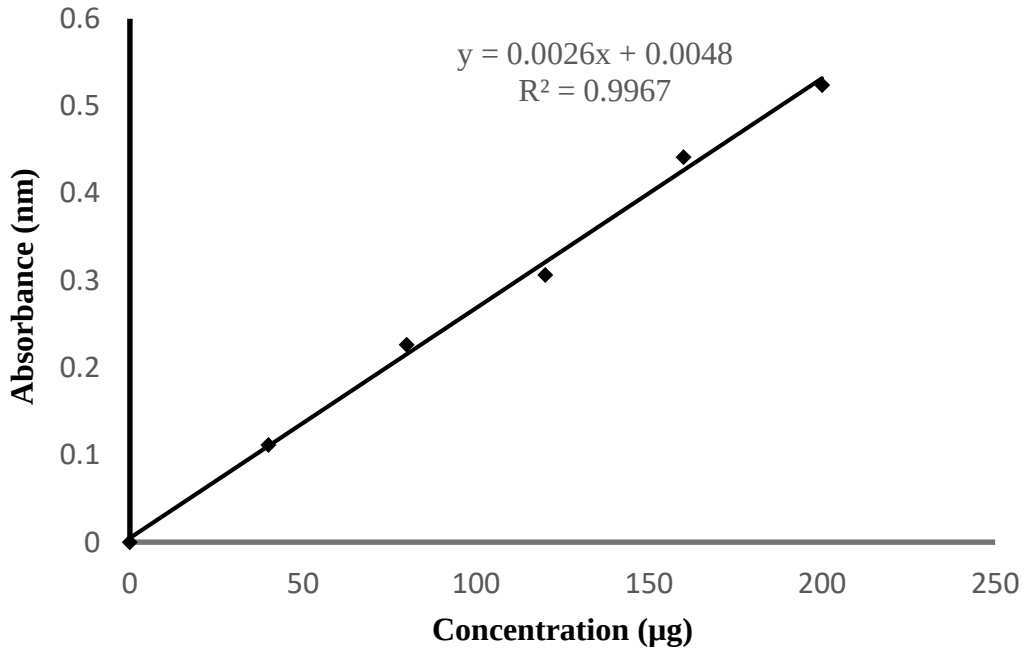

Fig. 1. Standard curve of bovine serum albumin (BSA) for protein estimation

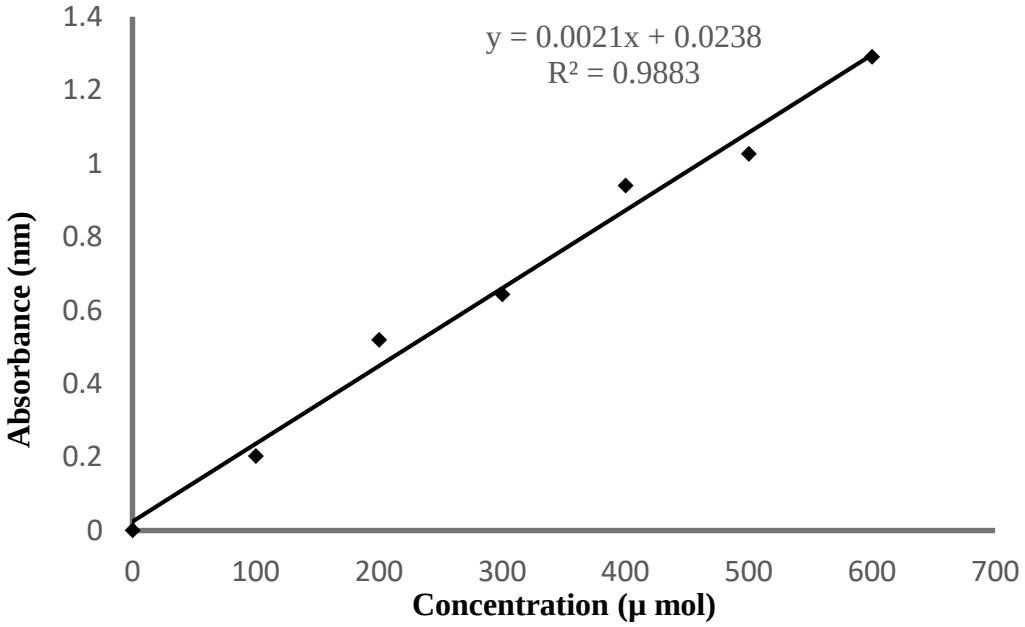

Fig. 2 Standard curve of α-naphthol for carboxyl esterase estimation

3.4.5 Estimation of Cytochrome P450

The cytochrome P450 assay was carried out according to the method of Brogdon *et al*. (1997) with slight modifications.

3.4.5.1 Preparation of Cytochrome C Standard

Pure cytochrome C from bovine heart (Sigma Aldrich) (3.081 mg) was dissolved in 10 ml of double distilled water to obtain a stock solution of 0.0025 mM concentration. Working standards of 0.025 nM to 0.2 nM were prepared from the stock solution. Hundred microliter each of working standard was taken in test tubes. To the working standards, 1 ml of 0.05% TMBZ (SRL, Mumbai) (3, 3', 5, 5'-Tetramethylbenzidine) (10 mg TMBZ dissolved in 5 ml absolute methanol mixed with 15 ml 0.25 nm sodium acetate buffer, pH 5), 400 µl of potassium phosphate buffer (pH 7.2) and 125 µl of 3% hydrogen peroxide were added. The reaction mixture was incubated for 30 min. The absorbance was recorded with UV spectrophotometer (Model – Agilent Cary 60 UV Vis) at 630 nm. Standard graph was drawn from the OD values and corresponding cytochrome C concentrations (Fig. 3).

3.4.5.2 Estimation of Cytochrome P450 Activity in Tribolium castaneum

To 50 µl of enzyme extract, 500 µl of TMBZ (0.05%), 200 µl potassium phosphate buffer (pH 7.2) and 62.5 µl of hydrogen peroxide (3%) were added. After 30 min. of incubation, absorbance was recorded using spectrophotometer at 630 nm. Cytochrome P450 activity was calculated from the standard graph and expressed in pmol mg protein^{-1} min^{-1}.

3.4.6 Statistical Analysis

Data were analysed by Analysis of variance (ANOVA) in SPSS 16.0 in CRD and means were separated by Tukey's test. Correlation of the activity of each enzyme and the LC_{50} of each insecticide was studied using SPSS16.0 and Pearson correlation coefficient was worked out.

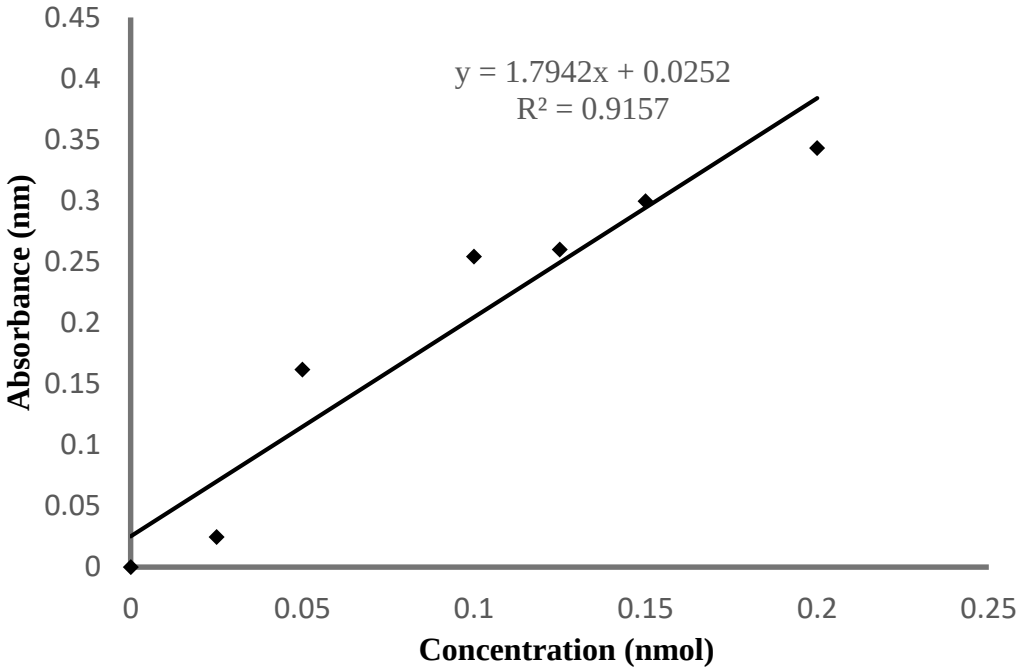

Fig. 3 Standard curve for cytochrome C for cytochrome P450 estimation

3.5 EVALUATION OF NEW INSECTICIDE MOLECULES AGAINST DIFFERENT STRAINS OF *Tribolium castaneum*

3.5.1 Bioassay with New Insecticide Molecules

Residual film method of bioassay (Plate 3) was carried out using bifenthrin, chlorfenapyr, spinosad and flubendiamide for all the strains of *T. castaneum* as described in section 3.3. Relative toxicity of each insecticide was calculated by the formula given below.

$$\text{Relative toxicity} = \frac{\text{LC}_{50} \text{ of malathion}}{\text{LC}_{50} \text{ of test insecticide}}$$

3.6 ESTIMATING THE RESIDUES OF INSECTICIDES IN RICE GRAINS

The most effective insecticides, selected based on the relative toxicity studies, were chosen for this experiment along with the FCI recommended pesticides. Twenty-seven small jute bags containing rice (1 kg) were arranged in a single layer (Plate 4) in an area of 1 m^2 for each insecticide. Insecticides were sprayed on these bags simulating the pesticide spray in FCI godowns. Twenty-seven such bags were kept for residue analysis, so as to obtain three bags (3 replications) each for residue analysis at different intervals of 2 h, 1, 3, 5, 7, 10, 15, 20 and 30 days after treating with insecticide (9 sampling intervals × 3 replications).

Concentration of pesticide for spraying rice bags was calculated based on the highest value of LC_{50} obtained during the bioassay with different strains of *T. castaneum*. Ten times LC_{50} value was calculated and sprayed over jute bags using a hand sprayer (Aspee ®). As recommended by FCI, the insecticide solutions were sprayed @ 30ml/m^2. Commercial formulations of the pesticides were used for spraying of jute bags (Table 1).

For treating jute bags with malathion and deltamethrin, the dose recommended by FCI was sprayed on bags. For the prophylactic treatment of food grains malathion (50% EC) was recommended @ 10ml/L and deltamethrin 2.5 WP @ 40 g/L. In the present study, deltamethrin (2.8% EC) was used and the spray

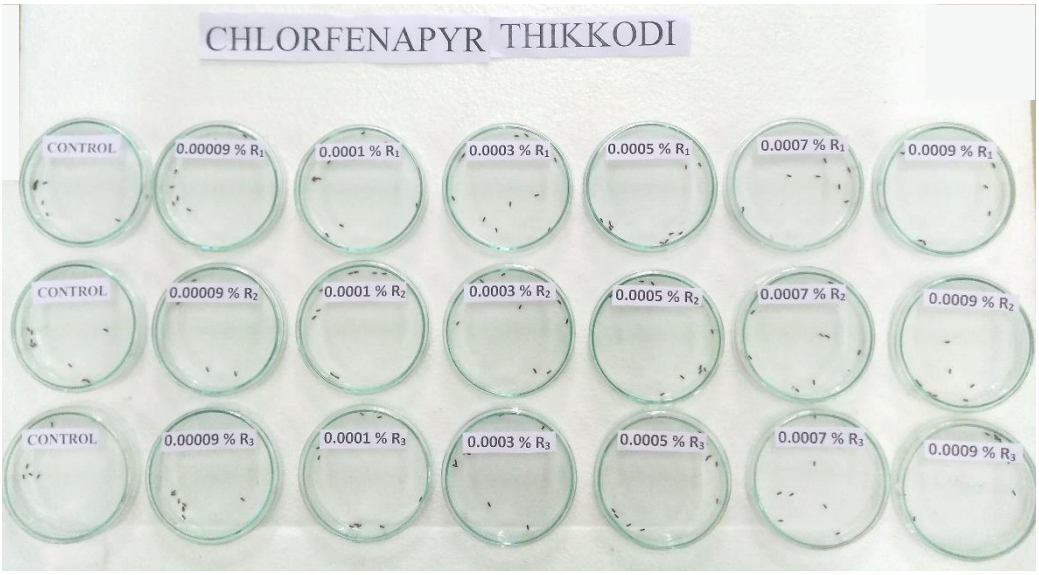

Plate 3. Residual film bioassay with chlorfenapyr in Thikkodi population of *Tribolium castaneum*

Plate 4. Jute bags containing 1 kg of rice arranged in a single layer in 1 m^2 for pesticide residue analysis

concentration was calculated based on the active ingredient content. Dichlorovos was recommended for spraying on walls/roof/floor of storage structures and not on storage bags. Hence, dichlorovos was not sprayed on jute bags and residue analysis was not carried out for dichlorovos.

3.6.1 Preparation of Pesticide Standards

Certified reference materials (CRM) of malathion, deltamethrin, chlorfenapyr and bifenthrin were procured from Sigma-Aldrich and stored in -20^{0}C deep freezer (Table 2). The glasswares used for residue analysis experiments were properly washed with a cleaning reagent (Extran MA 02) and rinsed with acetone. Washed glass wares were then dried in a hot air oven for 3 h at 50^{0}C.

Stock solution (1000 ppm) of each pesticide was prepared from the certified reference material. The weighed out quantity of pesticide was dissolved in acetone and made up with 1:1 hexane: toluene solvent in volumetric flasks. From the stock solution, 100 ppm working standard was prepared. From this working standard, required lower concentrations of each pesticide was prepared by serial dilution.

3.6.2 Method Development and Validation for Analysis of Pesticide Residues in Rice

3.6.2.1 Standardisation of Conditions of Gas Chromatograph

Residue analysis was carried out in Agilent 7890 B gas chromatograph equipped with electron capture detector (ECD). The operating parameters of gas chromatograph is given in Table 3.

3.6.2.2 Calibration and Linearity

In order to plot the calibration curve, six concentrations (0.05, 0.10, 0.20, 0.30, 0.40 and 0.50 ppm) of each pesticide were injected in triplicate into gas chromatograph. Calibration curves with pesticide concentration on x-axis and peak area on y-axis, were plotted. Slope and R^2 (coefficient of determination) were

Table 1. Insecticide formulations used for pesticide residue analysis

Sl. No.	Insecticide	Trade name	Toxicity label	Manufacturing company
1	Malathion 50EC	Killers	Blue	Jayakrishna Pesticides (P) Ltd.
2.	Deltamethrin 2.8 EC	Decis	Yellow	Bayer Crop Science Ltd
3.	Chlorfenapyr 10 SC	Lepido	Blue	PI Industries Ltd.
4	Bifenthrin 10 EC	Talstar	Yellow	FMC India Private Ltd.

Table 2. Certified reference materials used for pesticide residue analysis

Sl. No.	Pesticide group	Certified Reference material (CRM)	Purity (%)
1	Organophosphate	Malathion	99.20
2	Synthetic pyrethroid	Deltamethrin	99.70
3		Bifenthrin	99.10
4	Pyrrole	Chlorfenapyr	99.20

Table 3. Operating parameters of gas chromatograph

Parameters	Details
Gas chromatograph	Agilent 7890 B
Colum	Agilent HP-5 column (30 m x 320 μm)
Film thickness	0.25μm
Carrier gas	Nitrogen (99.9995% purity)
Flow rate	1 ml/min
Injector temperature	260^0C
Injector mode	Splitless
Oven temperature	70^0C ramped to 120°C @ 40^0C/min (held for 5 min.), then ramped to 300°C @ 50^0C/min (held for 8 min.)
Detector temperature	300^0C

calculated by carrying out linear regression analysis. All the solvents used for residue analysis were of HPLC grade.

