Development of insecticide resistance in Black legume aphid, *Aphis craccivora* Koch. to thiamethoxam

Snehasish Routray

Adm. No.: 02ENT/Ph.D./15

DEPARTMENT OF ENTOMOLOGY
COLLEGE OF AGRICULTURE
ODISHA UNIVERSITY OF AGRICULTURE AND TECHNOLOGY
BHUBANESWAR, ODISHA-751003
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Snehasish Routray
Adm. No. 02ENT/Ph.D./15

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2020

ODISHA UNIVERSITY OF AGRICULTURE AND TECHNOLOGY
DEPARTMENT OF ENTOMOLOGY
COLLEGE OF AGRICULTURE
BHUBANESWAR

Dr. H. P. Misra
Former Professor and Head,
Department of Entomology
College of Agriculture, O. U. A. T.
Bhubaneswar-751003

Bhubaneswar
Date: 02.09.2020

CERTIFICATE - I

This is to certify that the thesis entitled **"Development of insecticide resistance in Black legume aphid, *Aphis craccivora* Koch. to thiamethoxam"** submitted in partial fulfilment of the requirements for the award of the degree of **DOCTOR OF PHILOSOPHY IN AGRICULTURE (ENTOMOLOGY)** to the Odisha University of Agriculture and Technology is a faithful record of *bona fide* and original research work carried out by **SNEHASISH ROUTRAY, Adm. No. 02ENT/Ph.D./15** under my guidance and supervision. No part of this thesis has been submitted for any other degree or diploma.

It is further certified that the assistance and help received by his from sources during the course of investigation have been duly acknowledged.

(Dr. H. P. Misra)
CHAIRMAN
ADVISORY COMMITTEE

CERTIFICATE - II

This is to certify that the thesis entitled **"Development of insecticide resistance in Black legume aphid, *Aphis craccivora* Koch. to thiamethoxam"** submitted by **Snehasish Routray, Adm. No. 02ENT/Ph.D./15** to the Odisha University of Agriculture and Technology, Bhubaneswar in partial fulfilment of the requirements for the degree of **DOCTOR OF PHILOSOPHY IN AGRICULTURE (ENTOMOLOGY)** has been approved by the student's advisory committee and the external examiner.

ADVISORY COMMITTEE

CHAIRMAN:

Dr. H. P. Misra
Former Professor and Head
Department of Entomology,
College of Agriculture,
O.U.A.T., Bhubaneswar

08.04.21

MEMBERS:

1. **Dr. L. K. Rath**
Professor and Head
Department of Entomology,
College of Agriculture,
O.U.A.T., Bhubaneswar

08/04/20

2. **Dr. M. K. Mishra**
Professor
Department of Plant Pathology,
College of Agriculture,
O.U.A.T., Bhubaneswar

08/4/2021

3. **Dr. I. C. Mohanty**
Professor
Department Agricultural Biotechnology,
College of Agriculture,
O.U.A.T., Bhubaneswar

8/4/21

EXTERNAL EXAMINER:

DR. S. V. S. RAJU
(Name and Designation)
Professor

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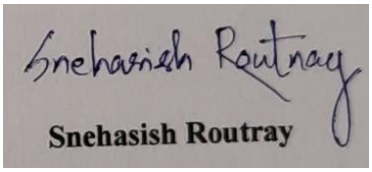

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ABBREVIATIONS USED

%	**:**	Per cent
@	**:**	At the rate of
a.i.	**:**	Active ingredient
***et al*.,**	**:**	And others
***viz*.,**	**:**	Namely
cv.	**:**	Cultivated variety
etc	**:**	And so on; and other people/ things
SE(m)	**:**	Standard Error of mean
CD (p=0.05)	**:**	Critical Difference at 5 per cent level
DMRT	**:**	Duncan's Multiple Range Test
rpm	**:**	Revolutions per minute
F_n	**:**	n^{th} filial generation
LC_{50}	**:**	Median lethal concentration
DAT	**:**	Days after treatment
w/v	**:**	Weight/ volume
mg	**:**	milligram
µl	**:**	microliter
pH	**:**	Potential of hydrogen ion concentration
°C	**:**	Degree Celsius
mM	**:**	Millimolar
ppm	**:**	Parts per million
ml	**:**	Millilitre
nm	**:**	Nano meters
r	**:**	Correlation coefficient
>	**:**	More than
SD	**:**	Standard deviation
±	**:**	Plus or minus
RH	**:**	Relative humidity

ABSTRACT

A laboratory study was undertaken to assess the magnitude of resistance development to thiamethoxam in black legume aphid, *Aphis craccivora* Koch. reared in cowpea crop at the Department of Entomology, College of Agriculture, Odisha University of Agriculture and Technology, Bhubaneswar during 2015-2017. Adult apterous aphids were collected from untreated cowpea crop from Central Research Station, OUAT, Bhubaneswar and reared in the insectary of Department of Entomology for several generations in cowpea plants (cv. Gomati) without thiamethoxam spray to obtain a susceptible parental line. The baseline toxicity of thiamethoxam to *A. craccivora* was determined to be 2.62 ppm using leaf-dip bioassay method. Further selection progressed with a selection pressure of LC_{60} of thiamethoxam every alternate generation. The degree of resistance to thiamethoxam was estimated to be 1.32 (F_2), 2.71 (F_4), 8.96 (F_6), 11.95 (F_8), 17.96 (F_{10}), 18.61 (F_{12}), 28.97 (F_{14}), 69.36 (F_{16}), 75.02 (F_{18}), 76.17 (F_{20}), 79.08 (F_{22}) and 86.19 (F_{24})- folds when compared with the parental line (F_0). The projected rate of resistance development to thiamethoxam calculated from realized heritability (h^2) studies revealed 13 to 5 generations would be required for a 10-fold increase in the resistance as the selection intensities increase from 50 to 95 per cent, respectively. While studying the reversion pattern of resistance, taking off the selection pressure of thiamethoxam from F_{24} generation resistant strain of aphids, it was found that only 18 generations are required by the resistant strain to revert to susceptibility indicating that thiamethoxam resistance in *A. craccivora* is unstable and acquired one. The persistent toxicity studies revealed that different doses of thiamethoxam persisted in cowpea plants for 11 to 23 days. The relative efficacy of insecticides evaluated in terms of persistency was thiamethoxam @ 35 g a.i./ha > imidacloprid @ 25 ml a.i./ha > thiamethoxam @ 30 g a.i./ha > thiamethoxam @ 25 g a.i./ha > diamethoate @ 300 ml a.i./ha > thiamethoxam @ 20 g a.i./ha when mortalities of aphids were studied 24 hours after release. Elevated levels of the detoxifying enzymes, Carboxylesterase (×42.02), Glutathion-s-transferase (×3.01) and Mixed function oxidases (×7.05) in the resistant strain of *A. craccivora* compared to susceptible strain indicated the role of detoxifying enzymes in resistance development in the black legume aphid, more particularly the Carboxylesterases whose activity was maximum in 'R-strain'. The fitness of the thiamethoxam strain of aphids decreased in terms of reduced fecundity which was 16.00±5.34 in F_{24} compared to 54.71±7.63 in F_0. The pre-oviposition period (0.57±0.18 days in F_0 to 1.60±0.34 days in F_{24}), oviposition period (7.78±1.40 days in F_0 to 8.89±1.27 days in F_{24}) and post-oviposition period (1.14±0.24 days in F_0 to 2.00±0.37 days in F_{24}) was found increased in R-strain of aphids compared to its susceptible counterparts indicating reduced fitness of resistant aphids.

INTRODUCTION

Aphids are small, soft bodied insects belonging to the Family Aphididae, Order Hemiptera. Around 4702 species of aphids are known to occur worldwide and 653 species are reported from India (Rajendran, 2002). Usually they damage various plants in three major ways. Firstly, they remove plant sap from phloem which weaken the plants resulting in lower quality and quantity of yield. Higher infestations can result in plant death. The second type of damage is evidenced from the fact that phloem is an amino acid poor substrate, aphids must process a large quantity of phloem extracts to gain the products necessary for protein synthesis which they need to produce offspring. Thirdly, with a modification of their gut into a filter chamber, aphids are able to shunt large quantities of fluid from plants which is excreted in a carbohydrate rich exudate termed as honeydew. Aphids produce large quantities of honeydew which can cover the leaves and fruits. The sugary substrate provides a suitable medium for the growth of black sooty mold fungi *viz.*, *Capnodium*, *Fumago*, *Scorias*, *Antennariella*, *Aureobasidium* and *Limacinula* (Schepers, 1988; Kotadia and Bhalani, 1992; Abbot, 2015).

Amongst the aphid species reported, *Aphis craccivora* Koch. is a highly polyphagous pest known by several names *viz.,* cowpea aphid, black legume aphid, groundnut aphid etc. Its host range covers about 80 plant families but, prefers to feed on plants belonging to family Fabaceae. This insect not only causes direct damage by sucking sap from plants, but also is involved in transmission of several viral diseases e.g. rosette virus in groundnut, poty virus in sunflower etc. (Singh *et al.,* 2005). It also causes significant damage to green gram and black gram foliage and pods along with other related legumes (Swaminathan *et al.*, 2012).

In cowpea (Fabacaea), the insect causes about 20 to 40 per cent loss in Asia and about 35 per cent loss in Africa (Singh and Allen, 1980). Black legume aphid may cause up to cent per cent yield loss in different varieties of country bean (*Lablab purpureus* (L.)), barbate (*Vigna sesquipedalis* (L.)), black gram (*Vigna mungo* (L.) Hepper), mung bean (*Vigna radiata* (L.) R. Wilczek) and cowpea (*Vigna unguiculata* (L.) Walp.) in different places (Ganguli and Roychaudhury, 1984).

The current aphid management strategies primarily focus on the use of various synthetic chemical insecticides *viz.*, organophosphates, carbamates, pyrethroids and neonicotinoids (Shetlar, 2001; Tang *et al*., 2013). Neonicotinoid insecticides are the most important chemical class of insecticides introduced to global market after the synthetic pyrethroids. Now-a-days neonicotinoids are registered globally in more than 120 countries and found to be effective against sucking pests such as aphids, leafhoppers, planthoppers, thrips, whiteflies etc. (Kundoo *et al*., 2018). But repeated and continuous use of a single effective insecticide leads to insecticide resistance which is one of the best examples of rapid micro-evolution found in recent past (Silva *et al.*, 2012). Selection by an insecticide allows some insects with resistance genes to survive and pass the resistance trait on to their offspring.

About 100 aphid species have successfully exploited agro-ecosystems to become economically important pests, and of them approximately twenty species have developed at least one known insecticide resistance mechanism (Simon, 2008; Van Emden and Harrington, 2007). Insecticide resistance is one of the major problems the entomologists are facing all over the world in crop protection, affecting the quantity and quality of the final product and sometimes causing economic disasters. Insecticide resistance also adds to the total insecticide cost due to additional treatment. Dense treatments with insecticides to combat resistance, increase environmental pollution and affects human health to a great extent. Reports revealed that insecticide resistance mainly occurs due to the development of either insecticide insensitive target sites such as insect nervous system or due to enhanced activity of insect detoxification enzymes (Whalon *et al.*, 2008). Target site insensitivity is often caused by highly specific point mutations. Mutated target sites do not bind to insecticides but perform their normal physiological functions. The alteration of acetylcholinesterase (AChE) to an insensitive form has been demonstrated as an important mechanism for insecticide resistance in many agricultural and medical pests (Mutero *et al*., 1994).

It is a well-known fact that acetylcholinesterase (AChE), encoded by *ace* genes, is a key enzyme in the insect central nervous system, and the target for inhibition by two groups of insecticides *viz*., Organophosphates (OPs) and Carbamates. These insecticides bind to the active site of AChE, and inactivate the enzyme by phosphorylating or carbamylating a serine residue in the enzyme's catalytic center (Fukuto, 1990), leading to repetitive firing of the postsynaptic nerve, desensitization of

the nervous system and eventually causing the death of the insect (Fournier and Mutero, 1994).