3.6.2.3 Determination of Limit of Detection (LOD)

Limit of detection (LOD) is the lowest concentration of a pesticide that can be detected reliably with the given analytical method. It is the lowest concentration of the pesticide that can be detected, but cannot be quantitated as an exact value. In order to find out LOD, working standards (0.05, 0.10, 0.20, 0.30, 0.40 and 0.50 ppm) were prepared in matrix (rice) solution and 1µl each of the matrix matched standards were injected in triplicate into gas chromatograph under conditions already stated. Each concentration was injected in triplicate and average standard deviation was worked out. Limit of detection was calculated for each pesticide using the formula given below.

$$\text{Limit of Detection (LOD)} = 3 \times \frac{\text{Average standard deviation}}{\text{Slope of linearity graph}}$$

3.6.2.4 Determination of Limit of Quantification (LOQ)

Limit of quantification (LOQ) is the lowest concentration of a pesticide that can be measured in a sample matrix with acceptable mean recovery (70-120%) and relative standard deviation (RSD) (<20%). Limit of quantification was calculated for each pesticide using the formula given below.

$$\text{Limit of Quantification (LOQ)} = 10 \times \frac{\text{Average standard deviation}}{\text{Slope of linearity graph}}$$

3.6.2.5 Determination of Recovery

In order to ensure the reliability of the method and to know the efficiency of extraction and clean up steps adopted in the present investigation, recovery studies were conducted. Pesticide free rice was homogenised and spiked with pesticides at three levels, LOQ, 5×LOQ and 10×LOQ and the residues were estimated.

3.6.3 Sample Preparation

QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) is a solid phase extraction method for extraction of pesticide residues in food (Anastassiades *et al.* 2003). A modified QuEChERS method as described below was employed for the current study.

Whole rice sample (1 kg) was ground in a mixer and 5 g of the crushed sample was transferred to a 50 ml centrifuge tube. Double distilled water (15 ml) was added to the centrifuge tube and mixed thoroughly. After 30 min. of incubation, 10 ml of acetic acid (1%) in acetonitrile/ethyl acetate was added to each centrifuge tube. The contents were vortexed for 30 sec. Afterwards 4 g of magnesium sulphate (activated at 500°C for 5-6 h.) and 1 g of anhydrous sodium acetate were added to each tube. Sample mixtures were homogenized at 13000-14000 rpm for 3 min. and the contents were centrifuged at 3000 rpm for 5 min. at 10°C in a refrigerated centrifuge. After centrifugation, 7 ml of supernatant was transferred to separate 15 ml centrifuge tubes containing 0.35 g of primary secondary amine (PSA) and 1.05 g magnesium sulphate. Again, the contents were vortexed and centrifuged at 5000 rpm for 5 min. at 10°C. After centrifugation, 3 ml of the supernatant from each centrifuge tube was transferred to labelled test tubes and the solvent was evaporated using nitrogen concentrator at 35-40°C temperature. Residue was reconstituted with 3 ml of hexane after the complete evaporation of solvent in the test tube. The mixture was sonicated for 1 min. to dissolve the residues. The contents were filtered through 0.2 µ PVDF membrane filter and filtrates were added to labelled vials for residue estimation by GC.

3.6.4 Calculation of Pesticide Residues

Residues of each pesticide was calculated based on the peak area of sample and peak area of standard using the following equation.

$$\text{Residue (ppm)} = \frac{\text{Area of sample}}{\text{Area of standard}} \text{ X } \frac{\text{Conc. of standard}}{\text{Wt. of sample}} \text{ X Final vol. of extract}$$

Results

4. RESULTS

Red flour beetle, *Tribolium castaneum* is an important secondary pest infesting stored food and processed products. Susceptibility of *T. castaneum* population collected from various FCI godowns of Kerala to recommended pesticides like malathion, dichlorovos and deltamethrin were assessed and the biochemical mechanisms of resistance were investigated. Susceptibility of *T. castaneum* to new insecticide molecules and the level of pesticide residues in rice grains were evaluated in this study. The results thus obtained have been depicted in this chapter

4.1 SUSCEPTIBILITY OF VARIOUS POPULATION OF *Tribolium castaneum* TO INSECTICIDES COMMONLY USED IN FOOD CORPORATION OF INDIA GODOWNS

Malathion, dichlorovos and deltamethrin are the insecticides recommended and commonly used in FCI godowns throughout the country. Malathion and deltamethrin are sprayed on the surface of jute sacks while dichlorovos is recommended for spraying on floors, walls and roofs of storage godowns.

4.1.1 Susceptibility of Different Population of *Tribolium castaneum* to Malathion

Residual film bioassay was carried out to evaluate the susceptibility of various population of *T. castaneum* to malathion. Susceptibility levels were determined based on LC_{50} value calculated by probit analysis.

Angamaly population of *T. castaneum* showed the least susceptibility to malathion with an LC_{50} value of 6949.80 ppm. The second highest LC_{50} was shown by Mulangunnathukavu population (6157.30 ppm), followed by Valiyathura population (5873.02 ppm), Olavakkode population (5727.94 ppm) and Thikkodi population (5703.48 ppm). The susceptible strain from IARI had the lowest LC_{50} of 520.76 ppm (Table 4).

Table 4: Susceptibility of different population of *Tribolium castaneum* to malathion

Strain	Heterogeneity		LC_{50} (ppm) (95 % fiducial limit)	LC_{90} (ppm) (95 % fiducial limit)	Slope
	d.f.	χ^2			
Mulangunnath-ukavu	3	2.24	6157.30 4923.70- 8716.10	18540 11897 - 46370	2.68
Valiyathura	3	0.64	5873.02 4931.60–7583.60	14030 9945 - 30177	3.39
Thikkodi	3	2.58	5703.49 4679.50 -7574.90	15525 10613 - 33139	2.95
Olavakkode	3	2.64	5727.94 4648.70 -7769.90	16121 10792 - 36288	2.85
Angamaly	3	0.94	6949.80 5353.90-10987.00	23042 13547 - 75302	2.46
Susceptible	4	1.79	520.76 434.20 – 613.10	1392.740 1085.29-2122.24	3.000

LC_{50} = Concentration (ppm) calculated to give 50 per cent mortality; LC_{90} = Concentration (ppm) calculated to give 90 per cent mortality

Comparing the LC_{90} values, Angamaly population had the highest LC_{90} value (23042 ppm) and the susceptible strain from IARI had the lowest (1392.74 ppm). LC_{90} values of Mulangunnathukavu, Valiyathura, Thikkodi and Olavakkode population were 18540 ppm, 14030 ppm, 15525 ppm and 16121 ppm, respectively (Table 4).

Chi-square values of all the probit regression analysis were below the table values, which indicated that the populations were homogenous. If the fiducial limits of LC_{50} was compared, it was observed that, all the field collected strains had a significantly higher level of LC_{50} when compared with the susceptible strain.

4.1.2 Susceptibility of Different Strains of *Tribolium castaneum* to Dichlorvos

Tribolium castaneum strain from Olavakkode exhibited highest tolerance to dichlorvos, a widely used insecticide in FCI godowns with LC_{50} value of 6010.06 ppm. LC_{50} of dichlorvos in susceptible strain was the lowest (3150.84 ppm). Mulangunnathukavu, Valiyathura, Thikkodi and Angamaly strains registered values of 5494.84 ppm, 5807.46 ppm, 5912.71 ppm and 5533.35 ppm, respectively (Table 5).

Highest LC_{90} value was recorded by Olavakkode strain (9721.0 ppm) followed by Mulangunnathukavu (8885.7 ppm), Valiyathura (8684.6 ppm), Thikkodi (9368.2 ppm) and Angamali strains (8080.4 ppm) of *T. castenum* and the lowest by IARI strain (6869.5 ppm).

The fiducial limits of all the strains at LC_{50} was significantly higher over the IARI strain. The chi-square values of all the bioassays were below the table value.

4.1.3 Susceptibility of Different Strains of *Tribolium castaneum* to Deltamethrin

Susceptibility of various strains of *T. castaneum* to deltamethrin is presented in Table 6. The highest LC_{50} value (290.60 ppm) was recorded by Thikkodi strain. Susceptible strain from the IARI had the lowest LC_{50} of 98.40 ppm.

Table 5. Susceptibility of different population of *Tribolium castaneum* to dichlorvos

Strain	Heterogeneity		LC_{50} (ppm) (95 % fiducial limit)	LC_{90} (ppm) (95 % fiducial limit)	Slope
	d.f.	χ^2			
Mulangunn-athukavu	4	0.91	5494.84 4890.20 –6143.40	8885.70 7626.60 - 11812	6.14
Valiyathura	4	0.39	5807.46 5251.10 –6411.70	8684.60 7612.20-11079.00	7.33
Thikkodi	4	0.97	5912.71 5307.60 –6618.90	9368.20 8016.30 - 12693	6.41
Olavakkode	4	0.54	6010.06 5386.20 –6773.50	9721.00 8232.30–13516.00	6.14
Angamaly	4	1.53	5533.35 5016.50 –6062.00	8080.40 7174.20 – 9980.30	7.79
Susceptible	4	4.46	3150.84 2394.90 –3991.70	6869.50 5221.60 –11230.00	3.79

LC_{50} = Concentration (ppm) calculated to give 50 per cent mortality; LC_{90} = Concentration (ppm) calculated to give 90 per cent mortality

Table 6: Susceptibility of different population of *Tribolium castaneum* to deltamethrin

Strain	Heterogeneity		LC_{50} (ppm) (95 % fiducial limit)	LC_{90} (ppm) (95 % fiducial limit)	Slope
	d.f.	χ^2			
Mulangunnat-hukavu	3	2.75	144.127 98.20 – 206.80	1095.900 559.20–5772.10	1.455
Valiyathura	3	2.66	147.570 118.90 – 184.80	492.424 349.40–887.80	2.449
Thikkodi	3	0.71	290.604 203.70 – 545.50	2337.800 985.70-20710.00	1.415
Olavakkode	3	1.57	187.754 143.90 – 258.90	921.572 547.90–2580.60	1.855
Angamaly	3	3.84	208.126 148.40 – 349.70	1990.400 861.50–13854.00	1.307
Susceptible	4	2.85	98.401 66.90– 224.30	1120.200 382.80–7088.00	1.213

LC_{50} = Concentration (ppm) calculated to give 50 per cent mortality; LC_{90} = Concentration (ppm) calculated to give 90 per cent mortality

Mulangunnathukavu, Valiyathura, Olavakode and Angamaly strains had LC_{50} values of 144.13 ppm, 147.57 ppm, 187.75 ppm and 208.13 ppm, respectively.

The order of LC_{90} values of different strains (Table 6) were Thikkodi (2337.80 ppm) > Angamaly (1990.40 ppm) > IARI (1120.20 ppm) > Mulangunnathukavu (1095.90 ppm) > Olavakkode (921.57 ppm) > Valiyathura (492.42 ppm).

The LC_{50} values of different strains were not significantly different from each other, as the fiducial limits of all the strains were overlapping with that of each other.

4.2 STUDIES ON BIOCHEMICAL BASIS OF INSECTICIDE RESISTANCE

4.2.1 Estimation of Total Protein in Different Strains of *Tribolium castaneum*

Total protein content in various strains of *T. castaneum* was estimated by Lowry's method and shown in Table 7. The highest protein content (6.95 mg/ ml) was observed in Thikkodi strain of *T. castaneum*, whereas the lowest protein content of 6.19 mg/ ml was recorded in susceptible strain from IARI. Protein content in field strains were at par with that of the susceptible strain and the protein content observed was, Mulangunnathukavu (6.44 mg/ml), Valiyathura (6.84 mg/ml), Olavakkode (6.61 mg/ml) and Angamaly (6.66 mg/ml).

4.2.2 Estimation of Carboxylesterase Activity in Different Strains of *Tribolium castaneum*

The highest carboxylesterase activity of 0.71 µmol min^{-1} mg $protein^{-1}$ was observed in *T. castaneum* strain from Mulangunnathukavu. It was followed by Thikkodi (0.69 µmol min^{-1} mg $protein^{-1}$), Valiyathura (0.58 µmol min^{-1} mg $protein^{-1}$), Angamaly (0.58 µmol min^{-1} mg $protein^{-1}$) and Olavakkode (0.55 µmol min^{-1} mg $protein^{-1}$). Susceptible strain from IARI exhibited the lowest carboxylesterase activity of 0.4758 µmol min^{-1} mg $protein^{-1}$ (Table 8).

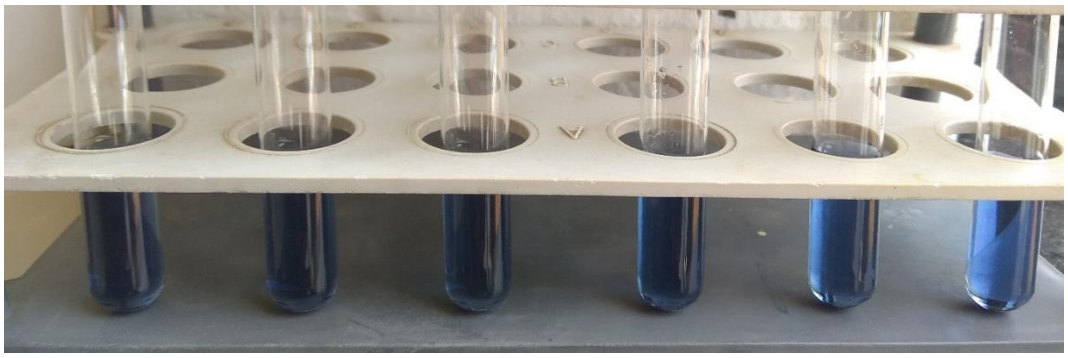

Plate 5. Estimation of total protein in different strains of *Tribolium castaneum*

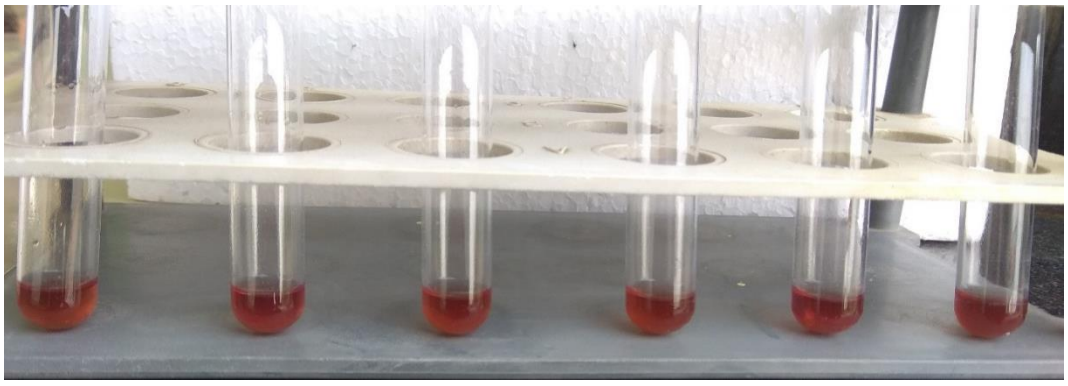

Plate 6. Estimation of carboxylesterase in different strains of *Tribolium castaneum*

Table 7: Total protein (mg/ ml) in various population of *Tribolium castaneum*

Population	**Total protein** (mg/ml)
Mulangunnathukavu	6.44[a]
Valiyathura	6.84[a]
Thikkodi	6.95[a]
Olavakkode	6.61[a]
Angamali	6.66[a]
Susceptible	6.19[a]

Figures followed by same letters are not significantly different at 0.05 % level of significance

Table 8: Carboxyl esterase activity (µmol min^{-1} mg $protein^{-1}$) in different population of *Tribolium castaneum*

Population	**Carboxyl esterase activity (µ mol min^{-1} mg $protein^{-1}$)**
Mulangunnathukavuavu	0.71[e]
Valiyathura	0.58[c]
Thikkodi	0.68[d]
Olavakkode	0.55[b]
Angamali	0.58[c]
Susceptible	0.47[a]

Figures followed by same letters are not significantly different at 0.05 % level of significance

Carboxylesterase activity was significantly higher in all the field collected strains than in the susceptible strain. Among the different field strains, significantly higher carboxylesterase activity was observed in Mulangunnathukavu strain. Thikkodi strain also had significantly higher level of carboxylesterase over Valiyathura, Olavakkode and Angamali strains. Valiyathura and Angamali strains demonstrated comparable carboxylesterase activity, which were significantly higher than that of Olavakkode strain

4.2.3 Estimation of Glutathione-S- transferase Activity in Different Strains of *Tribolium castaneum*

Table 9 shows that glutathione-S- transferase activity was the highest in Angamaly strain (0.39 μmol min^{-1} mg $protein^{-1}$) and the lowest in susceptible strain from IARI (0.28 μmol min^{-1} mg $protein^{-1}$). The order of glutathione-S- transferase activity in other strains was Olavakkode (0.37 μmol min^{-1} mg $protein^{-1}$) > Thikkodi (0.30 μmol min^{-1} mg $protein^{-1}$) > Mulangunnathukavu (0.28 μmol min^{-1} mg $protein^{-1}$) > Valiyathura (0.28 μmol min^{-1} mg $protein^{-1}$).