Increased metabolism of insecticides is often caused by qualitative and/or quantitative changes of esterase, glutathione-S-transferases and monooxygenes leading to quick development of insecticide resistance. Carboxylesterases are involved in resistance to ester-containing insecticides such as organophosphate, carbamate and pyrethroid insecticides. Resistance to OP insecticides as a result of enhanced carboxylesterase activity, has been demonstrated in many insects and mites including the green beach aphid (*Myzus persicae*). There are two general insecticide resistance mechanisms in which carboxylesterases are involved namely, esterase gene amplification and esterase mutation (Li *et al*., 2007). Another mechanism of insecticide resistance is increased cytochrome P-450 mediated oxidative metabolism which is a common resistance mechanism (Feyereisen, 1999; Scott, 1999).

Cytochrome P-450s (encoded by CYP genes) constitute a multigenic superfamily of enzymes (Nelson *et al*., 1996; Feyereisen, 1999), known for their ability to metabolize a wide range of endogenous and exogenous compounds thus contributing to numerous functions including nutrition, growth, development and xenobiotic detoxification (Scott *et al*., 1998; Feyereisen, 2005).

Glutathione-S-transferases (GST) are a family of enzymes that catalyze the nucleophilic attack of the sulfur atom of glutathione on the electrophilie center of many chemical compounds. The GSTs, in addition to their enzymatic activities, can bind with high affinity to a variety of hydrophobic compounds. In insects, they have been known to detoxify some organophosphorus (OP) and organochlorine insecticides, and play an important role in insect resistance to these compounds (Oppenoorth, 1985).

Since a stable resistance prevents the successful re-use of an insecticide for pest management, studies on the stability of insecticide resistance in resistant populations are essential in absence of further selection. Further, to increase the effectiveness of an insecticide, studies on resistance monitoring as well as stability study of the resistance must be conducted. This would generate information needed to establish sustainable and effective tactics of integrated pest management programs.

With the above facts in mind the following objectives were formulated to study the development of insecticide resistance in *A. craccivora* to thiamethoxam under laboratory conditions.

Objectives

1. Study of the development and build-up of resistance in *A. craccivora* to thiamethoxam in the laboratory.
2. Study of the reversion of resistance in resistant populations of *A. craccivora*.
3. Observation of the persistence toxicity of different dosages of thiamethoxam to *A. craccivora* in cowpea.
4. Study of the activity of detoxification enzymes (Cytochrome P-450 monoxygenases (MFO), esterases and glutathione-S-transferease (GST)) and target-site insensitivity (Acetylcholinesterase (AChE)) in resistant and susceptible population of *A. craccivora*.
5. Study of the fitness cost of the thiamethoxam resistance in *A. craccivora*.

REVIEW OF LITERATURE

Aphids are an interesting group of phytophagous insects which affect plants directly or indirectly through feeding on the plant's sap. Their host association is vast including plants in family Fabaceae and also many other plant families. They attack about 50 crops in 19 different plant families (Blackman and Eastop, 2007). About 1250 plant species belonging to 700 genera under 175 plant families are attacked by 653 species of aphids belonging to 208 genera. These aphids or plant lice are sap suckers and hence are serious agricultural pests. In addition to sap sucking from plants, they also damage host plants by transmission of viral diseases. About 300 species of aphids are vectors of equal numbers of different viruses (Eastop and Lambers, 1976).

Of the several aphid species, *Aphis craccivora* Koch. is a highly polyphagous pest feeding on about 80 plant families but prefers feeding on plants belonging to family Fabaceae (Blackman and Eastop, 2000). The synonyms of this pest are cowpea aphid, black legume aphid or, groundnut aphid. The damage by the pest ranges from deformed leaves, stunting and premature plant death, producing honeydew leading to growth of sooty mould fungus on plant parts, reduction in photosynthesis (Gonzáles *et al*., 2001) and transmission of major viruses such as, faba bean necrotic yellow virus (FBNYV), bean leaf roll virus (BLRV) (Laamari *et al*., 2008), cowpea mosaic virus (CMV), rosette virus and peanut stripe virus in groundnut, poty virus in sunflower (Singh *et al*., 2005) etc.

Cowpea (*Vigna unguiculata* Linn.) or Lobia belonging to Fabaceae family is mostly used as a green legume, fodder, vegetable and green manure. It is an important source of energy, minerals, vitamins and roughages. Among the sucking pests attacking cowpea, *A. craccivora* is the most important. It also attacks other crops *viz.*, alfalfa, beans, chickpea, lentils, lupins and groundnut. In cowpea, the losses in Asia range from 20 to 40% and about 35% in Africa (Singh and Allen, 1980). This pest may cause up to 100% yield loss in different varieties of country bean, barbate, black gram, mung bean and cowpea in different places (Ganguli and Roychaudhury, 1984). The pest reaches damaging numbers due to its short life-cycle leading to population explosion under favourable conditions inflicting heavy crop damage.

2.1 Current trends in aphid pest management

In the recent past and presently farmers heavily rely on the use of various synthetic chemical insecticides of various groups *viz*., organophosphates, carbamates, pyrethroids and neonicotinoids (Shetlar, 2001; Tang *et al*., 2013) for aphid pest management on their crops. In spite of several awareness campaigns farmers repeatedly and intensively use the same insecticides or insecticides with similar modes of action, for which resistance developed (Mokbel *et al*., 2017). About 20 aphid species are reported to at least one known insecticide resistance mechanism (Simon, 2008; Van Emden and Harrington, 2007). Han and Li (2004) opined that indiscriminate and large-scale use of synthetic chemical insecticides to control aphids leads to the development of insecticide resistance. The extensive use of neonicotinoids against aphids has resulted in the development of resistance in aphids (Srigiriraju, 2008). In response to intense and repeated anthropogenic pressures, such as insecticide treatments, use of insect pest resistant plants and biological agents, several aphid species have developed a series of evolutionary responses relying on adaptation and phenotypic plasticity (Simon and Peccoud, 2018).

2.2 History of insecticide resistance and neonicotinoids

Pest resistance to pesticides has been the most serious challenges the pest management scientists are facing all over the world concerned with crop production, human health and animal protection. History reveals that resistance to insecticides was first documented by Melander (1914) when Sanjose scale had shown resistance to the then used lime+sulfur. Between 1914 and 1946, 11 additional cases of resistance to inorganic insecticides were recorded (Melander, 1914). With the discovery of insecticidal properties of DDT, it was thought that insecticide resistance was an issue of the past. But, in vain, by 1947, housefly resistance to DDT was documented. Within next 2 to 20 years' resistance was reported to the then new insecticide classes *viz*., cyclodienes, carbamates, formamidines, organophosphates, pyrethroids and even *Bacillus thuringiensis.*

By 2008, there were more than 7747 cases of resistance involving more than 331 insecticides. About 553 species were reported with resistance to insecticides (Whalon *et al*., 2008). Insecticide resistance developed due to application of an insecticide which selects some insects with resistance genes to survive and pass the

resistance traits on to their offspring. As a result, in subsequent generations the proportion of resistant insects in a population continued to increase as the susceptible insects were eliminated by the application of insecticide. Eventually, resistant insects outnumbered susceptible insects and the insecticide was no longer effective.

During the early part of 1990s, a novel class of pesticides, the neonicotinoids was introduced for sucking insect pest control. The first neonicotinoid launched was imidacloprid in 1991, followed by nitenpyram and acetamiprid in 1995 and thiamethoxam in 1998. Thiamethoxam belongs to thianicotinyl class of compounds and is the first insecticide of the second generation of neonicotinoids. The neonicotinoids act as agonists at the postsynaptic nicotinic acetylcholine receptors (nAChR) of insects with much higher affinity (nanomolar level) than that of nicotine (micromolar level). The high-affinity binding site is conserved in neonicotinoid sensitivity and specificity (structure activity relationships) across a broad range of insects.

Within a few years of its extensive use in crop protection, resistance to neonicotinoids emerged as a significant threat and has been identified in several pest species (Zewen *et al*., 2003). The first report of neonicotinoid resistance was published in 1996, describing low efficacy of imidacloprid against Spanish greenhouse populations of cotton whitefly, *Bemisia tabaci* (Cahill *et al.*, 1996). Since then more than 500 peer-reviewed papers have been published on neonicotinoid resistance issues. The Arthropod Pesticide Resistance Database (APRD) lists more than 330 cases of imidacloprid resistance, followed by 130 and 50 cases of thiamethoxam and acetamiprid resistance, respectively. Till date, the number of arthropod species with resistance to neonicotinoids is increasing with time. Surprisingly, most cases of neonicotinoid resistance were reported in cotton whitefly, *B. tabaci* followed by the green peach aphid, *M. persicae*, the cotton aphid, *A. gossypii* and the rice brown planthopper, *Nilaparvata lugens*.

Senn *et al.* (1998) indicated that thiamethoxam @ 10 and 200 gm a.i./ha was sufficient for controlling target insect pests, such as aphids, rice hoppers, rice bugs, mealy bugs and some lepidopterous species under laboratory and field conditions. Thiamethoxam is widely used by the growers for its high efficacy and accordingly various studies has indicated the level of resistance in wide range of insect pests. Aphids are a group of insects that have become global pests in agriculture and

frequently exhibit insecticide resistance (Silva *et al*., 2012). Resistance ratio of 900-fold in a B-type strain of *B. tabaci* (Rauch and Nauen, 2003), 19.35-fold in *A. gossypii* (Pan *et al.*, 2015), 48.01 fold in *A. craccivora* (Abdallah *et al.*, 2016) and 60 fold in *B. tabaci* (Feng *et al*., 2009) to thiamethoxam has been reported.

2.3 Resistance in *A. craccivora* to non-neonicotinoid group of insecticides

Dhingra (1994) reported that a considerable shift in the level of susceptibility of *A. craccivora* to common insecticides after nearly a quarter century. The LC_{50} values of malathion, dimethoate, phosphamidon, methyl demeton, lindane, pyrethrin, endosulfan, fenitrothion, methyl parathion and nicotine sulphate increased 46, 28, 22, 21, 20, 15, 14, 12, 11 and 9 times, respectively when tested against it.

A comparison of LC_{50} values for pyrethroids (lambda-cyhalothrin, alphametrhin, decamethrin, cypermethrin, fenvalerate, fenproprathrin) were determined during the last one and a quarter decade by Dhingra (1993) and it was revealed that there had been a considerable shift in the level of susceptibility to these pyrethroids. There was about 33.9, 14.8 and 14.5-fold increase in the LC_{50} values of emulsifiable concentrate formulation of cypermethrin, decamethrin and fenvalerate, respectively. Pyrethrin also showed 14.7-fold increase but after a lapse of twenty-three years *i.e.*, nearly twice the period during which resistance developed to synthetic pyrethroids.

Mokbel *et al.* (2017) selected a field strain of *A. craccivora* for 24-generations under laboratory conditiuons to achieve a resistance factor of 82.3-fold to chlorpyrifos-methyl compared with a susceptible strain. The initial LC_{50} value was 0.18 ppm (for the 1st generation) and the resistance level was increased proportionally by continuous selection. Resistance ratio increased from 0.78 fold in the 1^{st} generation to 82.3 fold after the 24th generation. Ten-fold resistance required six successive generations of intense selection. Resistance increased gradually until the 12^{th} generation and further elevated dramatically up to the 24^{th} generation.

Fouad *et al.* (2016) monitored the resistance level of three field populations of *A. craccivora* to seven insecticides belonging to three different chemical classes *viz*., organophosphates, carbamates and neonicotinoids. The three populations were collected from three governorates in Egypt namely Dakahlia, Qalyobia and Beni Suef. Diagnostic concentrations (LC_{90} values for susceptible strain) for each insecticide were established using a leaf dipping technique. Resistance monitoring showed that the field

population from Dakahlia was highly resistant to all the tested insecticides. In a similar manner, the population from Qalyobia was also resistant to all insecticides except for fenitrothion to which only moderate resistance was observed. The field population from Beni Suef exhibited a lower level of resistance to all the seven evaluated insecticides.

2.4 Resistance in other aphids to non-neonicotinoid group of insecticides

Wang *et al.* (2002) reported >29,000-fold resistance in *A. gossypii* when selected on cotton to fenvalerate for 17 generations. On changing the host plant to cucumber, the same insect showed 700-fold resistance to fenvalerate.