Glutathione-S-transferase activity was significantly lower in the susceptible strain when compared to the field strains. Among the field strains, lowest GST activity was recorded in Mulangunnathukavu and Valiyathura strains, which was statistically different from that of other strains. Highest and significant GST activity was observed in Angamali strain. Olavakkode and Thikkodi strains also had high levels of GST activity but they were significantly lower than Angamali strain. Glutathione-S-transferase activity was significantly higher in Olavakkode strain compared to Thikkodi strain.

4.2.4 Estimation of Cytochrome P450 Activity in Different Strains of *Tribolium castaneum*

Cytochrome P450 activity was comparatively low in all the strains of *T. castaneum* and the highest activity among them was noticed in Mulangunnathukavu strain (1.02 pmol min^{-1} mg $protein^{-1}$). Cytochrome P450 activity in Valiyathura, Thikkodi, Olavakkode and Angamaly strains were 0. 88, 0.93, 0.85 and 0.93 pmol

min^{-1} mg $protein^{-1}$, respectively. Susceptible strain from IARI had the lowest cytochrome P450 activity of 0. 83 pmol min^{-1} mg $protein^{-1}$ (Table 10).

Cytochrome P450 activity was significantly lower in IARI strain and significantly higher in Mulangunnathukavu strain. Thikkodi and Angamali strains also had statistically significant cytochrome P450 activity over all the other strains, except Mulangunnathukavu strain. Among the field strains, Valiyathura strain had significantly lower cytochrome P450 activity, but the enzyme activity was significantly higher than the susceptible strain.

4.3 EVALUATION OF NEW INSECTICIDE MOLECULES AGAINST DIFFERENT POPULATIONS OF *Tribolium castaneum*

4.3.1 Susceptibility of Different Strains of *Tribolium castaneum* to Bifenthrin

Residual film bioassay was carried out to evaluate the susceptibility of different strains of *T. castaneum* to new insecticide molecule bifenthrin. Olavakkode strain was the most susceptible (LC_{50} = 15.12 ppm) and Valiyathura strain was least susceptible (LC_{50} = 56.19 ppm) to bifenthrin. LC_{50} values of bifenthrin for Mulangunnathukavu, Thikkodi, Angamaly and susceptible strains were 38.62 ppm, 35.73 ppm, 28.61 ppm and 15.66 ppm, respectively (Table 11).

Valiyathura strain had the highest LC_{90} value of 336.77 ppm and IARI strain had the lowest LC_{90} value of 57.37 ppm. The order of LC_{90} value in other strains was Angamaly (257.84 ppm) > Thikkodi (239.42 ppm) > Mulangunnathukavu (126.89 ppm) > Olavakkode (100.97 ppm).

Based on fiducial limit values, only Mulangunnathukavu and Valiyathura strains had significantly higher LC_{50} value over the susceptible strain.

Table 9: Glutathione S- transferase activity (µmol min^{-1} mg $protein^{-1}$) in different population of *Tribolium castaneum*

Population	Glutathion S- transferase activity (µmol min^{-1} mg $protein^{-1}$)
Mulangunnathukavu	0.28^{b}
Valiyathura	0.28^{b}
Thikkodi	0.30^{c}
Olavakkode	0.37^{d}
Angamali	0.39^{e}
Susceptible	0.28^{a}

Figures followed by same letters are not significantly different at 0.05 % level of significance

Table 10: Cytochrome P450 activity (pmol min^{-1} mg $protein^{-1}$) in different population of *Tribolium castaneum*

Population	Cytochrome P450 activity (pmol min^{-1} mg $protein^{-1}$)
Mulangunnathukavu	1.02^{d}
Valiyathura	0.88^{b}
Thikkodi	0.93^{c}
Olavakkode	0.85^{a}
Angamaly	0.93^{c}
Susceptible	0.83^{a}

Figures followed by same letters are not significantly different at 0.05 % level of significance

Table 11: Susceptibility of different population of *Tribolium castaneum* to bifenthrin

Population	Heterogeneity		LC_{50} (ppm) (95 % fiducial limit)	LC_{90} (ppm) (95 %Fiducial limit)	Slope
	d.f.	χ^2			
Mulangunnath-ukavu	3	1.07	38.62 24.90 – 48.10	126.89 95.20– 48.1	2.48
Valiyathura	3	2.33	56.19 32.30 – 78.00	336.77 221.10-776.70	1.65
Thikkodi	3	1.74	35.73 17.70 – 50.50	239.42 147.70–785.80	1.55
Olavakkode	4	3.65	15.12 7.70 – 21.90	100.97 69.00 – 202.40	1.55
Angamaly	4	3.41	28.61 16.90 – 39.80	257.84 143.20 –986.30	1.34
Susceptible	4	3.49	15.66 12.50 – 19.50	57.37 42.00 – 92.90	2.27

LC_{50} = Concentration (ppm) calculated to give 50 per cent mortality; LC_{90} = Concentration (ppm) calculated to give 90 per cent mortality

4.3.2 Susceptibility of Different Strains of *Tribolium castaneum* to Chlorfenapyr

All strains of *T. castaneum* were highly susceptible to chlorfenapyr compared to other test insecticides. The highest susceptibility (Table 12) was exhibited by Thikkodi strain with LC_{50} value of 2.55 ppm and the lowest by Olavakkode strain with LC_{50} of 4.72 ppm. The order of susceptibility of other strains to chlorfenapyr was susceptible strain from IARI (LC_{50} = 3.21 ppm) > Valiyathura (LC_{50} = 3.24 ppm) > Mulangunnathukavu (LC_{50} = 3.87 ppm) > Angamaly (LC_{50} = 4.47 ppm).

The highest LC_{90} value was observed in Mulangunnathukavu strain (10.53 ppm) and the lowest was observed in Valiyathura strain (7.12 ppm). Thikkodi, Olavakkode, Angamaly and IARI strains had LC_{90} values of 7.44 ppm, 10.50 ppm, 10.48 ppm and 9.01 ppm, respectively.

All the strains had overlapping fiducial limits at LC_{50} as well as LC_{90} levels.

4.3.3 Susceptibility of Different Strains of *Tribolium castaneum* to Spinosad

Susceptibility to spinosad was very low in all the strains of *T. castaneum* and hence LC_{50} values were very high with all the strains evaluated. Valiyathura strain showed the least susceptibility with LC_{50} of 9607.80 ppm. Susceptible strain from IARI had the lowest LC_{50} of 3013.3 ppm. LC_{50} of spinosad in Mulangunnathukavu, Thikkodi, Olavakkode and Angamaly strains were 7172.0 ppm, 7251.6 ppm, 6462.2 ppm and 7947.5 ppm, respectively.

LC_{90} values were also high for all the strains of *T. castaneum* and the highest value of 46283 ppm was recorded in Valiyathura strain. The lowest LC_{90} of spinosad was observed in Olavakkode strain (29716 ppm). LC_{90} values of 32589 ppm, 31379 ppm, 32098 ppm and 29803 ppm were observed in Mulangunnathukavu, Thikkodi, Angamaly and IARI strains, respectively (Table 13).

Table 12: Susceptibility of different population of *Tribolium castaneum* to chlorfenapyr

Population	Heterogeneity		LC_{50} (ppm) (95 % fiducial limit)	LC_{90} (ppm) (95 % fiducial limit)	Slope
	d.f.	χ^2			
Mulangunnat-hukavu	4	6.58	3.87 2.90– 5.90	10.53 7.0 – 25.3	2.95
Valiyathura	4	7.99	3.24 1.80– 4.40	7.12 5.1 – 13.6	3.75
Thikkodi	4	2.91	2.55 2.10 – 3.10	7.44 5.8 – 10.4	2.76
Olavakkode	3	0.65	4.72 3.90 – 5.50	10.50 8.4 – 15.5	3.69
Angamaly	4	4.24	4.47 3.40 – 5.60	10.48 7.8 – 18.6	3.46
Susceptible	3	1.88	3.21 2.50 – 3.90	9.01 7.0 – 13.3	2.86

LC_{50} = Concentration (ppm) calculated to give 50 per cent mortality; LC_{90} = Concentration (ppm) calculated to give 90 per cent mortality

Table 13: Susceptibility of different population of *Tribolium castaneum* to Spinosad

Population	Heterogeneity		LC_{50} (ppm) (95 % fiducial limit)	LC_{90}	Slope
	d.f.	χ^2			
Mulankunn-athukavu	4	1.34	7172.0 5564.50-9168.60	32589.0 19600-122360	1.949
Valiyathura	4	2.27	9607.8 7612.90-14188.00	46283.0 24682-274180	1.877
Thikkodi	4	2.22	7251.6 5697.30-9195.80	31379.0 19315-106000	2.014
Olavakkode	4	1.36	6462.2 4837.30-8160.10	29716.0 18217-106340	1.934
Angamaly	4	1.35	7947.5 6437.00-10195.00	32098.0 19968-99273	2.114
Susceptible	4	0.68	3013.3 1764.40-4227.50	29803.0 15955-128190	1.288

LC_{50} = Concentration (ppm) calculated to give 50 per cent mortality; LC_{90} = Concentration (ppm) calculated to give 90 per cent mortality

Based on the comparison of fiducial limits at LC_{50}, the field collected strains had significantly higher LC_{50} values over the susceptible strain.

4.3.4 Susceptibility of Different Strains of *Tribolium castaneum* to Flubendiamide

Residual film bioassay revealed that, susceptibility of different strains of *T. castaneum* to flubendiamide is very low. Mortality in all the strains could not be observed even at a dose of 20000 ppm after 48 h of exposure.

4.4 ESTIMATION OF RESIDUES OF INSECTICIDES IN RICE GRAINS

4.4.1 Method Development and Validation for Analysis of Pesticide Residues in Rice

For the analysis of pesticide residues in rice, the development of a method satisfying the requirements of linearity, limit of detection (LOD), limit of quantification (LOQ) and recovery is essential. The results of the method development and validation studies for estimation of pesticide residues in rice are given below.

4.4.1.1 Preparation of Calibration Curve and Checking of Linearity

Individual standards of 500 ppb concentration was prepared for malathion, chlorfenapyr, bifenthrin and deltamethrin in hexane. These standards were injected into gas chromatograph for identifying the retention time and for getting corresponding chromatograms (Fig. 4, Fig. 5, Fig. 6 and Fig. 7). Retention time of malathion, chlorfenapyr, bifenthrin and deltamethrin were 10.39, 11.03, 11.61 and 14.24 min. respectively (Table 14)

Each insecticide was injected six times into gas chromatograph at 50 ppb concentration to check the specificity of the method developed. Relative standard deviations of peak area and retention time were calculated from the obtained chromatogram. Relative standard deviations of peak area were 4.04, 3.51, 4.60 and

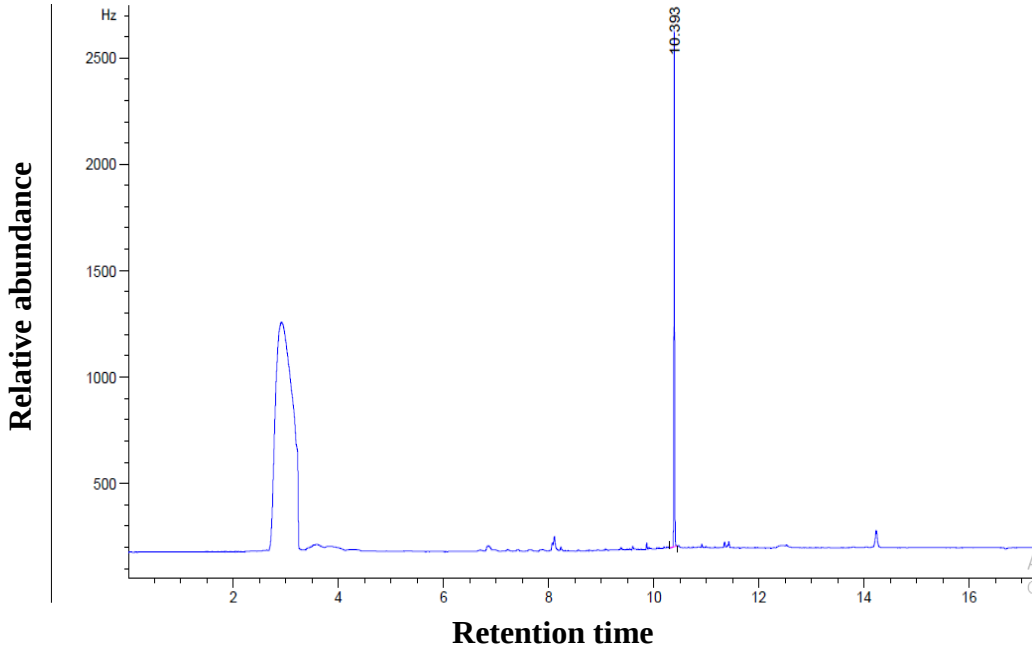

Fig. 4: Chromatogram of malathion at 500 ppb

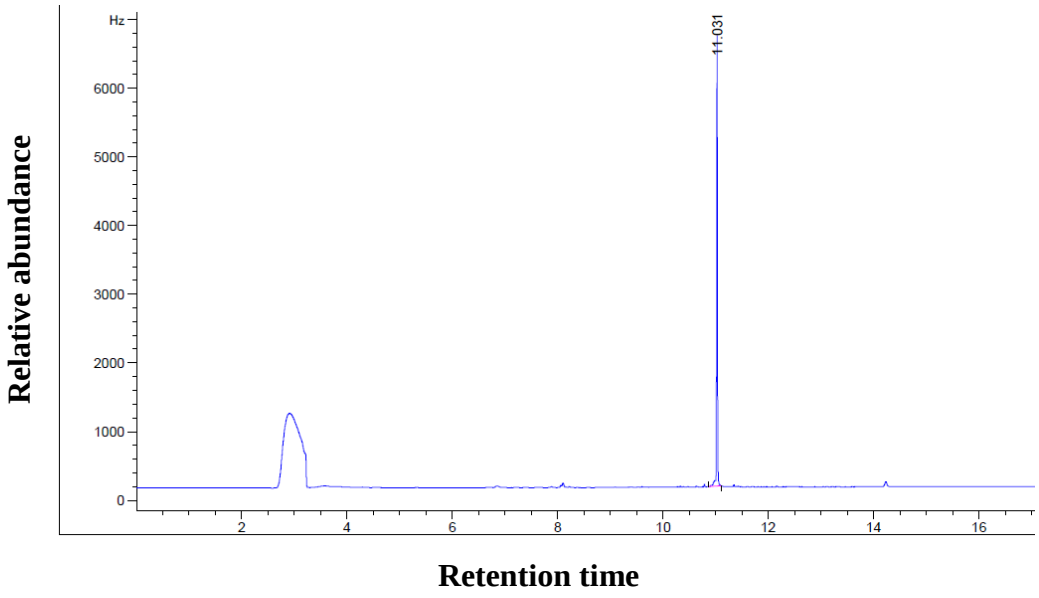

Fig. 5: Chromatogram of chlorfenpayr at 500 ppb

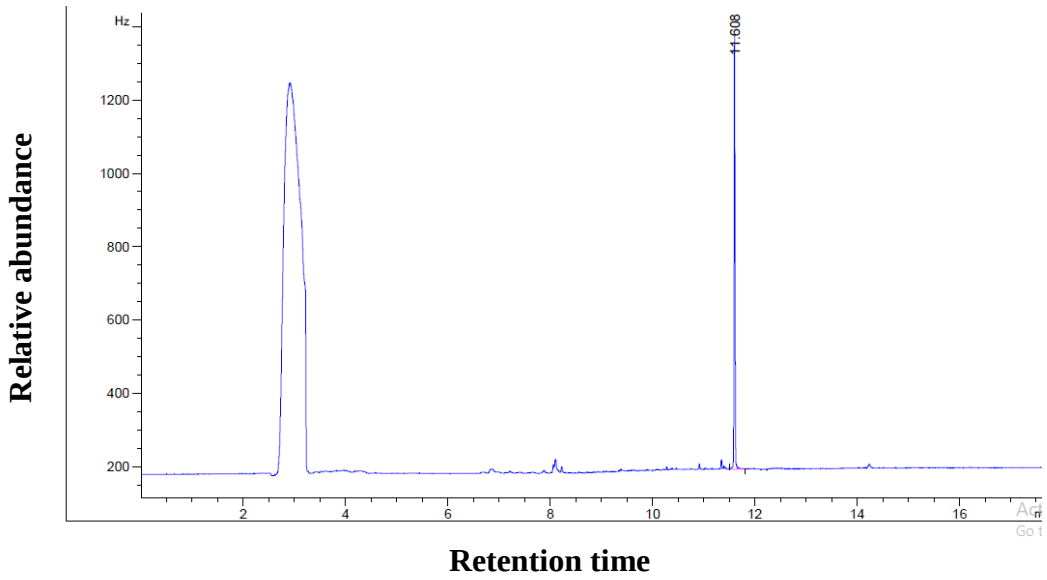

Fig. 6: Chromatogram of bifenthrin at 500 ppb

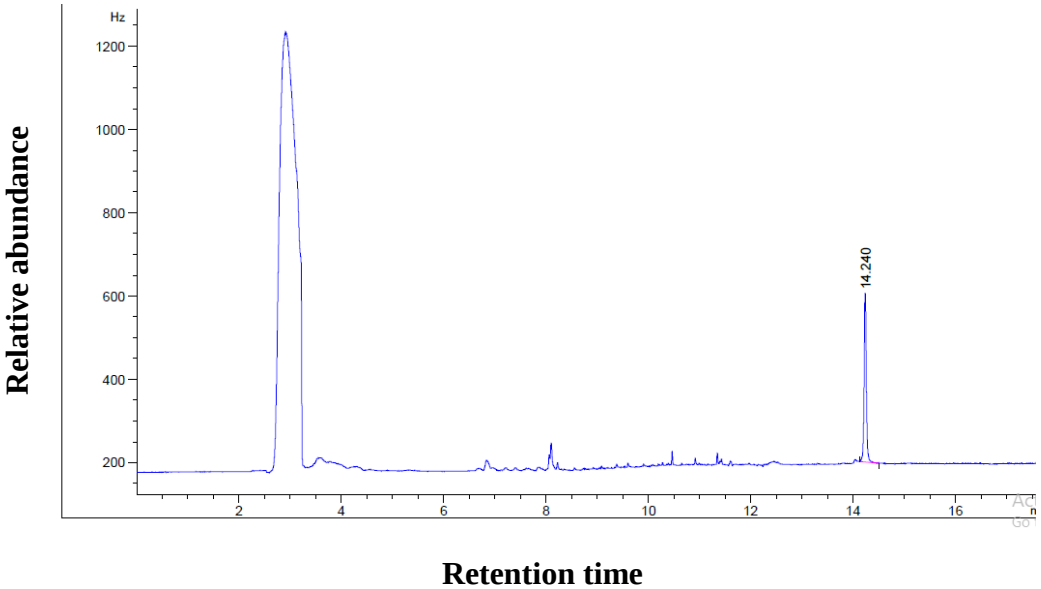

Fig. 7: Chromatogram of deltamethrin at 500 ppb

Table 14: Retention time of different insecticides

Insecticide	Retention time (min.)
Malathion	10.39
Chlorfenapyr	11.03
Bifenthrin	11.61
Deltamethrin	14.24

Table 15: Relative standard deviation (RSD) of peak area and retention time of insecticides

Insecticide	RSD of peak area (%)	RSD of retention time (%)
Malathion	4.04	0.000
Chlorfenapyr	3.51	0.005
Bifenthrin	4.60	0.004
Deltamethrin	2.80	0.024

2.80 per cent (Table 15) for malathion, chlorfenapyr, bifenthrin and deltamethrin, respectively which were within the acceptable range (<5%). The calculated RSD for retention time was 0.000, 0.005, 0.004 and 0.024 per cent for malathion, chlorfenapyr, bifenthrin and deltamethrin, respectively which again were within the acceptable range (<2%) (Table 15).

A calibration curve was prepared by plotting different concentrations (0.05, 0.1, 0.2, 0.3, 0.4 and 0.5 ppm) on x-axis and peak area on y- axis. Slope and R^2 of calibration curve of malathion were 3030.8 and 0.997, respectively (Fig .8). Calibration curve of chlorfenapyr had a slope and R^2 of 9596.3 and 0.996, respectively (Fig. 9). Corresponding values for bifenthrin and deltamethrin were 27029, 0.993 and 3091.7, 0.992 respectively (Fig. 10 and Fig. 11). Response was linear for all the four pesticides within the range of 0.05 to 10 ppm.

4.4.1.2 Determination of Limit of Detection (LOD)

Limit of detection was calculated for malathion, chlorfenapyr, bifenthrin and deltamethrin from the matrix matched calibration data by using the formula given in section 3.6.2.3. The limits of detection were 0.02, 0.01, 0.02 and 0.02 ppm for malathion, chlorfenapyr, bifenthrin and deltamethrin, respectively (Table 16).

4.4.1.3 Determination of Limit of Quantification (LOQ)

Limit of quantification for all the test insecticides was estimated by the formula described in section 3.6.2.4. Limit of quantification for malathion, chlorfenapyr, bifenthrin and deltamethrin were 0.07, 0.04, 0.08 and 0.08 ppm, respectively (Table 16). Limit of quantification of all the insecticides were in the range of 0.04 to 0.08 ppm.

4.4.1.4 Determination of Recovery

Recovery of malathion and deltamethrin was better when ethyl acetate was used as the solvent, while that of chlorfenapyr and bifenthrin were better in acetonitrile. The recovery of each pesticide from rice was estimated by comparing the peak area of spiked standards with those of the pure standards. Good recovery

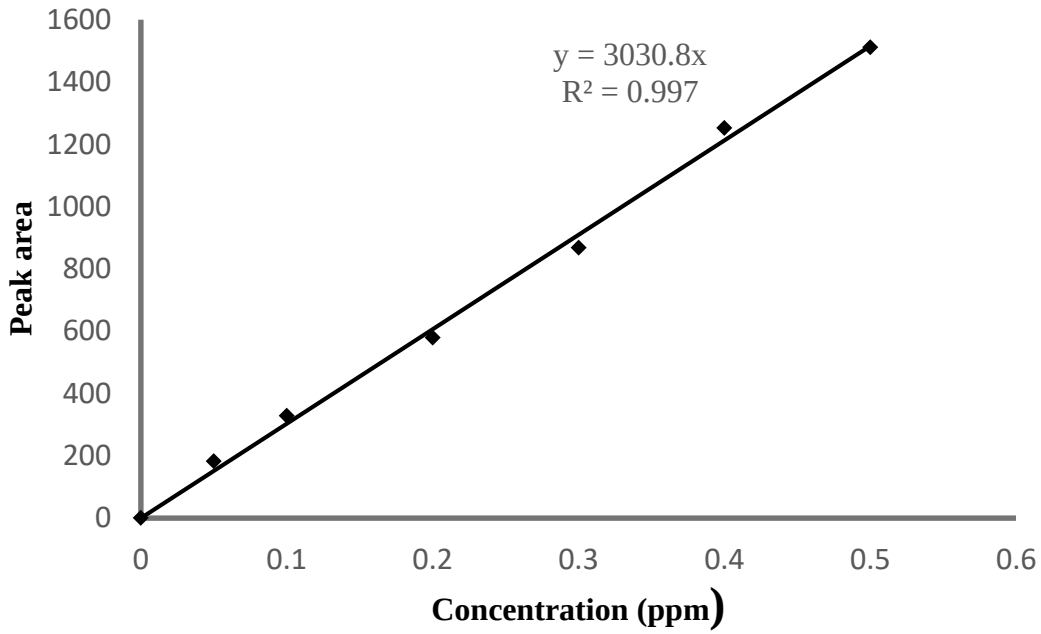

Fig. 8: Calibration curve of malathion

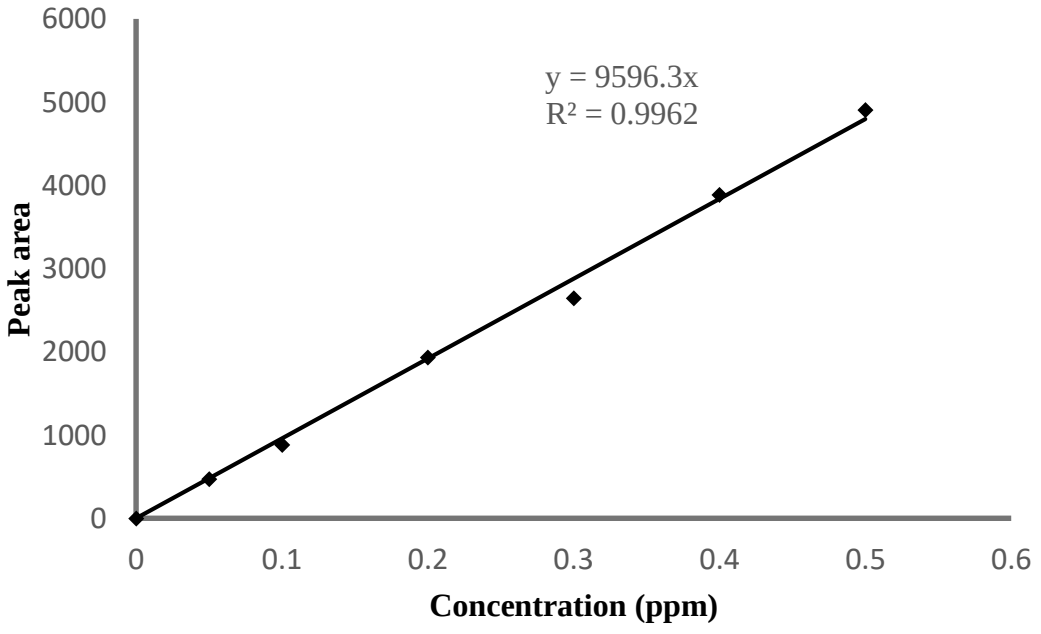

Fig. 9: Calibration curve of chlorfenapyr

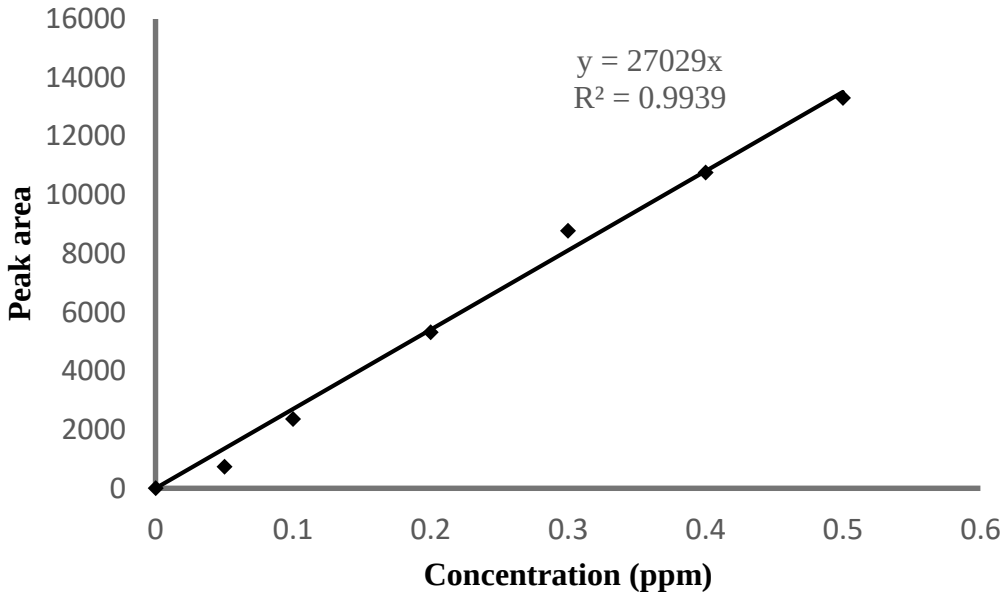

Fig. 10: Calibration curve of bifenthrin

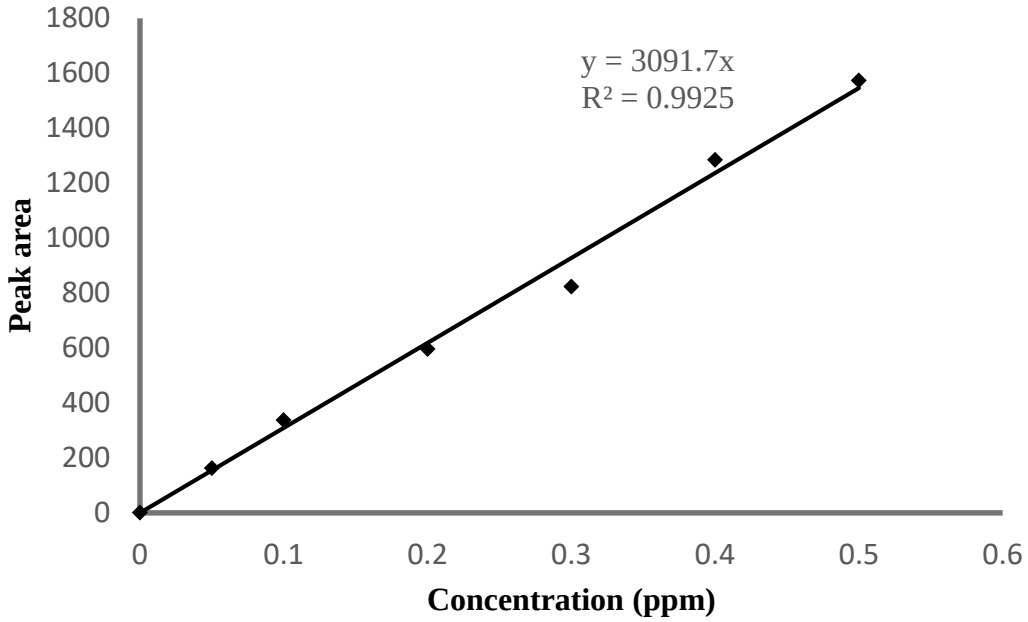

Fig. 11: Calibration curve of deltamethrin

Table 16: Limit of detection and limit of quantification of insecticides in ppm

Insecticide	LOD (ppm)	LOQ (ppm)
Malathion	0.02	0.07
Chlorfenapyr	0.01	0.04
Bifenthrin	0.02	0.08
Deltamethrin	0.02	0.08

of the target residues was obtained by the method validated for the estimation of different insecticides in rice.

Determination of per cent recovery was done at three fortification levels *viz*., 0.05 ppm (LOQ), 0.25 ppm (5 X LOQ) and 0.50 ppm (10 X LOQ). When the matrix was fortified at 0.05 ppm, the mean percentage recoveries of malathion, chlorfenapyr, bifenthrin and deltamethrin were 111.08, 94.46, 95.18 and 106.82 per cent, respectively (Table 17). Relative standard deviation for malathion, chlorfenapyr, bifenthrin and deltamethrin were 5.13, 2.80, 8.48 and 6.39, respectively (Table 17) which were within the acceptable limit of ≤20 per cent.