Lokeshwari *et al.* (2016) investigated the development of resistance in three different strains of *A. gossypii* (namely LKR-1 (collected from IIHR, Karnataka), LKR-2 (collected from Mangalagiri, Andhra Pradesh) and LKR-3 (collected from Guntur, Andhra Pradesh)) to dimethoate. Bioassay studies revealed that the LC_{50} values increased dramatically with dimethoate selection in resistant strains and the resistance ratio (RR) was 270-, 243- and 210-fold greater in LKR-1, LKR-2 and LKR-3, respectively than that of the susceptible strains by 30^{th} generation.

Moores *et al.* (1996) studied the level of resistance in three clones of *A. gossypii* (171B, 1081K, 968E) to different carbamate and organophosphorus insecticides *viz*., pirimicarb, triazomate, methomyl, demeton-S-methyl, methamidophos, monocrotophos, omethoate and pirimiphos methyl and reported that 171B showed high resistance (20-folds) to six of the eight insecticides evaluated. Resistance ratio of 968E was highest for pirimicarb (>380) and demeton-S-methyl (180) and lowest (5 or less) to methomyl and pirimiphos-methyl. In contrast, 1081K aphid strain showed strong resistance only to pirimicarb (74-fold) and triazomate (57-fold); moderate (9-to 18-fold) resistance to demeton-S-methyl and omethoate, and little or no resistance to the rest of the insecticides evaluated.

2.5 Insecticide resistance in *A. craccivora* to neonicotinoids

Neonicotinoid insecticides are highly effective against some of the world's most destructive crop pests *viz*., aphids, whiteflies, planthoppers and also some coleopteran, dipteran and lepidopteran species. Although many sucking insect species are still successfully controlled by the neonicotinoids, their popularity has imposed a mounting

selection pressure for resistance, which has now reached levels that compromise the efficacy of these insecticides (Bass *et al*., 2015).

Use of leaf dipping technique of bioassay has proved the high level of resistance in field populations of *A. craccivora* towards different class of chemicals belonging to organophosphates, carbamates and neonicotinoids (Fouad *et al.*, 2016). When *A. craccivora* adults were continuously selected with thiamethoxam for 12 generations, the resistance ratio (RR) was increased up to 48.01-fold (Abdallah *et al.*, 2016).

Mokbel and Mohamed (2009) studied the development of resistance in field strain of *A. craccivora* to another neonicotinoid, dinotefuran through 20 generations of selection. The LC_{50} value of the parent (first generation) was 5.39 ppm with slope value 1.06 ± 0.53 which indicated that this strain was not homogenous to this insecticide. The value of LC_{50} increased slowly with selection pressure to reach 22.24 ppm for the 10^{th} generation. Up to 15^{th} generation with the same selection pressure LC_{50} value increased to reach to 66.19 ppm, but by 20^{th} generation the LC_{50} was found to be 230.71 ppm. Resistance ratio values in relation to susceptible strain, increased slowly until the 10^{th} generation being 14.34-fold, fastly increased to 42.7-fold and 148.8-fold in 15^{th} and 20^{th} generation respectively.

2.6 Neonicotinoid resistance in other aphid pests

Wang *et al.* (2002) determined resistance of two strains of cotton aphid, *A. gossypii* Glover, to imidacloprid on cotton (*Gossypium hirsutum* L.) and cucumber (*Cucumis sativa* L.) after 12 consecutive generations of selection on cotton and cucumber in greenhouses. The resistance of *A. gossypii* to imidacloprid was 8.1-fold on cotton and 3.6-fold on cucumber after 12 consecutive generations of selection.

El-Kady (2007) studied the level of imidacloprid resistance in four different strains of *A. gossypii* using leadf dip bioassay and reported that out of four strains, maximum 32.55-fold resistance was observed in QAL strain (with LC_{50} of 1.595 ppm against the LC_{50} of 0.049 ppm in the susceptible strain).

Gore *et al.* (2013) continuously monitored the level of resistance in *A. gossypii* to thiamethoxam from 2008 to 2011. The concentration-mortality bioassays were conducted across the mid-southern United States. These bioassays suggest high levels

of resistance to thiamethoxam. Resistance ratios ranged from 0.9 to 562.6 at 48 hours and from 0.9 to 29.1 at 72 hours of treatment.

According to Koo *et al.* (2014), some field populations of *A. gossypii* showed high level of resistance to neonicotinoids when compared to pyrethroids, carbamates, niacines and sulfoxamines. Among the neonicotinoids, the aphids showed highest resistance to acetamiprids (2600-fold) and clothianidin (14000-fold), whereas, to dinotefuran and thiamethoxam they showed comparatively lower resistance (66-fold and 79-fold, respectively).

Chen *et al.* (2015) selected a strain of *A. gossypii* to imidacloprid for 15 generations and obtained a resistant strain with 75-folds of resistance. On the otherhand, after continuous selection of adult *A. gossypii* to thiamethoxam, Wei *et al.* (2017) got 13.79-folds of resistance. The LC_{50} value varied from 1.13 mg/L to 15.58 mg/L.

2.7 Neonicotinoid resistance in other sucking insect pests

Halappa and Patil (2016) conducted bioassay studies in cotton leafhopper, *Amrasca biguttula biguttula* (Ishida) to neonicotinoid groups of insecticides in low (Mundgod; MUD), medium (Davanagere; DVG), high (Haveri; HVR) and very high (Gulbarga; GLB) pesticide usage areas of Karnataka. The results depicted that imidacloprid, thiamethoxam, acetamiprid, thiacloprid and clothianidin showed varying levels of resistance for all the locations studied. The resistance ratio was high in imidacloprid (3.35, 8.57, 9.15 and 12.27 fold, respectively) and the lowest in dinoferuran (1.86, 5.13, 6.71 and 9.88-fold, respectively). The resistance ratio to thiamethoxam was 2.14, 7.74, 9.57 and 11.30 in MUD, DVG, HVR and GLB, respectively.

2.8 Baseline toxicity of thiamethoxam to aphids and black legume aphids

Using leaf-dip bioassay Pan *et al*. (2018) reported the baseline toxicity of *A. gossypii* to thiamethoxam was 2.85 ppm. Koo *et al*. (2014) reported that the baseline toxicity of cotton aphid *A. gossypii* to thiamethoxam was 0.14 ppm using the same leaf-dip bioassay method.

According to Abdallah *et al*. (2016) the LC_{50} value of the susceptible population of *A. craccivora* to thiamethoxam was 0.079 mg/L. Using a different modified dipping

method, Chen *et al.* (2015) reported that the baseline toxicity of *A. gossypii* to thiamethoxam was 1.88 ug/mL. But, according to Mokbel *et al*. (2017) it was found that the baseline toxicity of *A. craccivora* to thiamethoxam was 0.44 ppm using leaf-dip bioassay.

2.9 Reversion of insecticide resistance in aphids and other pests

Insecticide resistance persists over many generations after the withdrawal of selection pressure. Such cases have been noticed in many important crop pests. Since a stable resistance prevents the successful re-use of an insecticide for pest management, a study on the reversion of insecticide resistance assumes prime importance.

Hick *et al.* (1996) reported that the resistance in *M. persicae* to oxydemeton methyl was normally stable, but highly resistant aphid strains sometimes lost resistance when insecticidal selection pressure was removed. This loss of resistance, termed reversion results from a loss of elevated esterase enzyme activity through transcriptional control, *i.e.*, without loss of the amplified esterase DNA sequences. They also showed that loss of the elevated enzyme occurred simultaneously with loss of methylation at CCGG sites in the amplified DNA sequences. During reselection of resistance in these revertant clones, enzyme levels increased, but there was no corresponding return of methylation to DNA sequences when the selection pressure was taken off.

Rehan *et al.* (2011) studied the pattern of reversion of insecticide resistance in *Spodoptera litura* (Fabricius). The population collected from Dunyapur was reared for eleven generations under laboratory conditions without any insecticide exposure. The LC_{50} data was recorded through diet incorporation method against four insecticides such as emamectin benzoate, spinosad, imidacloprid and profenofos. For new chemistry insecticides the larval mortality data was taken after 72 hours while in case of conventional insecticides the mortality data was taken after 48 hours. Emamectin benzoate (1.59 ppm) was found to be most toxic on the basis of LC_{50} values (1.59 ppm) followed by spinosad (7.77 ppm), profenofos (686.5 ppm) and imidacloprid (258.75 ppm) in first generation. The decrease in the LC_{50} values after 11 generations as compared to the field population of *S. litura* was 4.81, 9.83, 9.3 and 13.82-folds against emamectin benzoate, spinosad, imidacloprid and profenofos, respectively. The estimated decrease in resistance was 11.36, 11.11, 16.67 and 9.61 for imidacloprid,

spinosad, emamectin benzoate and profenofos, respectively. The results suggest that spinosad can be included in the control program of *S. litura*, due to its lower stability and higher reversion rate with insecticides bearing novel modes of action and this baseline susceptibility data could be very helpful in future monitoring of insecticide resistance in *S. litura*.

Ninsin and Tanaka (2005) studied the stability of acetamiprid resistance (110 fold) in *Plutella xylostella* (Linnaeus). The resistant strain was developed by exposing third-instar larvae to acetamiprid-treated leaves of cabbage, *Brassica oleracea capitata* L. cv. *Chuseikanran*, for 72 hours, after which the surviving larvae were reared on untreated cabbage leaves and radish seedlings for the next selection. The reference strain was established by maintaining a part of the field-collected population over many generations without exposure to acetamiprid. The resistant strain was reared without further exposure to acetamiprid to determine the stability of resistance. Maintaining the resistant strain for seven generations in the absence of selection pressure resulted in a drop in resistance ratio from 110 to 2.42, indicating that acetamiprid resistance in *P xylostella* is not stable.

Fahmy and Miyata (1992) selected two strains such as TL (Tup Luang strain) and BK (Bang Khae) strain (from Thailand) of diamondback moth, *Plutella xylostella* (L.) with a chitin synthesis inhibitor- chlorfluazuron and they produced high levels of resistance in comparatively short time (9 and 10 generations). The resistance ratio values reached 276- and 216-fold, for TL and BK strains, respectively. The BK strain developed about 80-fold resistance level after only three selection generations with chlorfluazuron, suggesting that the resistance gene(s) has been kept in the population in large measure. On release from chlorfluazuron selection pressure for two generations after 8^{th} and 9^{th} generation of selection, both strains showed no reversion in susceptibility level.

Basit *et al.* (2013) studied the resistance levels in whitefly, *B. tabaci* (Gennadius) collected from cotton and sunflower (from four districts of Pakistan) for five neonicotinoids and two insect growth regulators (IGRs) for two consecutive years. Based on the LC_{50}(s), all collections showed slight to moderate levels of resistance for the insecticides evaluated compared with the laboratory susceptible population. The data also indicated that cotton and sunflower collections had similar resistance levels.

In comparison (four collections), Vehari collections showed higher resistance for acetamiprid, thiacloprid and nitenpyram compared with those of others. Average resistance ratios for acetamiprid, thiacloprid and nitenpyram ranged from 5- to 13-, 4- to 8-, and 9- to 13-fold, respectively. Multan and Vehari collections exhibited moderate levels (9- to 16-fold) of resistance to buprofezin. Furthermore, toxicity of neonicotinoids against immature stages was equal to that of insect growth regulators. Increase or decrease in resistance over a 1-year period for all tested insecticides was non-significant showing the resistance in the field populations was stable.

Reversion of resistance in the absence of selection to chloropyrifos- methyl was studied by Mokbel (2015) to investigate its stability in *A. craccivora***.** After ten generations without exposure to chloropyrifos methyl, resistance was unstable and LC_{50} decreased from16.34 ppm (91-fold) to 0.43 ppm (2.9- fold). Resistance level of cowpea aphid to chloropyrifos methyl decreased steadily from 1st generation to 9th generation with resistance factor of 12-fold. But, it tended to decrease sharply from 9th generation to 10th generation with resistance factor of 2.4- fold.

Gutiérrez-Olivares *et al.* (2007) studied the reversion of resistance to thiamethoxam (F2 to F6) and imidacloprid (F3 to F6) in *B. tabaci.* With respect to imidacloprid, resistance ratio (RR) of LC_{85} in F3 was 8.1-fold and descended to 4.8-fold in the following generation; from F5 to F6, RR decreased from 3.5 to 2.4-fold. RR to thiamethoxam was 6.9-fold in F2 and descended to 2.8-fold in F6. From the above findings they concluded that resistance to both insecticides was unstable in absence of selection pressure.