The mean per cent recoveries of malathion, chlorfenapyr, bifenthrin and deltamethrin were 96.44, 114.4, 89.58 and 92.66 per cent, respectively at the fortification level of 0.25 ppm (Table 17). Relative standard deviation values were 1.98, 2.89, 3.22 and 6.36 for malathion, chlorfenapyr, bifenthrin and deltamethrin, respectively, which were also within the acceptance limits (Table 17).

At a fortification level of 0.5 ppm, the mean per cent recoveries obtained were 102.66, 113.54, 85.42 and 109.3 per cent for malathion, chlorfenapyr, bifenthrin and deltamethrin, respectively (Table 17). Relative standard deviations of malathion, chlorfenapyr, bifenthrin and deltamethrin were 0.53, 2.50, 2.66 and 2.47, respectively (Table 17). The RSD values were within the acceptable limit of ≤20 per cent.

4.4.2 Pesticide Residue Analysis

Residues of malathion, chlorfenapyr, bifenthrin and deltamethrin in rice samples were analysed at different sampling intervals, after spraying the samples with commercial pesticide formulations. The results are given in Table 18. Two hours after insecticide spray, malathion at 0.084 ppm alone was detected which was below the MRL level of 4 ppm fixed by FSSAI, while chlorfenapyr, bifenthrin and deltamethrin were below quantification limit. Residue levels of all the four insecticides were below quantification levels one and third day after spraying.

Table 17: Recovery of insecticides in rice at different fortification levels

Insecticide	Level of fortification					
	0.05 ppm (LOQ)		0.25 ppm (5 X LOQ)		0.5 ppm (10 X LOQ)	
	Mean recovery (%) ± SD	Relative standard deviation (%)	Mean recovery (%) ± SD	Relative standard deviation (%)	Mean recovery (%) ± SD	Relative standard deviation (%)
Malathion	111.08 ± 2.85	5.13	96.44 ± 4.79	1.98	102.66 ± 2.76	0.53
Chlorfenapyr	94.46 ± 1.32	2.80	114.4 ± 8.28	2.89	113.54 ± 14.23	2.50
Bifenthrin	95.18 ±4.03	8.48	89.58 ± 7.21	3.22	85.42 ± 11.37	2.66
Deltamethrin	106.82 ± 3.41	6.39	92.66 ± 14.75	6.36	109.3 ± 13.52	2.47

Table 18: Pesticide residues in rice at different sampling intervals

Insecticide	Residue after 2 hours of spraying (ppm)	Residue after 1 day of spraying (ppm)	Residue after 3 days of spraying (ppm)
Malathion	0.08	BDL	BDL
Chlorfenapyr	BDL	BDL	-
Bifenthrin	BDL	BDL	-
Deltamethrin	BDL	BDL	-

BDL = Below Detection Limit

Discussion

5. DISCUSSION

The susceptibility of various population of *T. castaneum* collected from different FCI godowns of Kerala were compared with the susceptibile IARI strain to assess their susceptibility to commonly used insecticides in FCI godowns such as malathion, deltamethrin and dichlorovos. In order to determine the biochemical mechanism of resistance, the levels of various detoxifying enzymes in different strains of *T. castaneum* were also assessed. Effectiveness of various molecules such as bifenthrin, chlorfenapyr, spinosad and flubendiamide were also estimated by bioassay and the most effective insecticides were sprayed on jute bags containing rice to determine the presence/absence of pesticide residues in rice. The data obtained through these experiments is discussed with the help of pertinent literature under the following headings.

5.1 Susceptibility of different populations of *Tribolium castaneum* to commonly used pesticides in FCI godowns.

5.2 Role of detoxifying enzymes in contributing resistance in different strains of *Tribolium castaneum*

5.3 Susceptibility of different *Tribolium castaneum* populations to newer pesticide molecules

5.4 Estimation of pesticide residues of selected insecticides in jute bags containing rice

5.1 SUSCEPTIBILITY OF DIFFERENT POPULATION OF *Tribolium castaneum* TO COMMONLY USED PESTICIDES IN FCI GODOWNS

5.1.1 Susceptibility of *Tribolium castaneum* Strains to Malathion

Resistance ratio of various *T. castaneum* strains collected from different FCI godowns of Kerala was calculated with respect to the susceptible IARI strain. Among the various populations, Angamaly strain showed 13.34 fold resistance to

malathion (Table 19). Resistance ratio for Mulangunnathukavu strain was 11.82, for Valiyathura 11.27, for Olavakkode 10.99, and for Thikkodi strain it was 10.95.

If resistance ratio was calculated based on LC_{90}, there was no change in the order of resistance among the field collected strains of *T. castaneum*. Resistance ratio was 10.07 for Valiyathura strain, 11.14 for Thikkodi strain, 11.57 for Olavakkode strain, 13.31 for Mulangunnathukavu strain and 16.54 for Angamaly strain (Table 19).

Log dose – probit (ld-p) lines could be used as a tool to compare different populations of a test insect with respect to its susceptibility to a particular insecticide. When compared the ld-p lines of malathion, a significant rightward shift could be observed in the field collected population from that of laboratory maintained susceptible strain (Fig. 12). Log dose – probit lines of all the field collected populations were having the same slope and were cluttered together, which indicated that all the *T. castaneum* populations collected in the present study were homogenous in response to malathion.

Malathion resistance in *T. casatneum* was reported way back in 1962 by Parkin. Later on, resistance to malathion in red flour beetle, *T. castaneum* was detected from several parts of the world (Lemon, 1966; Speirs *et al*. 1967; Toppozada *et al*. 1969; Dyte and Blackman, 1970; Champ and Campbell-Brown, 1970, Greening, 1970, Pieters *et al*. 1972; Zettler, 1974). The FAO global survey on pesticide resistance in stored product pests revealed that malathion resistance was wide spread in *T. castaneum* all over the world (Champ and Dyte, 1976).

Malathion resistance in *T. castaneum* was reported for first time in our country by Bhatia *et al.* (1971). *T. castaneum* population from Naraina godown of FCI was 37.76-fold resistant to malathion. This report was followed by the studies of Rajak *et al.* (1973), which reported 16-21-fold resistance to malathion in *T. castaneum* populations from Uttar Pradesh. Resistance to malathion by *T. castaneum* was detected later by various workers such as Pasalu and Bhatia (1983), Dhaliwal and Chawla (1995) and Srivastava *et al.* (2001).

In a survey carried out throughout the country, 13 populations of *T. castaneum* were collected from National Seed Programme (NSP) centres (Srivastava *et al*., 2001). The resistance level to malathion varied from 0.725 to 24.53 in different populations. The resistance levels detected in the current study is in concurrence with the findings of the above survey.

Malathion specific resistance in *T. castaneum* is wide spread and resistant phenotype has almost completely replaced the susceptible phenotype. The frequency of resistant gene is stable in the natural population even after the withdrawal of pesticide exposure (Arnaud and Haubruge, 2002; Haubruge and Arnaud, 2001). It was argued that there was a little or no reproductive disadvantage between malathion resistant and susceptible strain (Arnaud and Haubruge, 2002; Haubruge and Arnaud, 2001).

This is the first report of malathion resistance in *T. castaneum* populations from FCI godowns of Kerala. When compared the fiducial limits of various field collected strains of *T. castaneum* against malathion at LC_{50}, there was no difference between the populations which indicated uniform development of malathion resistance in all the FCI godowns surveyed in the study. This might be due to the same level and frequency of exposure to malathion, as FCI follows a common protocol for pesticide sprays across FCI godowns.

5.1.2 Susceptibility of *Tribolium castaneum* Strains to Dichlorvos

There was significant difference in susceptibility of different strains to dichlorvos when the fiducial limits at LC_{50} were compared. Resistance ranged from 1.74 fold in Mulangunnathukavu strain to 1.90 fold in Olavakkode strain. The resistance ratio for other strains were 1.84, 1.88 and 1.76 in Valiyathura, Thikkodi and Angamaly strains, respectively (Table 20).

The resistance ratio, calculated based on LC_{90} values, was highest for Olavakkode strain (1.41), followed by Thikkodi strain (1.36), Mulangunnathukavu strain (1.29), Valiyathura strain (1.26) and the lowest for Angamaly strain (1.18).

Table 19: Resistance ratio of different population of *Tribolium castaneum* to malathion

Population	Resistance ratio	
	LC_{50}	LC_{90}
Mulangunnathukavu	11.82	13.31
Valiyathura	11.27	10.07
Thikkodi	10.95	11.14
Olavakkode	10.99	11.57
Angamaly	13.34	16.54

Table 20: Resistance ratio of different population of *Tribolium castaneum* to dichlorvos

Population	Resistance ratio	
	LC_{50}	LC_{90}
Mulangunnathukavu	1.74	1.29
Valiyathura	1.84	1.26
Thikkodi	1.88	1.36
Olavakkode	1.90	1.41
Angamaly	1.76	1.18

The ld-p lines for dichlorovs was identical to the ld-p lines of malathion (Fig. 13). There was a significant shift in the ld-p lines of all the field collected strains towards right, indicating the development of resistance to dichlorvos. In addition to it, all the field collected populations were homogenous in resistance to dichlorvos.

Though dichlorvs had been in use for stored product pest management for the last 4-5 decades (Toppozada, *et al.,* 1969; Zettler, 1982; Harein *et al.,* 1970; Zettler and Jones, 1977; Subramanyam *et al.,* 2014), reports of resistance to dichlorvos is not common. Halliday *et al.* (1988) reported dichlorvos resistance in only one out of 15 strains of *T. castaneum* infesting stored peanuts in United States. The resistant strain also showed cross resistance to pirimiphos methyl. Rahman *et al.* (2007), however, reported dichlorvos resistance in all the field collected populations of *T. castaeum* from Bangladesh, with resistance varying from 1.78 to 7.67 similar to resistant ratio obtained in the present study.

In India, dichlorvos resistance ranging from 3.56 to 10.51 fold in *T. castaneum* populations collected from FCI godowns of Mirsapur and Alhabad was reported by Saxena and Sinha (1989). Similarly, 2.2 to 12.3 fold resistance to dichlorvos was detected in 27 field collected strains of *T. castaneum* by Saxena *et al.* 1992.

In the present investigation, LC_{50} values were higher in all the field collected strains compared to the susceptible strain and the resistance ratios obtained were comparable to that reported by Saxena *et al.* (1992). Comparable to the present results, Madhumathi *et al.,* (2000) and Visalakshi *et al.* (2005) reported low level of dichlorvos resistance in *T. castaneum*.

5.1.3 Susceptibility of *Tribolium castaneum* Strains to Deltamethrin

Deltamethrin resistance in *T. castaneum* strains was not significantly higher when compared with the susceptible strain. Based on the calculated values of resistance ratio, Thikkodi strain had the highest resistance ratio of 2.95 followed by Angamaly (2.12), Olavakkode (1.91), Valiyathura (1.50) and Mulangunnathukavu (1.46) (Table 21).

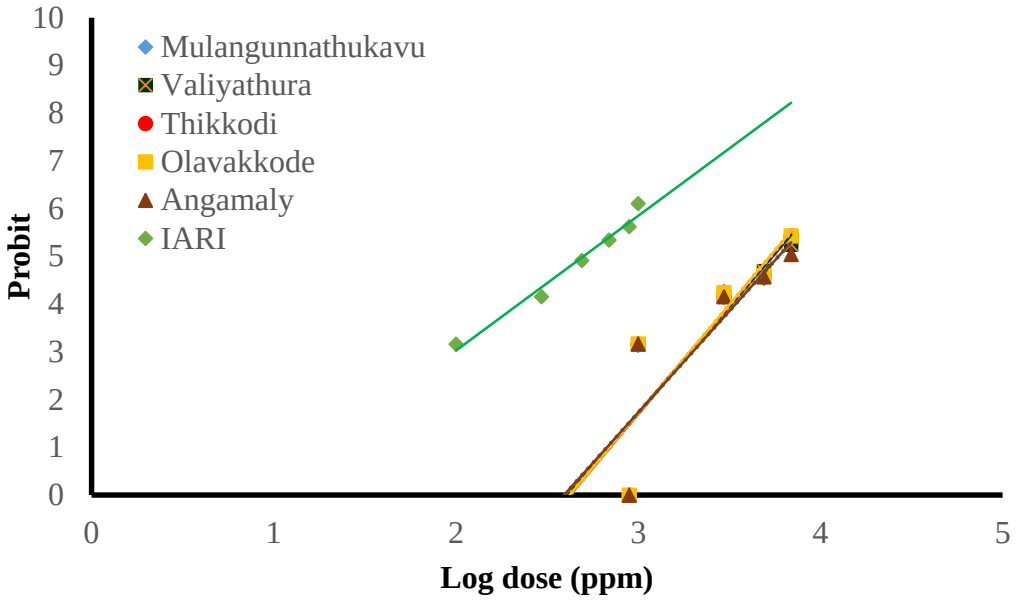

Fig. 12: Log dose – Probit line of malathion

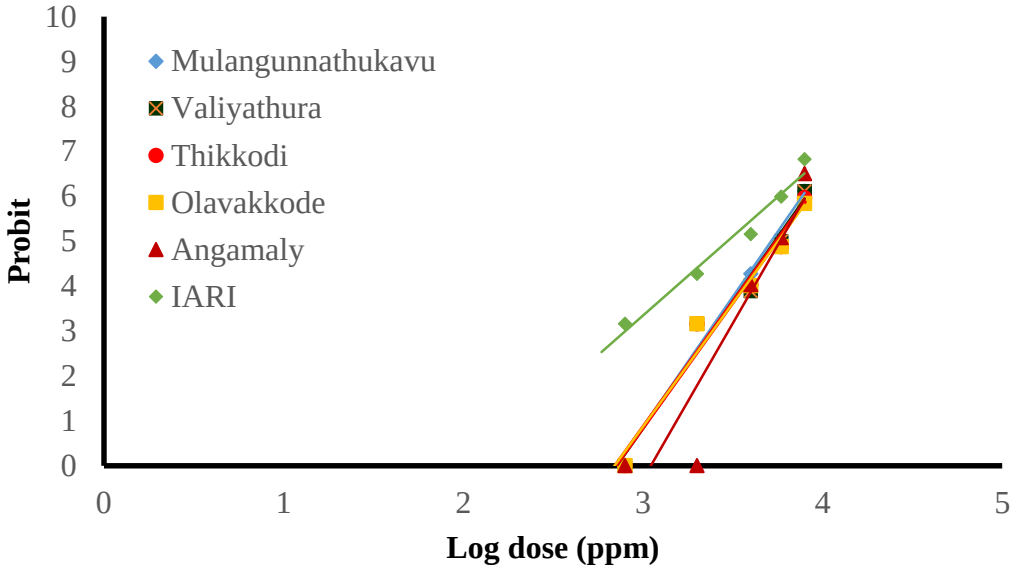

Fig. 13: Log dose – Probit line of dichlorvos

Based on LC_{90} values, Mulangunnathukavu, Valiyathura and Olavakkode strains were susceptible to deltamethrin (Table 21) as indicated by resistance ratios of 0.97, 0.43 and 0.82, respectively. However, Angamaly and Thikkodi strains had a slightly lowered susceptibility to delatamethrin with the resistance ratios being, 1.77 and 2.08, respectively.

The susceptible and field collected strains had parallel regression lines, which confirmed the susceptibility of all the *T. castaneum* strains to deltamethrin (Fig. 14).

Compared to the widespread malathion resistance in *T. castaneum*, resistance to deltamethrin in *T. castaneum* was relatively less frequent. Deltamethrin resistance was reported from field collected populations of *T. castaneum* (Collins, 1990; Rossi *et al*. 2010 and Riaz *et al.* 2018) and *R. dominica* (Lorini and Galley; 2000). As in the present study, Babu *et al.* (2017) reported a maximum of 1.6-fold resistance to deltamethrin in Bapatla strain of *R. dominica*.