Raymond *et al.* (1993) while studying the reversion of OP resistance due to amplification of esterase B1 or B2 in unselected strains of *Culex pipiens* showed that the resistance and amplification are both lost when strains are not homozygous for the presence of the amplified genes, due probably to fitness differences. In homozygous strains, both esterases remained amplified and expressed, and resistance was stable during at least 60 generations.

Sawicki *et al.* (1980) while carefully monitoring the changes in activity of esterase between parents and offspring of the OP and carbamate resistant *M. persicae* variant (clone G6) found that complete loss of resistance can occur either in a single step or over several generations (without insecticidal selection on Chinese cabbage

plants) and that below a threshold value, reversion to higher levels is very rare. Such type of reversion could only be detected by selection, with insecticide, of large clonal populations from an individual that had lost activity. High esterase activity was not stabilised by breeding for 16 generations only from individuals with high esterase activity.

In the absence of selection pressure, the stability of acetamiprid resistance in *A. gossypii* was studied by Mokbel (2018). After 16 generations of selection, there was a 22.55-fold increase in LC_{50} value. However, resistance to acetamiprid in the cotton aphid was unstable and resistance reversed in five generations without exposure to acetamiprid. This results exhibited that cotton aphid can develop resistance to acetamiprid under continuous selection pressure. The instability of acetamiprid suggests that *A. gossypii* can be managed by rotation with pymetrozine. Reversion of resistance could be attributed to the inability of the resistant individuals to compete effectively with the susceptible ones in terms of reproductive potential and other biotic factors (Georghiou, 1963; Ninsin and Tanaka, 2005). Sometimes reversion is cited as a pre-requisite for the success of rotational strategies for resistance management in the field (Tabashnik, 1990).

2.10 Inheritance of resistance

Realized heritability (h^2) is an index to quantify pushing degree to a trait in a population by selection. It's defined as the ratio of genetic variance to total phenotypic variance; this number can range from 0 (no genetic contribution) to 1 (all differences on a trait reflect genetic variation). It provides effective mean for prediction of future evolutionary response to selection (Tabashnik, 1992).

The calculated h^2 due to the laboratory selection tests might be higher than that in the field because of less ecological variation (Zhang *et al*., 2008) in the laboratory condition. Though laboratory trials do not absolutely indicate field circumstances, the approximated h^2 value provides proof for the prospective of further improvement in the level of resistance (Tabashnik, 1992). Estimates of h^2 in conjunction with estimates of selection intensity can be used to project rates of resistance development. Prediction based on h^2 must be interpreted cautiously because h^2 of resistance to a particular insecticide can vary between conspecific populations as well as within populations due to variation in the allele frequencies and environmental variation over time. So, the

predictions made from quantitative genetic theory on the basis of $G= R^{-1}$ gives valuable information regarding formulation of resistance management tactics (Tabashnik, 1992). Estimating h^2 from laboratory selection experiments for resistance is thus necessary to assess the risk of insecticide resistance in pests (Lai and Su, 2011) especially for evaluating the sustainability of a chemical pesticide on a pest population (Sayyed *et al*., 2005).

The degree of heritability was estimated in a field strain of *A. craccivora* to chloropyrifos-methyl by Mokbel (2015) throughout twenty-four generations of laboratory selection which resulted in 105-fold increase in median lethal concentration (LC_{50}) compared to the parental level. The estimated realized heritability (h^2) of chloropyrifos-methyl resistance was found to be 0.35. The projected rate of resistance development indicated that, if slope (3.38) and h^2 (0.35), then 11–5 generations are required for ten-fold increase in LC_{50} at 50–95% selection intensity.

Sethi *et al.* (2008) investigated the development of resistance in *B. tabaci* against imidacloprid, bifenthrin and fenalerate by selecting upto 8 generations. After 8^{th} generation, strains selected with these three insecticides exhibited 21.90, 7.12 and 4.13-fold increase in tolerance for these three insecticides, respectively. The realized heritability (h^2) of insecticide resistance was very high in imidacloprid selected strains compared to bifenthrin and fenvalerate selected strains. The results indicated high level of risk in the field populations for the development of resistance to imidacloprid.

Fahmy and Miyata (1992) selected two strains such as TL (Tup Luang strain) and BK (Bang Khae) strain (from Thailand) of diamondback moth, *Plutella xylostella* (L.) with chlorfluazuron which showed high levels of resistance in comparatively short time (9 and 10 generations). The resistance ratio values reached 276- and 216-fold, for TL and BK strains, respectively. The degree of heritability (h^2) for the TL strain was found significantly higher than that for the BK strain (0.27 for the TL strain and 0.064 for the BK strain).

After 14 generations of selection with the neonicotinoid imidacloprid under laboratory conditions, *Spodoptera litura* developed 137.48-fold resistance (Abbas *et al.*, 2012). The estimated realized heritability (h^2) of imidacloprid resistance was found to be 0.15 in the resistant strain.

Mokbel (2018) studied resistance risk in *A. gossypii*, to acetamiprid and acetamiprid resistance stability in the absence of selection. After 16 generations of selection, there was a 22.55-fold increase in LC_{50} and the realized heritability (h^2) of resistance was 0.17. Projected rates of resistance indicated that, if $h^2 = 0.17$ and 50% of the population was killed at each generation, then a ten-fold increase in LC_{50} would be expected in 12.2 generations. If h^2 was 0.27 then 7.63 generations would be needed to achieve the same level. In contrast, with h^2 of 0.07 it necessitates about 30 generations of selection to reach the same level of resistance development.

2.11 Persistent toxicity of neonicotinoids in cowpea

Patil and Lingappa (2001) reported that imidacloprid (40gm a.i/ha) as plant hole treatment (PTH) in tobacco was the most persistent to aphids (*M. nicotianae*) followed by acephate (0.075%) foliar spray. These two insecticides required more than 25 days to lose their effectiveness completely while oxy-demeton methyl lost its toxicity completely in 11-12 days.

Studies on the relative toxicity of some conventional insecticides against mustard aphids revealed that phosphamidon was the most effective insecticide followed by dimethoate, lindane, thiometon and chlorpyriphos (Sinha *et al.,* 2001). Phosphamidon remained effective upto 14 days followed by dimethoate. On the basis of relative persistent toxicity, the insecticides showed the order of efficacy as phosphamidon> dimethoate> lindane> thiometon> carbaryl> malathion> chlorpyrifos> endosulfan> quinalphos. On the basis of PT values, dimethoate, lindane, thiometon, carbaryl, malathion, chlorpyrifos, endosulfan and quinalphos were 0.88, 0.76, 0.67, 0.63, 0.59, 0.59, 0.59 and 0.52 times less toxic respectively than phosphamidon. Among the granules, disulfoton and phorate were found to be most toxic to the aphid, upto 78 days and the residual toxicity of both the chemicals persisted for 102 days in mustard.

Amongst the neonicotinoids, acetamiprid, imidacloprid, thiamethoxam and dinotefuran registered significantly high per cent reduction of *A. craccivora* on faba bean under field conditions at one, seven, fifteen and 21 days after treatment. The LT_{50} for acetamiprid, imidacloprid, thiamethoxam and dinotefuran were 5.8, 6.2, 6.95 and 4.2 days, respectively (Abd-Ella, 2014).

Preetha *et al.* (2009) studied the persistent toxicity of imidacloprid 17.8 SL, thiamethoxam 25WG and methyl demeton 25 EC against *A. gossypii* on Bhindi and reported that the higher dose of imidacloprid showed longest persistence up to 27 days for aphids and lower dose up to 21 days whereas, methyl demeton showed shorter persistence for a period of 13 days. The order of relative efficacy of the insecticides based on the persistent toxicity index was imidacloprid @50 g a.i. ha^{-1} > imidacloprid @25 g a.i ha^{-1} > imidacloprid (Tatamida®) @25 g a.i. ha^{-1} > thiamethoxam @25 g a.i. ha^{-1} > imidacloprid @15 g a.i. ha^{-1} > and methyl demeton @125 g a.i. ha^{-1}. Mohamed *et al*. (2015) reported that under field condition, both thiamethoxam and dinotefuran caused reduction in the cabbage aphid populations after 1, 3, 7, 15 and 21 days of treatments but thiamethoxam was more efficient than dinotefuran.

Dhandapani *et al.* (2009) studied the persistent toxicity of nine different insecticide treatments as foliar spray against the sucking pests of chillies *viz*., *A. gossypii* G., *Scirtothrips dorsalis* H. and *Hemitarsonemus latus* Banks. It was observed that monocrotophos 0.1% and pirimicarb 0.1% persisted upto 21 days after treatment and recorded high percentage mortality of aphids and thrips. Against mites, phosalone 0.07% and monocrotophos 0.1% recorded higher percentage mortality and also persist4ed upto 21 days after treatment.

Dimethoate at 0.03 per cent showed highest PT values of 909.16 and 861.7 on leaves and shoots of safflower and LT_{50} values to the tune of 7.68 and 7.07 days, respectively against nymphs of *Uroleucon compositae* (Theoblad) when compared to other insecticides (Gaikwad *et al*., 2015).

2.12 Mechanisms of resistance

Several studies have been made in the past for understanding pesticide resistance, more specifically on biochemical and physiological mechanisms, and the molecular genetics. Molecular genetic has revealed many details of the mechanism of resistance both at the individual and population levels. These mechanisms mainly occur due to the development of insecticide insensitive targets sites and / or due to enhanced activity of insect detoxification enzymes. In general, three major groups of detoxifying enzymes have been identified to play a major role in specific cases of insecticide resistance. They are cytochrome P-450 monooxygenases, esterases, and glutathione-S-transferases (Taniai *et al*., 2003). The metabolism of almost all insecticides in insects

has been found to be catalysed mainly by monooxygenases, hydrolases and gluthathione-S-transferases. Generally, in resistant insects, the enzymatic detoxification is believed to be so rapid that the toxic molecule does not reach its site of toxic action (Kranthi, 2005).

2.12.1 Role of proteins in insecticide resistance

Mokbel (2013) quantified the total protein content (mg/ g.bw) in the adults of susceptible, field and acetamiprid resistant strains (38.60 folds of resistance after selecting for 24 generations) of *A. craccivora* using leaf dipping method. The mean protein content was 71.90±5.27 mg/ g.bw, 105.00±1.82 mg/ b.bw and 98.70±2.55 mg/g. bw in susceptible, field and acetamiprid resistant strains respectively.

There is ample relationship between protein content and carboxylesterase activity of the individual apterous adults of *A. gossypii*. It is seen that high carboxylesterase activity is associated with aphids collected from areas where insecticides had been applied (Hama and Hosoda, 1988). Modification of the target protein affinity, or an increase of detoxification of some enzymes systems plays an important role in pesticide resistance (Berge, 1989). Rufingier *et al.* (1999) reported that the resistance of lettuce aphid *Nasonovia ribisnigri* to the carbamate insecticide pirimicarb and the cyclodiene, endosulfan were associated with increased detoxification enzyme activities and modification of the target proteins.

Shuai and Wang (2005) while investigating on the resistance of *M. persicae* to alpha-methrin found that, enzyme protein content increased significantly and there is significant correlation between LD_{50}, enzyme protein content and esterase activity, respectively.

2.12.2 Role of detoxifying enzymes in insects against insecticides

Ninsin and Tanaka (2005) studied the role of metabolic enzymes in the resistance of a laboratory strain of diamondback moth, *Plutella xylostella* (L) to the neonicotinoid insecticide acetamiprid with a synergists piperonyl butoxide (PBO), which suppresses the activity of cytochrome P-450 monooxygenases, and S,S,S-tributyl phosphorotrithioate (DEF), an inhibitor of esterases, using the leaf-dip method. Both PBO and DEF enhanced the insecticidal activity of acetamiprid significantly in the resistant *P. xylostella* strain but not in a reference strain, suggesting that cytochrome P-

450 monooxygenases and esterases play an important role in the resistance of *P. xylostella* to acetamiprid.