Development of resistance to deltamethrin in *T. castaneum* was obtained after six generations of selection in laboratory (Padhee *et al.* 2002). While, a strain of *T. castaneum* with 370.54 resistance to deltamethrin was developed by Singh and Prakash (2013). Field level resistance to deltamethrin in *T. castaneum* has not been reported from India. Our study also confirmed that deltamethrin resistance in *T. castaneum* was not wide spread in FCI godowns of Kerala.

Resistance development to deltamethrin in stored product pest was low when compared to malathion in different parts of the world (Guedes *et al*. 1994; Guedes *et al*. 1995; Subramanyam and Haugstrum, 1996; Perez-Mendoza, 1999). The low levels of tolerance to deltamethrin by *T. castaneum* in the present study indicates that use of deltamethrin has not resulted in any resistance development in *T. castaneum* strains from FCI godowns of Kerala to deltamethrin.

5.2 ROLE OF DETOXIFYING ENZYMES IN CONTRIBUTING RESISTANCE IN DIFFERENT STRAINS OF *Tribolium castaneum*

Esterases, monoxygenases and glutathione S- transferases are the three major groups of enzymes generally involved in insecticide resistance. Increased production of metabolic enzymes can aid in insecticide resistance by binding to the pesticide and/or by metabolising the pesticide into non-toxic compounds (Bass and Field, 2011).

5.2.1 Role of Carboxylesterases in Insecticide Resistance in Different Strains of *Tribolium castaneum*

Carboxylesterase activity was significantly higher in all the field collected strains over the susceptible IARI strain. The level of esterase enzyme was 1.15 to 1.49-fold higher in field collected strains of *T. castaneum* compared to the latter (Fig. 15). Increase in esterase activity was 1.49-fold, 1.22-fold, 1.44-fold, 1.15-fold and 1.22-fold in Mulangunnathukavu, Valiyathura, Thikkodi, Olavakkode and Anagamaly strains, respectively, over the susceptible strain.

Esterases are important in imparting resistance to organophosphorous insecticides in insects (Dyte and Rowlants, 1968) by hydrolysing ester, thioester and amide bonds in pesticides. Detoxification by esterases had been associated in resistance to malathion in *T. castaneum* populations by several authors (Navarro *et al*. 1986; Subramanyam *et al*. 1989; Wool and Front, 2001; Haubruge *et al*. 2002).

The resistance observed in *T. castaneum* strains in this current study to malathion could be due to the higher esterases activity in these strains. Elevated levels of esterases also contribute to pyrethroid resistance in many insects (Boyer *et al*. 2012). But, such a relationship between esterase activity and delamethrin was not evident in the present study.

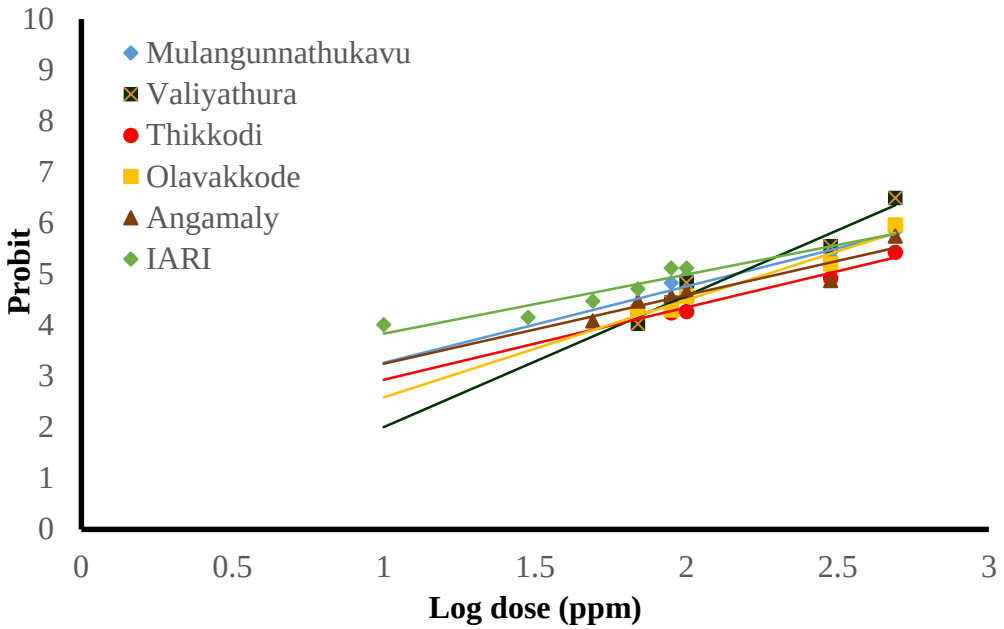

Fig. 14: Log dose – Probit line of deltamethrin

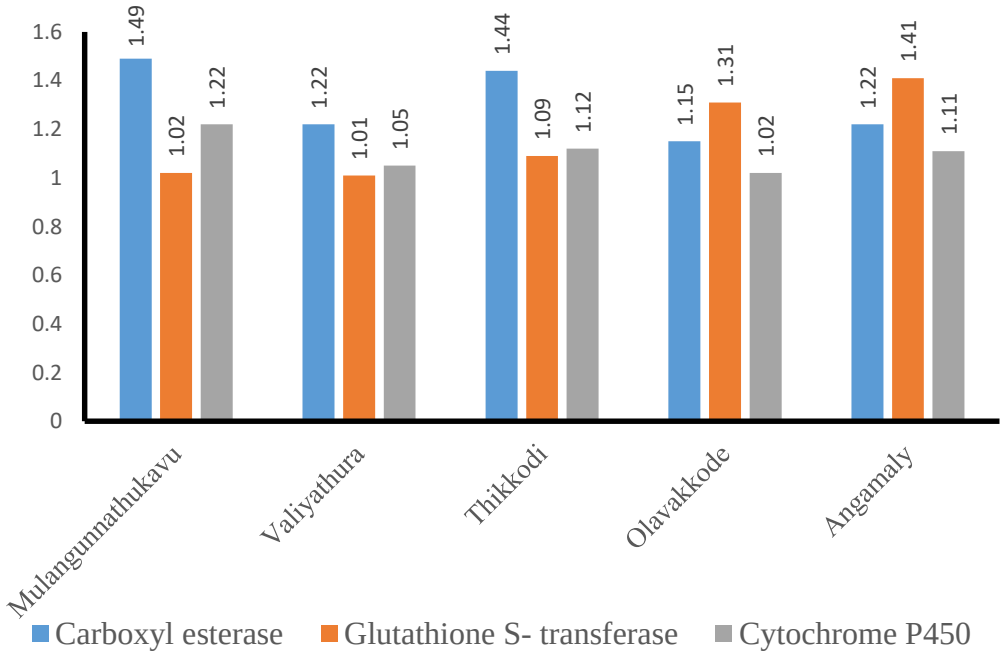

Fig. 15: Increase in enzymatic activity in the field strains of *T. castaneum* over susceptible strain

Carboxyl esterase mediated malathion specific resistance in a strain of *T. castaneum* without cross resistance to natural pyrethrins had been reported by Lloyd and Ruczkowski (1980).

Carboxylesterases are enzymes which catalyses the hydrolysis of carboxylic esters into the component acid and alcohol (Yan *et al*. 2009, Hatfield *et al*. 2016). Comparing the structure of malathion and dichlorvos, it would be evident that the carboxylic ester bond is present in malathion and absent in dichlorvos (Fig. 16 and 17) and that could be the possible reason for resistance to malathion in *T. castaneum* and not contributing to wide–spread cross resistance to dichlorvos .

5.2.2 Role of Glutathione-S-transferases in Insecticide Resistance in Different Strains of *Tribolium castaneum*

Glutathione-S-transferases (GST) are a family of multifunctional enzymes, ubiquitous in aerobic organisms and involved in the detoxification of xenobiotics like pesticides. They catalyse the conjugation of reduced glutathione and xenobiotics, rendering them water soluble which can be easily excreted (Enayati *et al*. 2005).

The increase in GST levels over susceptible strain was highest in Angamaly strain (1.41) and lowest in Valiyathura strain (1.01). Glutathione-S-transferase levels were 1.02, 1.09 and 1.31 fold higher over IARI strain in Mulangunnathukavu, Thikkodi and Olavakkode strains, respectively (Fig. 15).

GST activity was higher by 1.4 to 1.8-fold in a fenitrothion resistant strain of *Oryzaephilus surinamensis*, though it did not contribute to fenitrothion resistance (Rose and Wallblank, 1986). Reidy *et al*. (1990) also reported elevated levels of GST in cyfluthrin resistant *T. castaneum*, which showed resistance to organophosphates.

More than 2-fold increase in GST level lead to pyrethroid resistance in *S. zeamais* (Fragoso e*t al*. 2003). In the present study, though there was a significant

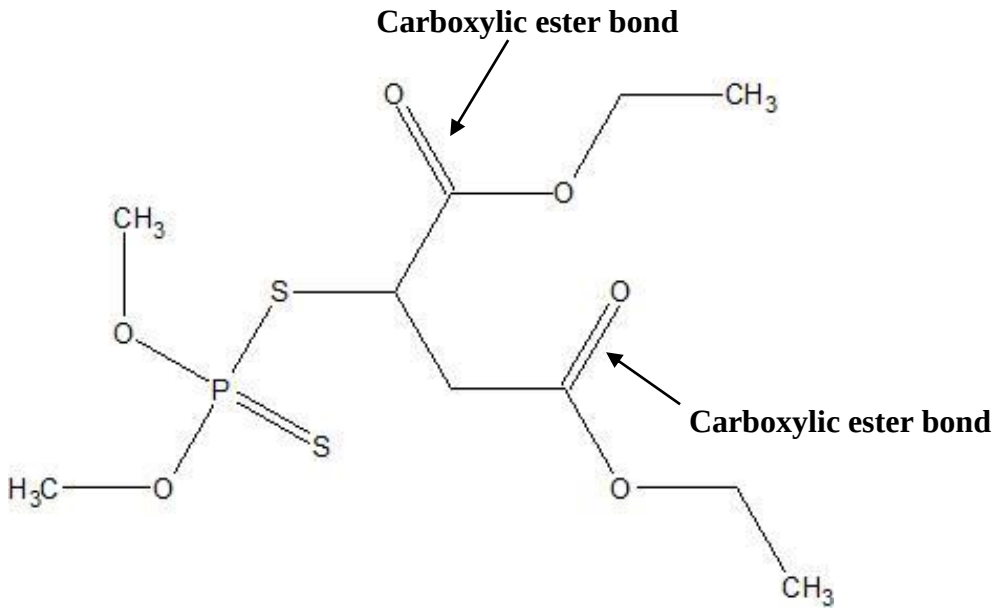

Fig. 16: Structure of malathion

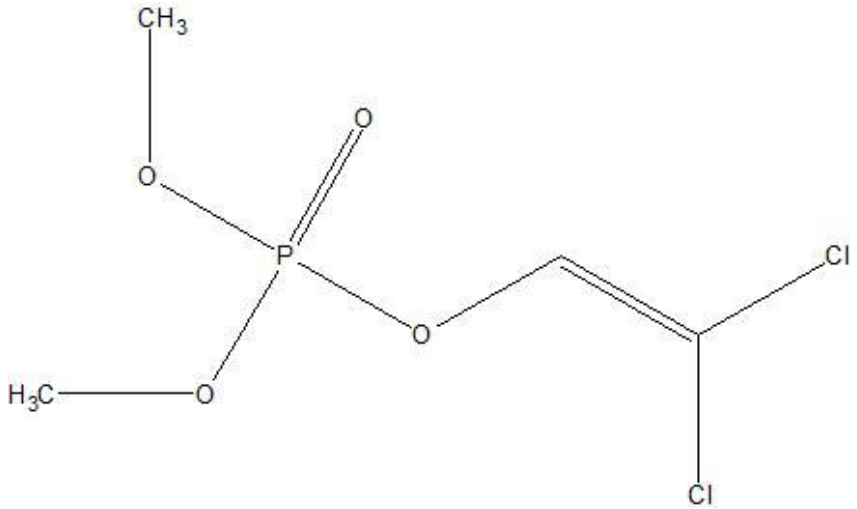

Fig. 17: Structure of dichlorvos

increase in the level of GST, resistance to deltamethrin, a synthetic pyrethroid, could not be observed.

5.2.3 Role of Cytochrome-P450 monooxygenases in Insecticide Resistance in Different Strains of *Tribolium castaneum*

Cytochrome P450 monooxygenases are membrane bound enzymes in endoplasmic reticulum, which catalyse hydroxylation reactions. They are involved in catabolism and anabolism of xenobiotics. They oxidise pesticides rendering them water soluble or may readily form complexes with pesticides so that the pesticides can be transported and excreted (Scott, 1999; Lee and Lees, 2001).

The increase in cytochrome P450 levels over the susceptible strain was highest in Mulangunnathukavu strain (1.22-fold), followed by Thikkodi (1.12-fold), Angamaly (1.11-fold), Valiyathura (1.05-fold) and Olavakkode strains (1.02-fold) (Fig. 15).

Cytochrome P450 levels were 3 and 10-fold higher in fenitrothion resistant larvae and adults of *O. surinamensis*, respectively (Rose and Wallblank, 1986). Cytochrome P450 was also involved in the conversion of malathion to malaoxon, a more toxic metabolite when compared to malathion (Lee and Lees, 2001). But, in the current investigation, the field strains were resistant to malathion. Even though, there was an increase in the level of cytochrome P450 in the field strains, which could have led to the increased conversion of more toxic malaoxon, there was a decrease in susceptibility to malathion. Scott (1999) was of the opinion that resistance to organophosphates could also be achieved by decreased activation of organophosphates to more toxic compounds.

Cytochrome P450 monooxygenases were involved in fenitrothion (an organophosphorous insecticide) resistance in a strain of *O. surinamensis* (Collins *et al.* 1992; Kotze and Wallblank, 1996). Similarly, there was a significant increase in the activity of cytochrome P450 and reduced susceptibility of field collected populations of *T. castaneum* to malathion and dichlorovos, as in the present study.

Increased levels of cytrochrome P450 in a multi-resistant strain of *T. castaneum* was detected by Cohen (1982). Even though the level of increase in cytochrome P450 in the field strains collected in the present study was significant, it was not contributing to multiple insecticide resistance as deltamethrin resistance could not be detected in any of the field strains.

5.2.4 Correlation between LC_{50} Values and Enzyme Activity in *Tribolium castaneum*

Carboxylesterase and cytochrome P450 levels were significantly correlated with the LC_{50} values (Table 22), but there was no correlation between the increase in GST levels and LC_{50} values.

There was significantly higher correlation (at 1% level) with malathion and dichlorovos resistance and activity of carboxylesterase and cytochrome P450 monooxygenase. Though the LC_{50} values of deltamethrin were correlated to both esterases and cytochrome P450 monooxygenases, correlation was more pronounced between cytochrome P450 levels and LC_{50} of deltamethrin (significance at 1% level) than to carboxylesterases (significance at 5% level).

These results are in consonance with the findings of Lee and Lees, (2001) and Cohen (1986). Resistance to malathion and chlorpyriphos-methyl in *O. surinamensis* was found to be associated with carboxylesterases and cytochrome P450 monooxygenases (Lee and Lees, 2001). But, Cohen (1986) found that GST activity in a malathion resistant *T. castaneum* strain was not significantly higher than the susceptible strain.

Similar to the present findings, carboxylesterase was found to be responsible for resistance to organophosphorous insecticides (Singh, 2014; Cui *et al*. 2007; Liu *et al*. 2011; Li *et al.* 2007) as well as pyrethroids (Hemingway *et al*. 1990; Kranthi *et al.* 1997; Gunning *et al*. 1999; Li *et al.* 2007; Feng *et al.* 2018). Hence, resistance to malathion and dichlorovos and the reduced susceptibility to deltamethrin in the present study could be correlated to the increased levels of carboxylesterases.

Table 21: Resistance ratio of different population of *Tribolium castaneum* to deltamethrin

Population	Resistance ratio	
	LC_{50}	**LC_{90}**
Mulangunnathukavu	1.46	0.97
Valiyathura	1.49	0.43
Thikkodi	2.95	2.08
Olvakkode	1.90	0.82
Angamaly	2.11	1.77

Table 22: Correlation between LC_{50} value and enzyme activity

	Malathion	Dichlorovos	Deltamethrin
Carboxyl esterase	0.898**	0.621**	0.526**
Glutathione S-transferase	- 0.149	- 0.149	- 0.149
Cytochrome P450	0.65**	0.898**	0.898**

* Significant and 5% level

** Significant at 1% level

Cytochrome P450 monooxygenases were involved in the resistance of almost all groups of insecticides (Berge *et al.* 1998; Scott, 1999; Feyereisen, 2005). Enhanced levels of cytochrome P450, which lead to detoxification of organophosphates as well as synthetic pyrethroids, had been reported by several authors (Hemingway *et al*. 1990; Yu and Nguyen, 1992; Zhao *et al*. 1996; Ahmad *et al*. 2008). Similarly, in the present study, increased levels of cytochrome P450 is correlated to resistance against organophosphates, malathion and dichlorovos, and in reducing the susceptibility to deltamethrin.

There was no correlation with the increase in GST activity and the LC_{50} values of the insecticides tested. In the same way, Kranthi *et al*. 1997 found elevated levels of all the three detoxifying enzymes, esterases, monooxygenases and GST, in *Helicoverpa armigera*. But, elevated levels of GST could not be attributed to resistance to cypermethrin and fenvalerate.

5.3 RELATIVE TOXICITY OF VARIOUS INSECTICIDES TO DIFFERENT STRAINS OF *Tribolium castaneum*

The relative toxicity of selected insecticides to *T. castaneum* was worked out with respect to malathion in order to assess the superiority of insecticides over malathion.

5.3.1 Relative Toxicity of Dichlorovos and Deltamethrin to Different Strains of *Tribolium castaneum*

Dichlorovos was as toxic as malathion against *T. castaneum* strains except for the susceptible strain, where malathion was more toxic than dichlorovos (Table 23). For the field strains, relative toxicity values were 0.95, 0.96, 1.01, 1.12 and 1.25 for Olavakkode, Thikkodi, Valiyathura, Mulangunnathukavu and Angamaly strains, respectively, whereas, it was 0.16 for the susceptible strain. This indicates that, the field strains were not as susceptible to dichlorovos as IARI strain, suggesting possibility of cross resistance to malathion and dichlorovos in the field collected strains of *T. castaneum*.

Zettler (1982), who studied insecticide resistance in *T. castaneum* strains collected from peanut storage and processing facilities in United States could not detect any cross resistance to dichlorovos in malathion resistant *T. castaneum*. However, Halliday *et al*. (1988), who studied the same population six years later reported resistance to dichlorovos in 8 strains of *T. castaneum* which were resistant to malathion. Similarly, malathion resistant *T. castaneum* strains collected from various parts of Bangladesh were resistant to dichlorovos too. But the susceptible strain was more susceptible to dichlorovos than to malathion (Rahman *et al*. 2007). These studies are in consonance with the findings of the present study in that, dichlorovos was more toxic to the susceptible strain but less toxic to the field strains.

Deltamethrin, the pyrethroid insecticide recommended in FCI godowns, was toxic to all the strains of *T. castaneum*. With the susceptible IARI strain, deltamethrin was 5.29-fold more toxic than malathion. But, with the field collected strains, relative toxicity varied from 19.62 to 42.72. Relative toxicity was 19.62 in Thikkodi strain, 30.50 in Olavakkode strain, 33.39 in Angamaly strain, 39.79 in Valiyathura strain and 42.72 in Mulangunnathukavu strain (Table 23). This shows that there is no multiple resistance associated with resistant populations collected from FCI godowns of Kerala. It also showed that there was some fitness cost associated with resistance to organophosphates in the resistant populations. Mulangunnathukavu strain, which had significantly higher levels of carboxylesterase and cytochrome P450 monooxygenase, had the highest relative toxicity value, making it the most susceptible strain to deltamethrin.

A multiresistant strain of *T. castaneum* was released on peanuts treated with insecticides and the development as well as mortality of were observed by Daglish *et al.* (1992). Mortality of *T. castaneum* adults were below 1.3 per cent even at the highest dose of malathion (100 mg/kg), while, with deltamethrin, there was 96.60 per cent mortality, even at the lowest dose of 5 mg/kg, indicating the lack of cross-resistance between deltamethrin and malathion.

A *T. castaneum* strain (T_ca_43), collected from Italy was 14-fold resistant to deltamethrin but was highly susceptible to malathion (Rossi *et al*., 2010) which indicated the lack of cross resistance between deltamethrin and malathion in *T. castaneum*.

5.3.2 Relative Toxicity of *Tribolium castaneum* to New Molecules

5.3.2.1 Relative Toxicity of Tribolium castaneum to Bifenthrin

Bifenthrin, a synthetic pyrethroid, exhibited high toxicity to all the strains of *T. castaneum*. It was 33.25-fold toxic to susceptible strain with respect to malathion (Table 24). Among the field strains, bifenthrin was most toxic to Olavakkode strain (relative toxicity-378.85) followed by Angamaly strain (relative toxicity-242.94), Thikkodi strain (relative toxicity-159.61), Mulangunnathukavu strain (relative toxicity-159.43) and Valiyathura strain (relative toxicity-104.53) (Table 24).

The ld-p lines of different strains with bifenthrin also confirmed that all the strains *T. castaneum* had a similar response to bifenthrin (Fig. 18). Thus, the present study clearly brought out that, there is no cross resistance to pyrethroids in the strains of *T. castaneum* collected from FCI godowns of Kerala. Bifenthrin, though a synthetic pyrethroid similar to deltamethrin, is toxic to all strains of *T. castaneum* in the present study

Kljajic *et al.* (2006) had reported the high potential of bifenthrin for the management of *S. oryzae*, *S. granarius* and *S. zeamais.* In the present investigation, the field collected strains did not exhibit any significantly higher tolerance to deltamethrin and they were also susceptible to another synthetic pyrethroid bifenthrin.

A comparison of the LC_{50} values of bifenthrin and deltamethrin showed bifenthrin to be 2.62 to 12.42 fold more toxic to *T. castaneum* than deltamethrin. The highest toxicity to bifenthrin was exhibited by Olavakkode strain (12.42) followed by Angamaly strain (7.28), susceptible strain (6.28), Thikkodi strain

Table 23: Relative toxicity of dichlorvos and deltamethrin to different population of *Tribolium castaneum* with respect to malathion

Population	Dichlorvos	Deltamethrin
Mulangunnathukavu	1.12	42.72
Valiyathura	1.01	39.79
Thikkodi	0.96	19.62
Olavakkode	0.95	30.50
Angamaly	1.25	33.39
Susceptible	0.16	5.29

Table 24: Relative toxicity of different population of *Tribolium castaneum* to new molecules with respect to malathion

Population	Bifenthrin	Chlorfenapyr	Spinosad
Mulangunnathukavu	159.43	1650.75	0.86
Valiyathura	104.53	1896.97	0.61
Thikkodi	159.61	2236.65	0.79
Olavakkode	378.85	1214.32	0.89
Angamaly	242.94	1554.76	0.87
Susceptible	33.25	162.28	0.17

Table 25: Relative toxicity of bifenthrin to different population of *Tribolium castaneum* with respect to deltamethrin

Population	Relative toxicity
Mulangunnathukavu	3.73
Valiyathura	2.63
Thikkodi	8.13
Olavakkode	12.42
Angamaly	7.28
Susceptible	6.28

(8.13), Mulagunnathukavu strain (3.73) and least toxic to Valiyathura strain (2.63) (Table 25).

These results were contrary to the findings of Reddy (2002), who observed that both EC and WP formulations of bifenthrin were less toxic against the susceptible strain of *T. castaneum* than to deltamethrin EC.

Similarly, working with susceptible and phosphine resistant strains of *T. castaneum*, Pathrose *et al.* (2005), found bifenthrin to be less toxic when compared to deltamethrin with film residue method of bioassay.

The variability in results could be due to the use of technical material of bifenthrin in the present study as against the use of commercial formulations in the above studies.

5.3.2.2 Relative Toxicity of Tribolium castaneum to Chlorfenapyr

Chlorfenapyr, a pyrrole insecticide which affects ATP synthesis, was the most toxic among all the insecticides evaluated in this study. The relative toxicity of chlorfenapyr in comparison with malathion was 162.28 in susceptible strain, 1214.32 with Olavakkode strain, 1554.76 with Angamaly strain, 1650.75 with Mulangunnathukavu strain, 1896.97 with Valiyathura strain and 2236.65 against Thikkodi strain (Table 24). The ld-p lines of all the strains with chlorfenapyr were parallel to each other, confirming uniform susceptibility of all the strains to chlorfenapyr (Fig. 19).

This extreme toxicity of chlorfenapyr may be due to its entirely different mode of action affecting ATP synthesis by uncoupling oxidative phosphorylation in mitochondria, and thus leading to the loss of energy and subsequent death of the organism (Raghavendra *et al*. 2011).

Several studies had reported of high toxicity of chlorfenapyr against *T. castaneum* (Arthur, 2008; Arthur and Fontenot, 2012; Arthur, 2013). After applying one organophosphate pirimiphos methyl, two pyrethroids *i.e.,* α-cypermethrin and deltamethrin and a pyrrole insecticide chlorfenapyr on to woven polypropylene,

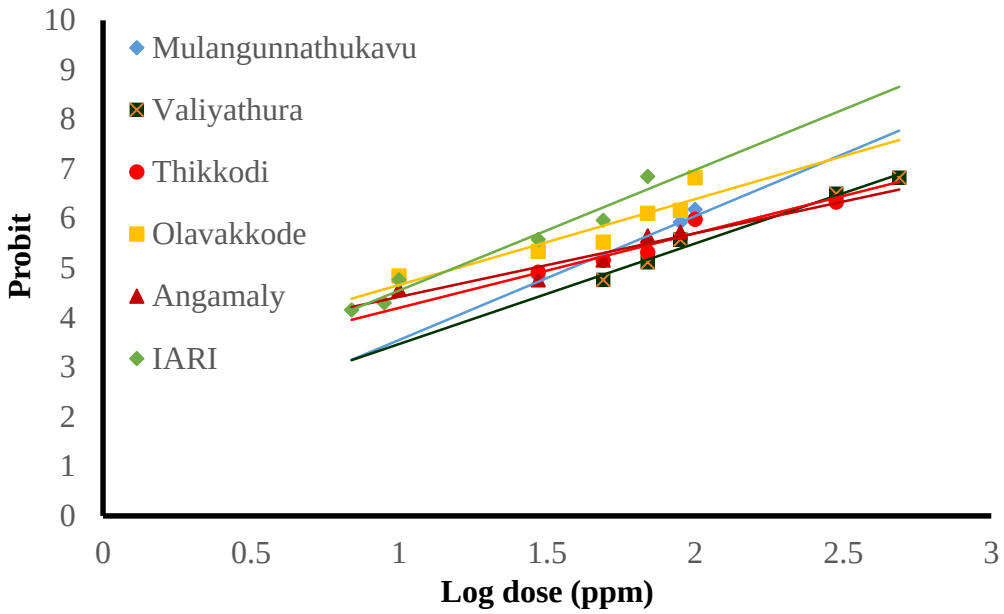

Fig. 18: Log dose – Probit line of bifenthrin

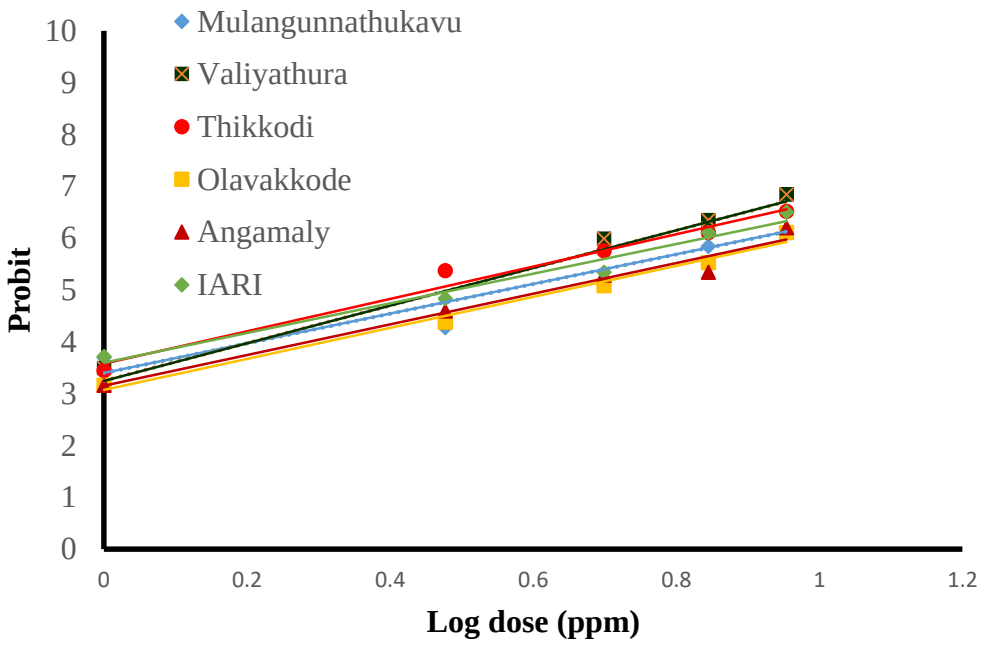

Fig. 19: Log dose – Probit line of chlorfenapyr

biaxially oriented polypropylene and craft paper bags, Kavallieratos and Boukouvala (2018) found chlorfenapyr and pirimiphos methyl as the most effective insecticides against adults and larvae of *T. granarium*. Both these insecticides resulted in more than 90 per cent mortality of *T. granarium* even after fifth day of exposure.

Exposing *T. castaneum* for two hours on chlorfenapyr treated concrete surfaces resulted in a survival of only 2.5 per cent adults by sixth day of exposure (Arthur, 2008). There was no progeny production for up to eight weeks when *T. castaneum* was exposed for a period of 24 h to concrete surface treated with chlorfenapyr (1.1 g a.i./ m^2) (Arthur, 2013). This indicates that chlorfenapyr could be a choice for spraying on walls, floors, and roofs of FCI godowns for the management of *T. castaneum*.

Chlorfenapyr was also found to be effective against insects which are already resistant against conventional insecticides such as pyrethroids (Raghavendra *et al*. 2011; N'guessan *et al.* 2007; Pimprale, 1997; Sheppard and Joyce, 1998; Scott *et al*. 2004).

5.3.2.3 Relative Toxicity of Tribolium castaneum to Spinosad

Spinosad, a mixture of spinosyn A and D, is a natural product having insecticidal properties obtained by fermentation of *Saccharopolyspora spinosa*. Spinosad has been found to be effective for the management of stored product pests (Toews and Subramanyam, 2003; Toews *et al.* 2003; Subramanyam, 2006; Huang *et al.* 2007; Subramanyam *et al.* 2007; Nikpay, 2007; Huang and Subramanyam, 2007; Athanassiou *et al.* 2010; Athanassiou *et al.* 2011).