Abdallah *et al.* (2016) reported that carboxylestersae activity was 30 times greater in the resistant strain of *A. craccivora* than in the susceptible strain towards thiamethoxam. In addition, the enzyme activity of glutathione-S-transferase (GST) and mixed function oxidases (mfo) increased only in the resistant strain 3.7 and 2.7 times, respectively, in relation to the susceptible (the control) strain. This indicated significant activity of the detoxifying enzymes, particularly carboxylesterase, in the resistant strain of *A. craccivora.*

In a chorpyriphos methyl resistant strain of *A. craccivora* with 82.3 folds of resistance in 24 generations was observed compared to S-strain. Furthermore, R-strain exhibited a slight change in glutathione-S-transferase activity with a ratio of 1.33 compared with S-strain (Mokbel *et al*., 2017).

Koo *et al.* (2014) studied the activities of the cytochrome P-450, glutathione-S-transferase and esterase detoxification enzymes in the susceptible and neonicotinoid resistant strains of *A. gossypii* and found no significant differences between the susceptible and resistant strains. In a thiamethoxam resistant strain of *A. gossypii*, no target site mutation was found involved, rather overexpression of P-450s and esterases were seen (Wei *et al*., 2017).

In another study by Lokeshwari *et al*. (2016), the biochemical assays of *A. gossypii* revealed enhanced activities of carboxylesterases (CarE), glutathione-S-transferases (GSTs) and cytochrome P-450-mediated *p*-Nitroanisole *O*-demethylase (PNOD) in resistant strains supporting their role in dimethoate detoxification which is one of the mechanisms underlying dimethoate resistance in *A. gossypii* collected from South India.

Fouad *et al.* (2016) monitored the resistance of three field populations of *A. craccivora* to seven insecticides belonging to three different chemical classes *viz*., organophosphates, carbamates and neonicotinoids and detoxifying enzyme level in then. The activity of the glutathione-S-transferase and mixed function oxidases was moderate in the populations from Qalyobia and Dakahlia. But, the enzyme activity in *A. craccivora* collected from Beni Suef was variable and differed slightly from the activity measured in the susceptible strain. They opined that possible occurrence of

resistance in the cowpea aphid to the tested insecticides may be due to the higher activity of carboxylesterases.

2.12.3 Esterases

Damayanthi and Karunaratne (2005) studied insecticide resistance to malathion, chlorpyriphos, propoxur and permethrin and the underlying mechanisms of resistance in seven species of insect pests in vegetables *viz.*, aphids, *A. gossypii*, *M. persicae*, *A. craccivora*, *Toxoptera citricidus* and *Lipaphis erysismi*; diamond-back moth *Plutella xylostella*; leafminer *Liriomyza huidobrensis* etc. on the biochemical analysis. Elevated levels of esterases was found to be the major resistance mechanism in aphids and *P. xylosttella.* The highest esterase activity (1.006±0.64 µmol/min/mg) was found in *M. persicae.*

Carboxylesterases hydrolyze ester containing insecticides such as organophosphate (OP), carbamate and pyrethroid insecticides thus conferring resistance to them. In general, two insecticide resistance mechanisms in which carboxylesterase are involved occur either through, esterase gene amplification or esterase mutation (Li *et al*., 2007).

At first, mechanism of resistance to insecticides was described in *M. persicae* due to the enhanced production of carboxylesterases which confers broad spectrum of resistance to members of the organophosphate (mono-methyl), carbamate and to a much lesser extent pyrethroids. This mechanism was first implicated several years ago when it was demonstrated biochemically that the esterases of OP resistant aphids had an enhanced ability to hydrolyse a model substrate (1- naphthyl acetate) (Needham and Sawicki, 1971). Subsequent work showed that resistance was due to the overproduction of one of two possible carboxylesterases, E4 or FE4, that both hydrolyse and sequester the insecticide before it can reach the target site in the insect nervous system (Devonshire and Moores, 1982; Devonshire *et al.*, 1983). Further studies confirmed that overproduction of E4 and FE4 carboxylesterase genes through gene amplification is responsible for enhanced degradation and sequestration of various insecticides, including organophosphates, carbamates and pyrethroids (Field and Devonshire, 1998). High level of malathion resistance had been shown in *A. gossypii*, due to two site mutations (K14Q and N354D) with high frequency, which was found in the resistant strain (Yiou *et al*., 2009) signifying metabolic resistance may also occur through

selection of mutant caroxylesterases in insects and both a modified gene structure and an increase in expression of carboxylesterase is responsible for it.

Cao *et al*. (2008) proposed that increased carboxylesterase detoxification due to gene overexpression accounts for omethoate resistance in laboratory selected *A. gossypii*. Conversely, Shang *et al*. (2012) found that the activity of carboxylesterase showed no significant difference in omethoate resistant strain (231.2-fold) of the cotton aphid, *A. gossypii*.

The activity of Carboxylesterase (CbE) was increased (2.6-fold) significantly in the pirimicarb resistant strain of *M. persicae*, suggesting that enhanced CbE activity probably confers pirimicarb resistance as a supporting factor. Some of the carboxylesterase (CbEs) function as non-specific sequestration proteins for various groups of insecticides. Thus, this enhanced CbE activities may also be associated with the moderate levels of cross-resistance to neonicotinoids in *M. persicae* (Kwon *et al*., 2009).

Pan *et al*. (2015) suggested that the up-regulated ribosomal proteins, ecdysteroid UDP-glucosyltransferase, esterase and peroxidase may confer towards the tolerance of thiamethoxam-resistant strain of cotton aphid, which displayed a 19.35-fold greater resistance to thiamethoxam compared to a susceptible strain. But, Koo *et al*. (2014) reported the absence of resistant mechanism based on enhanced esterase in imidacloprid resistant strain of cotton aphid.

2.12.4 Monoxygenases

Enhanced Cytochrome P-450 monoxygenases activity is regarded as a major mechanism of resistance for various insecticide classes, including organophosphates, carbamates, pyrethroids and neonicotinoides (Li *et al*., 2007).

Chen *et al.* (2015) studied the activities of three detoxifying enzymes such as Cytochrome P-450 mediated O-demethylation (activity toward *p*-nitroanisole (PNOD)), carboxyeserases (CarE) and GSTs in an imidacloprid resistant population of *A. gossypii.* The activities of PNOD (ratio) was found highest (4.59), followed by CarE (2.33) and GST (1.13).

Insecticide bioassays using enzyme inhibitors suggested that P-450 mediated detoxification plays an important role in conferring resistance to neonicotinoids in *M.*

persicae, although additional mechanisms may be involved (Philippou *et al*., 2009; Puinean *et al*., 2010). Many studies have been conducted with P-450 inhibitors, particularly piperonyl butoxide (PBO), which synergizes compounds degraded by P-450. A reduction in the level of resistance to synergized insecticides is generally taken to be an useful diagnostic tool indicating the role of P-450 in endowing resistance (Sanchez-Arroyo *et al*., 2001).

Enhanced activity of cytochrome P-450 monooxygenases conferring pyrethroid and neonicotinoid resistance have been well documented in various insects *viz*., *Culex pipiens pallens* Caguillett (Shen *et al*., 2003), *Blattella germanica* (Linnaeus) (Pridgeon *et al*., 2003), *Plutella xylostella* (Linnaeus) (Bautista *et al*., 2009), *B. tabaci* Gennadius (Karunker *et al*., 2008) and *Tribolium castaneum* (Herbst) (Zhu *et al*., 2010). A clone of *M. persicae* collected from tobacco in Greece exhibing 30-60 fold resistance (through topical bioassays) to several neonicotinoids when compared with a reference susceptible strain using an array populated with probes (Microarray analysis) corresponding to all known detoxification genes in *M. persicae*, revealed constitutive overexpression (22-fold) of a single P-450 gene (CYP6CY3) (Philippou *et al*., 2009; Puinean *et al*., 2010) indicating its role in development of resistance to neonicotinoids in *M. persicae*.

Bass *et al*. (2014) reported that cytochrome P-450 affects imidacloprid resistance in the green peach aphid, *M. persicae*, as a result of their identification of a synergistic effect of piperonyl butoxide (PBO) used with imidacloprid. On the other hand, Koo *et al*. (2014) found no effect of P-450 in the imidacloprid resistant strains of *A. gossypii* by using the synergist test and enzyme activity.

2.12.5 Glutathione-S-Transferases

Glutathione-S-transferases are a family of enzymes that catalyse the nucleophilic attack of the sulfur atom of glutathione on the electrophilic center of many chemical compounds. The GSTs, in addition to their enzymatic activities, can bind with high affinity to a variety of hydrophobic compounds. In insects, GSTs have been known to detoxify some organophosphorus and organochlorine insecticides, and play an important role in insect resistance to these compounds. Seven GST genes have been implicated in insecticide resistance through gene amplification or overexpression (Oppenoorth, 1985).

The inactivation and excretion of a number of both endogenous and exogenous compounds takes place through glucosidation. In a study by Pan *et al.* (2018), two inhibitors of UGT enzymes *viz*., sulfinpyrazone and 5-nitrouracil, significantly increased the toxicity of thiamethoxam against the resistant strain of *A. gossypii*, indicating UGTs are involved in thiamethoxam resistance in the cotton aphid. In the resistant cotton aphids, the transcripts of 23 UGTs were elevated and the transcripts of 13 UGTs were almost doubled compared to the thiamethoxam-susceptible strain.

According to Halappa and Patil (2016), the enzyme activity ratio of glutathione-S-transferase in cotton leafhopper *Amrasca biguttula biguttula* was relatively greater (11.36 times than that of susceptible), and corresponded to the higher LC_{50} values of neonicotinoids for very high, high, medium and low pesticide usage areas. Their research also suggested that the higher activity of glutathione-S-transferase in the resistance population of cotton leafhopper had a significant role in conferring resistance to neonicotinoids.

Nasonovia ribisnigri, (Homoptera: Aphididae) the main pest of salad crops, has developed resistance to the carbamate (pirimicarb) and the cyclodiene (endosulfan), the two insecticides widely used for control of this aphid in France. It was observed that endosulfan resistance was mainly due to increased detoxification by glutathione-S-transferases (Rufingier *et al*., 1999). Kwon *et al*. (2009) reported that no significant differences in GST activities were found between the pirimicarb resistant and susceptible *M. persicae*. Similar results were found in omethoate resistant strain of *A. gossypii* (Shang *et al*., 2012) and in imidacloprid resistant strain of *A. gossypii* (Koo *et al*., 2014).

2.12.6 Target site Inhibitors (AChE)

Mokbel (2013) reported that in an acetamiprid resistant strain of *A. craccivora* the activity ratio of acetylcholinesterase was 0.92 with a mean value of 5.68×10^6 mg substrate/ g protein / min comparing to the susceptible strain with a mean value of 6.18×10^6 mg substrate/ g protein / min. In another case when *A. craccivora* was selected for 12 generations with 48.01-folds of resistance to thiamethoxam the activity ratio of acetylcholinesterase was 3.68 with specific activity of 37.55±1.18 mOD. min^{-1}. mg^{-1} (Abdallah *et al*., 2016).

Moores *et al.* (1996) studied the total esterase activity, and acetylcholinesterase (AChE) sensitivity to inhibition by insecticides in three clones of *A. gossypii* (171B, 1081K, 968E) resistant to various carbamate and organophosphorus insecticides (pirimicarb, triazomate, methomyl, demeton-S-methyl, methamidophos, monocrotophos, omethoate, pirimiphos methyl). On calculation of the biomolecular rate constants, which provide the most reliable measure of AChE sensitivity to inhibition by insecticides, the value was found to be 340-to 4500-fold higher for 171B than for 1081K and 968E, respectively for pirimicarb. Whereas, distribution of total esterase activity for 171B and 1081K aphids overlapped considerably and the difference between the mean values for these clones was not statistically significant.

The molecular bases of target-site mediated insecticide resistance provide useful information on how specific insecticides exert their lethal effects, and insensitivity due to point mutations in genes encoding for protein that are target site of insecticides. The mutant molecules include acetylcholinesterase for organophosphates, ɤ-aminobutyric acid (GABA) receptors for cyclodienes and voltage-gated sodium channels for synthetic pyrethroids and dichloro-diphenyl trichloro ethane (DDT). In addition, detoxification through alteration in the levels or enzyme activities that degrade or sequester insecticides is also responsible for insecticide resistance. Thus, understanding the molecular basis of insecticide resistance is important. It opens windows of understanding that may improve future pest control (Perry *et al*., 2011).

Target site insensitivity is often caused by highly specific point mutations. Mutated target sites do not bind to insecticides but perform their normal physiological functions. The alteration of AchE to an insensitive form has been demonstrated as an important mechanism for insecticide resistance in many agricultural and medical pests (Huchard *et al*., 2006).

Acetylcholinesterase (AChE), encoded by *ace* genes, terminates nerve impulses by catalyzing the hydrolysis of the neurotransmitter acetylcholine. As a key enzyme in the insect central nervous system, it was a target for the development of inhibiting insecticides. Two important classes of inhibitors, organophosphates and carbamates are analogous to the substrate acetylcholine (Huchard *et al*., 2006). At least 16 different mutations in AChE have been associated in insects with insensitivity to OPs and carbamates. These altered forms have wide differing spectra of insensitivity between

species, as well as a marked range of insensitivity to different compounds within species. Part of the complexity can be attributed to the finding that more than one *ace* gene is encoding the synaptic AChE target (Huchard *et al*., 2006).

Alteration of AChE to an insensitive form is an important mechanism for the development of insecticide resistance in *A. gossypii* toward OP and carbamate insecticides (Moores *et al*., 1996; Toda *et al*., 2004; Andrews *et al*., 2004; Benting and Nauen, 2004). Point mutations that confer insensitivity to OPs and carbamates were reported both in *ace1* and *ace2* (the two genes encoding AChE), while most mutations confer similar resistance level towards carbamate and OP insecticides, Gly119Ser in two sub-species of mosquitoes and Ser331Phe in two aphid species are associated with much higher resistance level towards carbamates than OPs (Russell *et al*., 2004).

Fei and Zhaojun (2004) found that the mutation F139L in *ace2* and the other A302S in Ace1 occurred in organophosphate resistant clones of cotton aphid (*A. gossypii*). In the potato peach aphid (*M. persicae*) an amino-acid mutation (S431F) occurred on the second AChE was proposed to cause pirimicarb resistance (Nabeshima *et al*., 2003).

Target-site insensitivity of AChE was characterized in field collected, green peach aphid, *M. persicae* (adapted to tabacco) from nine different states in the eastern United States during the period 2004 to 2007. The specific activity of the AChE among the 65 aphid colonies screened by Ellman's assay ranged from 0.017–0.259 U/min/mg protein (Srigiriraju *et al*., 2010). Eight colonies, with a wide range of specific activities were chosen to study the inhibition of AChE in the presence of two carbamate insecticides, methomyl and pirimicarb. The I_{50} values for methomyl ranged from 0.35 to 2.4 μM, while six out of eight colonies had lower values that ranged from 0.16 to 0.30 μM for pirimicarb. Two colonies that were inhibited by methomyl had very high I_{50} values for pirimicarb, *i.e.,* 40.4 and 98.6 μM, respectively. The target-site insensitivity in these two colonies that are resistant to pirimicarb could be due to an *ace*2 gene mutation. The results indicated that the possible insensitivity due to MACE (modified acytelcholinesterase) resistance in some colonies may render selected carbamate insecticides ineffective.

Pan *et al*. (2010) studied the levels of gene expression for *ace*1 and *ace*2 in omethoate resistant strains of the cotton aphids compared to a relatively susceptible

strain by using real- time quantitive PCRs. The results indicated that the relative transcription levels of *ace*1 and *ace*2 were 0.26 and 1.07-fold, respectively in the resistant strains.

Mutants in both *ace*1 and *ace*2 were combined with significantly lower specific AChE activity. Based on analysis of I_{50} indices, enzyme inhibition experiments showed that AChE from the omethoate resistant strain of cotton aphids was 10.6, 3.2, 6.2,10.5 and 4.4-fold more insensitive to inhibition by eserine, omethoate, paraoxon, paraoxon-methyl and malaoxon, respectively, compared to a susceptible strain associated with a decrease in activity of AChE (Shang *et al*., 2012).

2.13 Fitness cost of insecticide resistance in aphids and other pests

Fitness costs are studied to know any change in fitness-related traits such as reproduction, development time and adult body size in insecticide resistant insect strains (Fenton *et al*., 2010). It has vital role in sustainable utilization of pesticides because they restrain the progression of resistance in agroecosystems. This effect is prominent when fitness costs are partly dominant (Raymond *et al*., 2007). Biological characteristics evaluation of insecticide resistant populations is useful in formulating the insecticide resistance management strategies (Campanhola *et al.*, 1991). There are reports of fitness decline in insecticide resistant strains of *P. xylostella* (Cao and Han, 2006), *Nilaparvata lugens* (Liu and Han, 2006), *S. exigua* (Jia *et al*., 2009), *B. tabaci* (Feng *et al*., 2009), *H. armigera* (Wang *et al*., 2010), *S. litura* (Abbas *et al*., 2014; Zaka *et al*., 2014) and *P. solenopsis* (Afzal *et al*., 2015). The insecticide resistant strain of insects is observed to show reduction in reproductive performance, longer development times and a reduction in body size in several insect species in crop fields that are sprayed with insecticides and in crop fields that are sprayed with insecticides and in insecticide free environments (Berticat *et al*., 2008). May and Dobson (1986) opined that the fitness cost linked with insecticide resistance may affect the spread of resistance genes in insect population.

Liu and Han (2006) reported that laboratory selected imidacloprid resistant strain of *Nilaparvata lugens* having 250-fold resistance in 37 generations had reduced larval survival rate (78%), adult emergence rate (69.6%), copulation rate (64.9%), fecundity (217.9) and hatchability (57.3%) compared to that of the susceptible strain which had survival rate (93.7%), adult emergence rate (92.1%), copulation rate

(87.5%), fecundity (491.3) and hatchability (88.2%). According to Belinato and Martins (2016) an organophosphate and insect growth regulator (IGR) resistant population of *Aedes aegypti* had longer developmental time, lower longevity, problems with blood feeding and low reproductive traits.

According to Feng *et al.* (2009), a laboratory selected thiamethoxam resistant strain of *B. tabaci* (60-fold resistance in 36 generations) showed fitness disadvantages in their development, reproduction and morphology. They have also observed that the fitness of resistant B-type whiteflies decreased dramatically, to only one-half than that of the susceptible strain. The nymphal duration increased from 14.60 ± 0.20 days to 23.17 ± 0.72 days and the adult longevity decreased form 18.67 ± 0.77 days to 16.60 ± 0.53 days. Some changes in the morphological characteristics of the resistant strain were also observed. The lengths of first, second and third instars of the resistant strain were significantly smaller than those of the susceptible strain, and the width of the first and the fourth instar larvae were also significantly smaller in comparison to the susceptible strain.

An increase in the male and female nymphal duration, pupal duration, emergence rates, male and female generation time with decreased fecundity in a spinosad resistant strain of cotton mealybug, *Phenacoccus solenopsis* (282.45 resistance fold in 9 generations) was observed (Afzal and Shad, 2017). The longevity of male and female were significantly increased as compared to the susceptible populations (4.67 to 6.75 days in male and 10.77 to 23.23 days in female).

According to Gao *et al.* (2014), survival percentage of 1st instar larva of thiamethoxam resistant western flower thrips, *Frankliniella occidentalis* (15.1 fold of resistance in 55 generations) was significantly lower for the resistant strain (60.2 ± 5.2%) than for the susceptible strain (72.2 ± 6.0%). The percentage of pupation (75.4 ± 2.8%) and fecundity (47.7 ± 1.8 eggs per female) of resistant strain were significantly lower than those of susceptible strain (83.9 ± 2.0% and 54.0 ± 3.2% eggs per female, respectively).

Abbas *et al.* (2012) observed that a resistant strain of *S. litura* to imidacloprid (137.48-fold resistance in 14 generations) had a relative fitness of 0.38, with substantially lower rates of larval survival, larval duration, male pupal duration, development time, emergence rate of healthy adults, fecundity, hatchability and

prolonged larval and pupal duration. Mean relative growth rate of the larvae, intrinsic rate of population increase, and biotic potential was lower for the selected populations.

It is very often observed that insecticide resistance may cost significant fitness to the pest population. The biological parameters of insects, for example reduced fecundity and enhanced growth period, may bring changes in relative fitness. The pest survival rate decreases because of resistance, which finally leads to declining in fitness (Roush and McKenzie, 1987; Forrester *et al.*, 1993).

It is proposed that in resistant population the reduced fecundity might be the result of less energy utilized for reproduction, *i.e.* part of the metabolic energy is used in physiological and biochemical defences for insecticide detoxification (Ribeiro *et al.*, 2001).

Fitness reduction in resistant strain as compared to their counterpart susceptible strain may be utilized in resistance management tactics by eliminating heterozygotes and resistant homozygotes and also by conserving susceptible homozygotes (Leeper *et al*., 1986).

MATERIALS AND METHODS

To start the work on development of resistance in black legume aphid, *A. craccivora* Koch. to thiamethoxam under laboratory conditions, the first step was collection and rearing of the susceptible strain of the aphid in its natural plant cowpea. The work proceeded in the following steps.

3.1 Collection and rearing of *Aphis craccivora* on cowpea

The field strains of black legume aphids *A. craccivora* were collected from the Central Research Station, Odisha University of Agriculture and Technology, Bhubaneswar (20.2647° N, 85.8141° E) during July, 2015 from cowpea research plots. The strains were assured without prior exposure to any insecticides in the field. The adults were collected using camel hair brush and petri plates and were used for rearing under insectary condition. Cowpea seedlings (cv. Gomati) were used in this studies. The seeds were sown in pots (15 cm diameter) in staggered manner for the continuous availability of host plants for the rearing of aphids. About 15 to 20 days old seedlings of cowpea were used for the rearing of *A. craccivora* (Figure 3.1). The freshly collected aphid adults from cowpea field were released on the fresh foliage of cowpea seedlings (15 to 20 days old). At a time about 18 to 20 seedlings were used for the rearing of aphids in the insectary (Figure 3.2). Adults were allowed to complete their life cycles on the same cultivars (Figure 3.3 and 3.4). After the moulting of adults, they were further reared on fresh batch of cowpea seedlings. Without any exposure to pesticides, the population was reared and maintained.

3.2 Determination of the baseline toxicity of thiamethoxam to *A. craccivora*

For determining the baseline toxicity of thiamethoxam over period on adult *A. craccivora*, leaf-dip bioassay method was followed (Moores *et al*., 1996). Ventilated containers with small holes, too small for the aphids to escape were used (Figure 3.5). The containers were lined with moist blotting papers to keep the treated leaves fresh. Each time a batch of freshly moulted adult aphids were collected using hair brush and petri plates. Accurate dilutions of the test compound were prepared (Figure 3.7). For initial studies, five to six widely spaced concentrations of thiamethoxam were used and further the concentration ranges were narrowed till an appropriate dose response was identified. For doing that, cowpea leaves were dipped in the test liquid for 10 seconds with gentle agitation and were placed for surface-drying on paper towel (abaxial surface facing upwards) under shade (Figure 3.6). The dried leaves were kept upside

Figure 3.1 Cowpea seedlings aged 15 to 20 days old for rearing of *A. craccivora*

Figure 3.2 Ventilated cage for the rearing of *A. craccivora* over generations

Figure 3.3 Infestation of *A. craccivora* during rearing process (Susceptible strains)

Figure 3.4 Infestation of *A. craccivora* during rearing process (Susceptible strains)

down in the labelled containers. Ten numbers of adult aphids were placed carefully in each replication of each treatment concentration of thiamethoxam. For each concentration three replications were made. The insecticide treated insects were kept in a B.O.D. incubator under laboratory set of conditions (28±2°C; 75-80% relative humidity). The mortality (dead and live) counts were made after 24 hours. Aphids which were unable to right themselves within 10 seconds once turned on their back were considered as dead. Moribund were counted as dead. The mortality counts were corrected using Abbott's formula (Abbott, 1925).

Corrected mortality = $[(T - C)/(100 - C)] \times 100$

Where T = mortality in treatment, C = control mortality

Data were analyzed following probit analysis (Finney, 1971) using the software package Ldp-line. While rearing, susceptible *A. craccivora* population in untreated cowpea plants, in every alternate generation, bioassay as mentioned above was conducted and LC_{50} of thiamethoxam was found out. The method continued till a stable LC_{50} value of thiamethoxam was obtained. That stable value was accepted as the baseline toxicity.