Spinosad was found to be less toxic to all strains of *T. castanum* in the present study when compared to malathion. The relative toxicity of Mulangunnathukavu, Valiyathura, Thikkodi, Olavakkode, Angamaly and susceptible strains were 0.86, 0.61, 0.79, 0.89, 0.87 and 0.17, respectively (Table 24). A subtle shift in the ld-p lines of all the field collected strains was observed in comparison to the susceptible

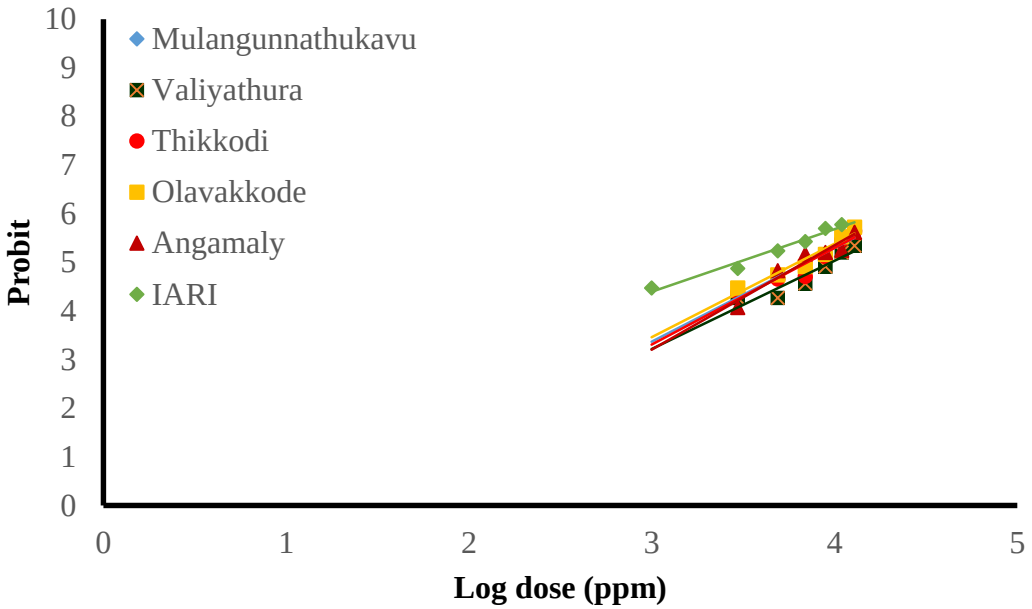

Fig. 20: Log dose – Probit line of spinosad

strain (Fig. 20). This indicates the possibility of the development of tolerance to spinosad in the field collected strains of *T. castaneum*.

Azinphosmethyl (an organo phosphate insecticide) resistant strain of leaf roller, *Choristoneura rosaceana* exhibited cross resistance to spinosad (Dunley *et al.* 2006). Hsu and Feng (2006) reported cross resistance between malathion and spinosad in oriental fruit fly, *Bactrocera dorsalis*. The above two studies indicates the possible chances of development of cross resistance to spinosad in malathion resistant strains of *T. castaneum* in the present investigation.

Most of the studies carried out with spinosad had indicated reduced susceptibility of *T. castaneum* to the insecticide. After evaluating eight species of stored product beetles by exposing them to different surfaces treated with spinosad, Toews *et al*. (2003) observed cent per cent mortality in all the species except *T. castaneum*. Similarly, *R. dominica* and *S. oryzae* adults were two times more susceptible to spinosad than *T. castaneum* (Toews and Subramanyam, 2003). *Rhyzopertha dominica* was found to be more susceptible to spinosad, compared to *T. castaneum* (Nayak *et al*., 2005). Reduced susceptibility and mortality of *T. castaneum* exposed to spinosad was also observed by Nikpay (2007), Sadeghi *et al.* (2011) and Mutambuki *et al*. (2012).

Chintzoglou *et al.* (2008b) observed that combining diatomaceous earth with spinosad increases the mortality of *T. confusum*. Similarly Vayias *et al.* (2009a) also found increased mortality of *T. confusum* with spinosad by increasing the rate of diatomaceous earth. This could be due to the higher uptake of spinosad when it was combined with diatomaceous earth which adheres to insect cuticle leading to loss of water and mortality by desiccation (Subramanyam and Roesli, 2000). Diatomaceous earth causes desiccation in insects, thereby increasing the metabolic stress. Increased metabolic stress caused by diatomaceous earth increases the activity of spinosad (Chintzoglou *et al.*, 2008b).

5.3.2.4 Relative Toxicity of Tribolium castaneum to Flubendiamide

Flubendiamide acts as a ryanodine receptor modulator and is highly effective against lepidopteran insects. Though Bhogeesh *et al*. (2014) and Raju and Jyothi (2016) found flubendiamide to be effective against bruchids namely *Caryedon serratus* and *Callosobruchus maculatus*. However, our study with *T. castaneum* found that it was least susceptible to flubendiamide without any mortality even at a dose of 20000 ppm.

5.4 DETERMINATION OF PESTICIDE RESIDUES ON RICE

The use of pesticide on any food or agricultural commodity would invariably lead to the presence of pesticide residues. Hence, pesticide residues were assessed after spraying the pesticides found as effective in the previous experiments on rice packed in jute bags.

QuEChERS method was also used by Kolberg *et al.* (2011) for the estimation of deltamethrin residues in wheat grains, flour and bran. But, the solvent used for extraction was acetonitrile, whereas recovery of deltamethrin was better with ethyl acetate in the present study. Similarly, acetonitrile was the better solvent for the extraction of pesticide residues in rice (Tsochatzis *et al*. 2010; Nguyen *et al*. 2008). While, acetone-methanol mixture (1:1) was used for the extraction of pesticide residues from rice grains (Uddin *et al*. 2011). But, similar to the present study, ethyl acetate was used for the extraction of pesticide residues in grains in other studies also (Dorea and Sobrinho, 2004; Ogah and Coker, 2012).

The method developed was validated and all the parameters were found to be within the acceptable limit and hence can be utilised for the estimation of residues of malathion, deltamethrin, chlorfenapyr and bifenthrin in rice grains (Sanco, 2017).

Summary

6. SUMMARY

The present investigation on "Susceptibility of red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) to insecticides" was carried out at Pesticide Residue Testing Laboratory and AINP on Agricultural Ornithology, at College of Horticulture, Vellanikkara during 2017-19. The study consists of evaluation of susceptibility of different population of *T. castaneum* collected from different FCI godowns of Kerala to recommended insecticides, investigation of biochemical basis of insecticide resistance by estimating activities of detoxifying enzymes in different population, evaluation of new insecticide molecules against different population of *T. castaneum* and method development and validation for residue analysis of selected insecticides in rice grain.

The important findings of the study are summarized here under

- Population of *T. castaneum* collected from the five FCI godowns of Kerala were resistant to malathion and dichlorvos. Resistance ratios of all the *T. castaneum* population with respect to malathion were more than 10.
- None of the population of *T. castaneum* were resistant to deltamethrin
- Activities of detoxifying enzymes *viz*., Carboxyl esterases, glutathione-S-transferases and cytochrome P450 were higher in all the population of *T. castaneum* collected from FCI godowns of Kerala.
- Activities of carboxyl esterase and cytochrome P450 were positively correlated with LC_{50} values of malathion, dichlorvos and deltamethrin.
- Glutathion-S-transferase activity had no correlation with LC_{50} values of malathion, dichlorvos and deltamethrin.
- Chloefenapyr was extremely toxic to all the population of *T. castaneum* at extremely low concentrations (2-4 ppm) followed by bifenthrin and deltamethrin.
- Dichlorvos and spinosad were less toxic to population of *T. castaneum*.
- Toxicity of spinosad was lower to that of malathion in all the population of *T. castaneum*.

- Method for assessing selected pesticides residues was developed and validated.
- Recoveries of malathion and deltamethrin were high in ethyl acetate, whereas it was high in acetonitrile for chlorfenapyr and bifenthrin.
- Retention times of malathion, chlorfenapyr, bifenthrin and deltamethrin were 10.39, 11.03, 11.61 and 14.24, respectively.
- Limit of detection (LOD) values of malathion, chlorfenapyr, bifenthrin and deltamethrin were 0.02, 0.01, 0.02 and 002 ppm respectively. Corresponding limit of quantification values were 0.07, 0.04, 0.08 and 0.08 ppm, respectively.
- Residues of all the selected insecticides were below the quantification limit after one day of spraying.
- The study revealed that Populations of *T. castaneum* collected from different FCI godowns of Kerala have evolved resistance to malathion and dichlorvos which could be due to the increased levels of carboxyl esterase and cytochrome P450 enzymes. Chlorfenapyr and bifenthrin can be used for the management of these resistant strains without any residues of these pesticides in rice

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Appendix

Table 1. Per cent mortality of *Tribolium castaneum* in bioassay with malathion

Dose (ppm)	Per cent mortality					
	Mulangunnathukavu	**Valiyathura**	**Thikkodi**	**Olavakkode**	**Angamaly**	**Susceptible-IARI**
100	-	-	-	-	-	3.33
300	-	-	-	-	-	20.00
500	-	-	-	-	-	46.66
700	-	-	-	-	-	63.33
900	0	0	0	0	0	73.33
1000	3.12	0	3.33	3.33	3.33	86.66
3000	23.33	20.00	20.00	22.58	20.00	-
5000	32.35	38.23	35.48	34.37	33.333	-
7000	61.29	60.00	68.75	66.66	51.61	-

Table 2. Per cent mortality of *Tribolium castaneum* in bioassay with dichlorvos

Dose (ppm)	Per cent mortality					
	Mulangunnathukavu	**Valiyathura**	**Thikkodi**	**Olavakkode**	**Angamaly**	**Susceptible – IARI**
600	0	0	0	0	0	0
800	0	0	0	0	0	3.33
2000	0	0	0	0	0	23.33
4000	23.33	13.33	16.66	16.12	16.66	56.25
6000	53.33	50.00	45.16	45.16	53.12	83.87
8000	86.66	86.66	83.33	80.00	93.33	96.66

Table 3. Per cent mortality of *Tribolium castaneum* in bioassay with deltamethrin

Dose (ppm)	Per cent mortality					
	Mulangunnathukavu	**Valiyathura**	**Thikkodi**	**Olavakkode**	**Angamaly**	**Susceptible-IARI**
10	-	-	-	-	-	16.12
30	-	-	-	-	-	20.00
50	-	-	-	-	18.18	30.00
70	23.33	16.66	22.50	24.24	30.00	38.70
90	43.33	29.03	26.66	33.33	36.66	54.83
100	48.27	43.33	41.37	40.00	43.28	61.29
300	63.33	70.96	58.06	58.06	62.16	-
500	80.00	93.33	66.66	83.33	77.14	-

Table 4. Per cent mortality of *Tribolium castaneum* in bioassay with bifenthrin

Dose (ppm)	Per cent mortality					
	Mulangunnathukavu	**Valiyathura**	**Thikkodi**	**Olavakkode**	**Angamaly**	**Susceptible-IARI**
7	-	-	-			20.00
9	-	-	-			24.13
10	-	-	-	43.75	33.33	40.62
30	43.33	-	46.66	63.33	40.62	71.87
50	56.25	40.62	54.54	70.00	56.66	83.33
70	70.00	54.83	62.50	86.66	76.66	96.87
90	82.35	71.87	-	87.87	80.00	-
100	88.23	-	83.87	96.66	86.66	-
300	-	90.90	90.90	-	-	-
500	-	93.93	-	-	-	-

Table 5. Per cent mortality of *Tribolium castaneum* in bioassay with chlorfenapyr

Dose (ppm)	Per cent mortality					
	Mulangunnathukavu	**Valiyathura**	**Thikkodi**	**Olavakkode**	**Angamaly**	**Susceptible-IARI**
0.9	3.22	3.03	6.06	-	0	-
1	9.67	6.66	15.15	0	0	10
3	23.33	23.33	58.06	26.66	34.37	43.33
5	58.06	58.06	77.41	53.33	61.29	63.33
7	80.00	80.00	86.66	70.00	63.63	86.66
9	93.33	93.33	93.54	86.66	88.57	93.33

Table 6. Per cent mortality of *Tribolium castaneum* in bioassay with spinosad

Dose (ppm)	Per cent mortality					
	Mulangunnathukavu	**Valiyathura**	**Thikkodi**	**Olavakkode**	**Angamaly**	**Susceptible**
1000	-	-	-	-	-	30.00
3000	26.66	23.33	26.66	30.00	17.64	45.16
5000	36.66	26.66	36.66	40.00	38.70	59.37
7000	45.16	33.33	38.70	46.66	43.33	66.66
9000	51.61	46.66	54.83	56.66	50.00	75.75
11000	66.66	58.06	67.74	70.00	58.06	78.12
13000	74.19	63.33	74.19	76.66	73.33	-

SUSCEPTIBILITY OF RED FLOUR BEETLE, *Tribolium castaneum* (Herbst) (COLEOPTERA: TENEBRIONIDAE) TO INSECTICIDES

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ABSTRACT OF THE THESIS

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Abstract

The red flour beetle, *Tribolium castaneum* (Coleoptera: Tenebrionidae) is a cosmopolitan stored grain pest, causing postharvest losses of more than 20 per cent in developing countries and up to nine per cent in developed countries. Even though several practices are available for management of *T. castaneum*, chemical control remains the most efficient, easy and economic method to reduce the insect pest populations to acceptable levels. Selection pressure from insecticides, however has led to development of resistance in *T. castaneum* to insecticides. *Tribolium castaneum* ranks 17th among the 20 most insecticide resistant arthropods in the world and it has already developed resistance against phosphine, methyl bromide, organophosphates, pyrethroids and insect growth regulators which are the commonly used insecticides for its management.

The present study entitled "Susceptibility of red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) to insecticides" was undertaken at the Department of Agricultural Entomology, College of Horticulture, Vellanikkara during October 2018 to July 2019. The objective of the study was to assess the susceptibility of red flour beetle populations to selected insecticides, to study the biochemical basis of resistance and to screen new molecules for the safe management of *T. castaneum*.

Different populations of *T. castaneum* were collected from five different godowns of Food Corporation of India (FCI), *viz*., Thikkodi, Olavakkode, Mulangunnathukavu, Angamaly and Valiyathura. These five strains, along with the susceptible strain of *T. castaneum* (procured from Division of Entomology, Indian Agricultural Research Institute (IARI), New Delhi) which was maintained without exposure to any insecticides for more than 35 years, were used to conduct the experiments.

Residual film bioassay with malathion, dichlorvos and deltamethrin, which were the recommended and commonly used insecticides in FCI godowns, revealed that susceptibility to malathion was lowest in the Angamaly strain of *T. castaneum*,

while susceptibility to dichlorvos and deltamethrin was lowest in Olavakkode and Thikkodi strains, respectively. While, all the three recommended pesticides were toxic to the susceptible strain. Resistance ratio for all the field collected strains, with malathion, ranged from 10.95 in Thikkodi strain to 13.34 in Angamaly strain. There was a significant decrease in susceptibility to dichlorvos also.

Biochemical basis of insecticide resistance was investigated by estimating the amount of detoxifying enzymes such as carboxyl esterase, glutathione-S-transferase and cytochrome P450 in different strains of *T.castaneum*. The activity of all the three detoxifying enzymes were significantly higher in field collected populations over that of the susceptible strain. Correlation studies indicated that carboxylesterase and cytochrome P450 levels were significantly correlated with the LC_{50} values.

Residual film bioassay was done to evaluate the susceptibility of different strains of *T. castaneum* to new insecticide molecules *viz*., bifenthrin, chlorfenapyr, spinosad and flubendiamide. Bifenthrin and chlorfenapyr was found to be most toxic to all the *T. castaneum* strains, while, spinosad and flubendiamide were not effective in controlling *T. castaneum*. When compared to malathion, bifenthrin was 104 to 378 times more toxic, while chlorfenapyr was 1214 to 2236 times toxic to the field collected strains.

The most effective insecticides, selected based on the relative toxicity studies along with FCI recommended pesticides, were sprayed on small jute bags containing 1 kg of rice. A method was developed and validated to analyse the residues of malathion, deltamethrin, chlorfenapyr and bifenthrin in rice samples with limit of detection and limit of quantification of 0.02 and 0.08 ppm, respectively. Pesticide residue analysis was carried out at different sampling intervals. When the sprayed sample was analysed after 2 hours of pesticide spray, 0.084 ppm of malathion was detected, which was below the MRL level of 4 ppm. However, residue levels, 1 and 3 days after spraying were below detection limit. In case of chlorfenapyr, bifenthrin and deltamethrin the residue levels were below detection limit throughout the study period.