3.3 Selection for resistance to thiamethoxam

For the selection of resistant aphid population in first generation, the lethal concentration (LC_{60}) of thiamethoxam based on the baseline data was used. Adult apterous aphids were released on 10 numbers of untreated cowpea plants in separate cages (Figure 3.8) in the insectary 24 hours prior to the insecticide spraying at LC_{60} of thiamethoxam. The plants with aphids were sprayed with LC_{60} of the parental susceptible generation. The neonates from the surviving adults were continued to be reared on the same treated plants till the next generation. A new LC_{50} was found out every two generations (*i.e.*, F_2, F_4, F_6, F_8 ……) and then for the next generation LC_{60} of the previous generation was used based on the progress of resistance. During bioassay in every even generation, bioassay of susceptible strain was also conducted following the same methodology. The magnitude of resistance development (resistance ratio) was calculated by dividing the LC_{50} of the selected generation by the LC_{50} of the susceptible strain. The selection continued up to 24 generations. The susceptible strains of *A. craccivora* were maintained in a separate cage (Figure 3.9).

Figure 3.5 Leaf dip bioassay using ventilated plastic containers

Figure 3.6 Surface drying of dipped treated leaves

Figure 3.7 Serial dilutions of thiamethoxam

Figure 3.8 Selection for resistance to thiamethoxam in separate cage

Figure 3.9 Maintenance of Susceptible strains of *A. craccivora*

3.4 Estimation of realized heritability (Inheritance of Resistance)

Realized heritability (h^2) was estimated by using the method described by Tabashnik (1992) as follows:

$$h^2 = \frac{\text{Response to the selection (R)}}{\text{Selection differential (S)}}$$

Response to selection (R) was estimated as follows

$$R = \frac{(\text{Log final } LC_{50} - \text{Log initial } LC_{50})}{n}$$

Where the final LC_{50} is the LC_{50} of population after n generations of selection and initial LC_{50} is for the parental population before selection.

The selection differential (S) was estimated as follows:

$S = i^{\delta}p$,

i, intensity of selection (Falconer, 1989) was estimated from 'p', which is the percentage of the population with values above the selection threshold (*i.e.* the percentage surviving selection) using Appendix of Falconer (1989), which is based on the properties of normal distribution

It was estimated as $i = 1.583 – 0.0193336p + 0.0000428 p^2 + 3.65194/p$

'p' is the average percentage of surviving rate of thiamethoxam Selected strain

'$^{\delta}p$' is the phenotypic standard deviation, calculated as:

$^{\delta}p = [1/2(\text{initial slope} + \text{final slope})]^{-1}$

Or, $(\text{mean slope})^{-1}$

To estimate either a change in R, S and h^2 during the selection pressure, each parameter was calculated for the first and second half of the experiment (equal generations in each half). The response to selection (R) can be estimated as follows:

$R = h^2 S$

S, selection differential ($S=i.\ ^{\delta}p$)

The number of generations required for a tenfold increase in LC_{50} was calculated as follows:

$G = R^{-1} = (h^2\ S)^{-1}$

3.5 Reversion of resistance

Once sufficient resistance was established in the strains of aphids, *A. craccivora*, reversion pattern was studied. The reversion/stability of insecticide resistance was studied by rearing the resistant population without any selection pressure (*i.e.* spraying insecticide). For this, the adult apterous aphids were collected from the resistant strains and were released on ten untreated cowpea seedlings (20 days old). After getting next generation from the released adults, again newly formed adults were shifted and released on new plants. At the interval of each two generations the LC_{50} of thiamethoxam was detected by the same leaf-dip method of bioassay. Likewise, the median toxicity level (LC_{50}) were detected to monitor the change in the median lethal concentration (LC_{50}). The study on reversal of resistance was continued till the toxicity reached the baseline value as found in susceptible strains.

3.6 Persistent toxicity studies

The experiment was designed in completely randomised design with six treatments and four replications. The 15 day old seedlings of cowpea were used to study the persistence toxicity of the insecticides such as, T_1 (Thiamethoxam 20g a.i./ha), T_2 (Thiamethoxam 25g a.i./ha), T_3 (Thiamethoxam 30g a.i./ha), T_4 (Thiamethoxam 35g a.i./ha), T_5 (Imidacloprid 25g a.i./ha), T_6 (Dimethoate 300g a.i./ha) and and Control (distilled water) (Table 3.1). All the insecticides were applied as a foliar spray. The spraying was done by using a hand sprayer. The insecticidal solution was prepared according to the following formula.

$$V = (C \times A)/(\%\ a.i.)$$

Where,

V= Volume of the insecticide

C = Concentration required

A = Amount of spray solution needed

% *a.i.* = Percentage of active ingredient of the insecticide

The persistent toxicity was studied in microcage method (Figure 3.10). Pre-counted adult apterous aphids were caged in well-ventilated plastic containers (6 cm diameter) on treated plants at 1st DAT, 3rd DAT, 5th DAT, 7th DAT and 9th DAT onwards. The observations regarding the mortality (24 hours and 48 hours after release) were taken using visual counting method. The observed mortality was corrected using Abbott's formula (Abbott, 1925) based on the mortality in control.

For determining the persistent toxicity of each insecticide, the product (PT) of average residual toxicity (T) and the period (P) for which the toxicity persisted was used as an index. The persistent (PT) values were calculated by the criterion developed by Pradhan (1967) as given below:

$$\text{Average residual toxicity(T)} = \frac{\text{Sum of corrected mortalities at different intervals}}{\text{Number of observations}}$$

Persistent toxicity (PT)= Average residual toxicity × period for which toxicity was Observed.

Accordingly, Relative Persistence (RP) values were calculated as per Bharti *et al.* (2015) as below.

Relative Persistence (RP) = (PT value of Insecticide/ Insecticide with lowest PT value)

The median lethal time (LT_{50}) was calculated using Ldp line software.

Figure 3.10 Persistence toxicity studies in *A. craccivora* using microcage method

Table 3.1 Details of the insecticides and dosages used in the persistence toxicity study

Treatments	Insecticides	Dosages	Original strength	Trade name	Manufacturer
T_1	Thiamethoxam	20g a.i./ha	25%WDG	Actara	Syngenta
T_2	Thiamethoxam	25g a.i./ha	25%WDG	Actara	Syngenta
T_3	Thiamethoxam	30g a.i./ha	25%WDG	Actara	Syngenta
T_4	Thiamethoxam	35g a.i./ha	25%WDG	Actara	Syngenta
T_5	Imidacloprid	25g a.i./ha	17.8%SL	A. One	Plant Remedies Pvt. Ltd
T_6	Dimethoate	300g a.i./ha	30%EC	Rogor	Plant Remedies Pvt. Ltd
T_7	Control	Distilled water	-	-	-

3.7 Study on Increased activity of detoxification enzymes in resistant and susceptible population

3.7.1 Determination of the total protein content

The Protein content was determined by the method of Bradford (1976), using bovine serum albumin (BSA) as the standard. For the preparation of Bradford reagent 100 mg Coomassie Brilliant Blue G-250 was dissolved in 50 ml 95% ethanol. To it, 100 ml 85% (w/v) phosphoric acid was added and then diluted to 1 liter when the dye had completely dissolved, and the solution was filtered through Whatman No. 1 paper just before use. Then, 25 adult aphids (~10 mg) from each strain (susceptible and resistant) were homogenized in 200 µl of ice-cold 0.1M phosphate buffer (pH 7.5), homogenates were centrifuged at 13000 rpm for 15 min at 4ºC and the supernatants were transferred to new tubes. Twenty µl of sample solution was added to 1000 µl of Bradford reagent and then mixed by vortex. A blank tube was prepared using 20 µl phosphate buffer and 1000 µl of Bradford reagent and mixed well. The absorbance was measured after 10 minutes at 595 nm using UV/ Vis spectrophotometer (V-530). The

weight of protein was plotted against the corresponding absorbance resulting in standard curve used to determine the protein in unknown samples.

The level of activity of different detoxifying enzymes were assayed by following standard protocols.

3.7.2 Carboxylesterase assay

The esterases activity were assayed with α-naphthyl acetate (α-NA), as substrate following the process described by Van Asperen (1962). The assay is based on the principle that **e**sterases split simple esters in biological systems and such activity can be estimated in terms of the products formed, using various substrates. The substrates used are α-naphthyl acetate or α-naphthyl butyrate and the formation of α-naphthol is monitored.

The first step in the assay is preparation of the following stock solutions.

a. **Substrate solution:** 0.3 mM α -naphthyl acetate. A stock solution of 30 mM α-naphthyl acetate was prepared in acetone and 1 ml was added to 99 ml of phosphate buffer (40 mM, pH 6.8).

b. **Staining solution** (prepared fresh). One per cent Fast blue BB salt w/v in 0.04 M, phosphate buffer pH 6.8 and 5 % Sodium dodecyl sulphate (SDS) w/v in double distilled water. The quantity of staining solution was determined depending on the number of samples being processed, and 2 parts of 1% Fast blue solution to 5 parts of 5% SDS was added.

c. **Enzyme stock.** Ten µL of the enzyme solution (10,000 X g supernatant of whole body homogenate) was added to 990 µL of phosphate buffer (40 mM, pH 6.8).

Further, the following assay mixture were prepared.

a. Enzyme stock-1.0 ml + 5.0 ml substrate solution.

b. Control-blanks were kept with 1.0 ml 0.04 M, phosphate buffer pH 6.8 + 5.0 ml substrate solution.

2. further incubated in dark for 20 min at 30ºC, with occasional shaking.

3. One ml of each of the staining solution were added to the sample and control blank tubes. Further incubated for 20 minutes at room temperature. About 3 ml of each of the blank solution were pipetted out into each of two 4 ml cuvettes.

4. The sample was then placed in reference slots of a double beam spectrophotometer. The reading was adjusted to zero at 590 nm.

5. The contents of the sample cuvette was replaced with 3 ml of the processed enzyme sample. The absorbance was recorded at 590 nm.

6. The enzyme activity was calibrated from the α-naphthol standard curve.

Preparation of α-naphthol standard curve

At first, 14.42 mg (100 µ moles) α-naphthol were dissolved in 5ml acetone (Stock A). Five, 10, 15, 20, 25, 30, 35, 40 and 45 µL of stock A was added to phosphate buffer (40 mM, pH 6.8) and the volume was made up to 1 ml, to get standard solutions of 1 ml phosphate buffer containing 0.10, 0.20, 0.30, 0.40, 0.50, 0.60, 0.70, 0.80 and 0.90 µmoles α- naphthol, respectively. A blank was maintained separately as control, in which α-naphthol was not added to 1 ml phosphate buffer. One ml of the blank and standard solutions was added separately to 5ml of phosphate buffer (40 mM, pH 6.8). One ml of staining solution was added to each of the above tubes. Further, those were incubated for 20 minutes in dark. Then, absorbance was read at 590 nm against the blank placed in the reference cuvette in a double beam UV spectrophotometer and graph was plotted putting µ moles of α-naphthol on the 'X' axis against absorbance on the 'Y' axis.

3.7.3 Mono-oxygenases assay

The Mixed Function Oxidases (MFO) activities (mono-oxygenases) were analyzed according to the protocol given by Hansen and Hodgson (1971). For the purpose, stock solutions *viz*., (i) 0.1 phosphate buffer (pH 7.8), (ii) 2 mM p-nitroanisole and (iii) 9.6 mM NADPH were prepared. Then, 10 adult aphids were homogenised in 500 µL of ice-cold 0.1M phosphate buffer (pH 7.8). The homogenates were centrifuged at 15000 rpm for 15 minutes at 4ºC and the supernatants were transferred to new tubes. To it, 100 µL of 2 mM p-nitroanisole and 90 µL enzyme stock solutions were mixed. The mixture was incubated at 27 ºC for two minutes. Then, 10 µL of 9.6 mM NADPH were added to this. Optical densities were immediately recorded at 405 nm at intervals

of 25 seconds for 10 minutes. For blanks, 100 µL of p-nitroanisole, 90 µL of phosphate buffer and 10 µL of NADPH solutions were mixed and used.

Protocols for standards

A stock solution of p-nitrophenol of 20 mM were prepared by dissolving 13.9 mg p-nitrophenol in 5 mL of 0.5N NaOH. Subsequent dilutions were made. The absorbance was read at 405 nm.

3.7.4. Glutathione-S-transferase assay

The glutathione-S-transferase was measured as described by Habing *et al*. (1974). Glutathione transferases catalyse the conjugation of 2,4- dinitro-chlorobenzene (CDNB) or 3,4-dichloronitrobenzene (DCNB) with reduced glutathione (GSH), to produce a yellow product that has an absorbance maximum at 340-360 nm and the rate of product formation, that indicates the enzyme activity, can be calculated by following the increase in absorbance at 340 nm.

For studying the GST activities in resistant and susceptible *A. craccivora* the following stock solutions were prepared.

1. Reduced glutathione 50 mM in phosphate buffer (100mM, pH 6.5).
2. CDNB 50 mM in ethanol
3. Enzyme stock: 10,000 x g supernatant of midgut homogenate
4. Sodium Phosphate buffer (100mM, pH 6.5), containing 1 mM EDTA.

Then 50 µL of 50mM CDNB, 150 µL of 50 mM reduced glutathione were added to 2.77 ml Phosphate buffer (100mM, pH 6.5, 0.1 mM PTU). To it, 30 µL of enzyme stock was added. The contents were shaken gently and incubated for 2-3 minutes at 25ºC. The contents were transferred into a 4ml cuvette and were place it in the sample cuvette slot of the spectrophotometer. Three ml of reaction mixture was added without the enzyme and was placed in the reference slot of the spectrophotometer. The absorbance was followed for 6-7 minutes at 340 nm. The increase in absorbance was considered over 5 minutes for calculations. The enzyme activity was calculated as follows:

CDNB GSH conjugate formed in µ moles min^{-1} mg^{-1} protein

$$= [\text{ABS(increase in 5 min)} \times 3 \times 1000]/(9.6 \times 5 \times \text{protein in mg})$$

3.7.5 Acetylcholinesterase assay

Acetylcholinesterase activity was measured following Ellman *et al.* (1961) in both resistant and susceptible *A. craccivora* population. Acetylthiocholine, which is an ester of thiocholine and acetic acid, is used as a substrate in the assay. As a result of hydrolysis of the ester a mercaptan is formed which reacts with DTNB (5,5'- thiobis-2-nitrobenzoic acid) to split it into two products one of which is 5-thio-2-nitrobenzoate. This 5-thio-2-nitrobenzoate shows peak absorbance at 412 nm and thus the acetylcholinesterase enzyme activity can be estimated by following the increase in absorbance at 412 nm.

Twenty-five numbers of adult aphids were homogenized in 0.05 M phosphate buffer (pH 7.2) containing 0.5 % triton X-100 and 2mM EDTA. The sample were centrifuged at 10,000 rpm at 40ºC for 20 min and the supernatant was used as enzyme source. Stock solutions *viz.*, acetylthiocholine iodide, 0.10 M in sodium phosphate buffer (0.1 M, pH 8.0), DTNB, 0.01 M in sodium phosphate buffer (0.1 M, pH 8.0 containing 1.5% sodium carbonate) and Sodium phosphate buffer (0.1 M, pH 8.0) were prepared. Then 100 µL of the supernatant incubated at room temperature for 5 minutes, 10 µL of the DTNB solution and 30 µL of acetylthiocholine iodide were added to 2.86 ml sodium phosphate buffer (0.1 M, pH 8.0) in a 4 ml cuvette (sample cuvette) and 10 µL of DTNB and 30 µL of acetylthiocholine iodide solutions were added to 2.96 ml sodium phosphate buffer (0.1 M, pH 8.0), which was used as blank in a double beam spectrophotometer. The absorbance was recorded in the sample cuvette at 412 nm for 30 min against the blank.

The acetylcholinesterase activity is calculated as

AChE activity in µmoles/min/ml of enzyme= $(E \times 1000 \times 3.0)/(1.36 \times 10^4 \times 0.10)$

where E is change in absorbance per minute.

1.0 is the total volume of reaction mixture (ml).

0.1 is the volume of enzyme (ml).

1000 is the factor to obtain µ moles.

1.36×10^4 is the molar extinction coefficient of the chromophore at 412 nm.

3.8 Fitness cost study

Different biological parameters *viz*., nymphal duration, adult longevity and fecundity were studied in different generations of selected *A. craccivora* (parental strain, F_2, F_4, F_6, F_8, F_{10}, F_{12}, F_{14}, F_{16}, F_{18}, F_{20}, F_{22} and F_{24}) under laboratory conditions (28±2^{o}C; 75-80%). The 2nd or 3rd opened fresh leaves of cowpea leaves at the peak vegetative stage were used for studying the biology of aphids. Fresh leaves were taken in glass petri plates of 9 cm diameter lined with moist blotting paper. The adult apterous aphids were kept in foliage to get freshly laid first instar nymphs. Subsequently, by the help of camel hair brush the first instar nymphs of aphids were released on excised cowpea leaves at the rate of one nymph per petri plate. The developmental characters of aphids were studied under binocular microscope (MAGNUS Stereoscopic binocular microscope Model MS 24 Alpha with objective (2x & 4x) and eyepiece 10x (F.N.22) having with in-built Light Stand (Incident: 6V15W Lamp/ Transmitted: 5W Fluorescence Lamp). Observations were taken at every 12 hours' duration, on moulting, change in instars, nymphal durations, adult longevity (pre-oviposition, oviposition and post-oviposition periods) and fecundity. Fresh leaves were provided as and when required. The moisture content of the blotting papers was also maintained accordingly. A total of 20 replications per selected generation were used in the study. Statistical comparisons were made using DMRT to find out significant differences in nymphal durations, adult longevity (pre oviposition, oviposition and post oviposition periods) and fecundity.

RESULTS

4.1 Development and build-up of resistance in *A. craccivora* to thiamethoxam in the laboratory

The results regarding the development of resistance in *A. craccivora* during various generations of selection to thiamethoxam have been presented in Table 4.1 to 4.14. The results obtained are as follows.

4.1.1 Determination of baseline toxicity of thiamethoxam to adult *A. craccivora*

The baseline toxicity of thiamethoxam to adults of *A. craccivora* under laboratory conditions was determined through bioassay in terms of LC_{50} values. Different diagnostic concentrations of thiamethoxam were used repeatedly in laboratory bioassay studies based on the response of adult apterous aphids. Finally, the concentrations of thiamethoxam chosen to determine the baseline LC_{50} were 0.50 ppm, 1.00 ppm, 3.00 ppm, 5.00 ppm, 7.00 ppm, 9.00 ppm and 10.00 ppm (Table 4.1). The linear mortality of aphids corresponding to each concentration of thiamethoxam from lower to higher were found to be 12.37, 25.03, 53.70, 67.35, 75.31, 80.49 and 82.46 per cent respectively. The control mortality was 3.33 per cent. The LC_{50} value was found to be 2.62 ppm with 1.91 ppm as fiducial lower limit and 3.43 ppm as fiducial upper limit. The probit mortality graph (Figure 4.12) depicted that the slope of the line (b) or, regression coefficient was 1.60 ± 0.22 with intercept value 4.327. The regression equation was determined to be Y= 1.60X + 4.32 and the Chi-square (X^2) value was 10.13 (P = 0.05) with 5 degrees of freedom.

4.1.2 Bioassay results of the selected adult *A. craccivora* in second generation (F_2)

After repetitive diagnostic bioassays of F_2 generation adult aphids, different concentrations of thiamethoxam were chosen for testing the response (Table 4.2). The concentrations chosen were 1.00 ppm, 3.00 ppm, 5.00 ppm, 7.00 ppm, 9.00 ppm, 10.00 ppm and 14.00 ppm. The linear mortality percentage among the tested aphids from lower to higher order were 17.82, 44.68, 59.21, 68.24, 74.37, 76.74 and 83.44 per cent with 3.33 per cent control mortality. The total number of tested adults in this bioassay were 240. The LC_{50} value was found to be 3.61 with fiducial limits 2.53 ppm (lower) and 4.68 ppm (upper). The regression equation was Y= 1.65X +4.07 with slope or the

regression coefficient value 1.65 ± 0.28. The probit mortality graph is depicted in Figure 4.13. The Chi-square (X^2) value was 4.69 (P = 0.05) with 5 degrees of freedom.

4.1.3 Bioassay results of the selected adult *A. craccivora* in fourth generation (F_4)

In this selected generation (F_4) various concentrations of thiamethoxam was chosen and tested against the adult apterous *A. craccivora viz*., 3.00 ppm, 5.00 ppm, 7.00 ppm, 10.00 ppm, 20.00 ppm, 30.00 ppm and 40.00 ppm (Table 4.3). The linear mortality percentage obtained among the tested aphids were 22.17, 37.71, 49.42, 61.86, 82.03, 89.91 and 93.71 per cent, respectively for those tested concentrations along with the control mortality of 3.33 per cent. The LC_{50} was found to be 7.11 ppm with fiducial limits 5.48 ppm (lower) and 8.82 ppm (upper). The Chi-square (X^2) value for this bioassay was 3.33 (P = 0.05) with 5 degrees of freedom. The slope value was found to be 2.04 ± 0.27 (Figure 4.14) with the regression equation Y= 2.04X + 3.35.

4.1.4 Bioassay results of the selected adult *A. craccivora* in sixth generation (F_6)

In sixth generation repetitive diagnostic tests were done with different concentrations of thiamethoxam to test the response of adults. Different concentrations chosen for the bioassay test were 7.00 ppm, 9.00 ppm, 20.00 ppm, 30.00 ppm, 40.00 ppm, 50.00 ppm and 60.00 ppm. The linear mortality percentage among the tested aphids were 12.45, 18.04, 43.90, 59.20, 69.38, 76.39 and 81.40 per cent with 3.33 per cent control mortality (Table 4.4). The total number of tested adults in this bioassay were 240. The LC_{50} value was found to be 23.49 ppm with fiducial limits 19.03 ppm (lower) and 28.65 ppm (upper). The Chi-square (X^2) value for this bioassay was 5.69 (P = 0.05) with 5 degrees of freedom. The slope value was found to be 2.19 ± 0.30 (Figure 4.15). The regression equation was Y = 2.19X + 2.09.

4.1.5 Bioassay results of the selected adult *A. craccivora* in eighth generation (F_8)

After repetitive diagnostic bioassays, various concentrations of thiamethoxam were chosen and tested against the adult apterous *A. craccivora viz*., 10.00 ppm, 20.00 ppm, 40.00 ppm, 60.00 ppm, 80.00 ppm, 100.00 ppm and 140.00 ppm to test in eighth selected generation. The linear mortality percentage among the tested aphids were 14.39, 33.81, 59.02, 72.76, 80.88, 86.02 and 91.84 per cent, respectively for those tested concentrations with 3.33 per cent control mortality (Table 4.5). The LC_{50} was found to be 31.32 ppm with fiducial limits 24.10 ppm (lower) and 38.72 ppm (upper).

The Chi-square (X^2) value for this bioassay was 3.72 (P = 0.05) with 5 degrees of freedom. The regression equation was Y = 2.14X + 1.85 with slope value 2.14 ± 0.28 (Figure 4.16).

4.1.6 Bioassay results of the selected adult *A. craccivora* in tenth generation (F_{10})

In 10th generation different concentrations of thiamethoxam tested for the response of adult *A. craccivora* were 10.00 ppm, 30.00 ppm, 50.00 ppm, 70.00 ppm, 100.00 ppm, 120.00 ppm and 140.00 ppm. The linear mortality percentage among the tested aphids were 5.76, 32.33, 52.43, 65.66, 77.82, 82.93 and 86.61 per cent, respectively with 3.33% control mortality (Table 4.6). The total number of tested adults in this bioassay were 240. The LC_{50} value was found to be 47.08 ppm with fiducial limits 37.42 ppm (lower) and 56.97 ppm (upper). The Chi-square (X^2) value for this bioassay was 4.54 (P = 0.05) with 5 degrees of freedom. The regression coefficient or slope value was 2.34 ± 0.32 (Figure 4.17). The regression equation was Y= 2.34X + 1.25.

4.1.7 Bioassay results of the selected adult *A. craccivora* in twelfth generation (F_{12})

Various concentrations of thiamethoxam were tested against the adult apterous *A. craccivora viz*., 10.00 ppm, 30.00 ppm, 50.00 ppm, 70.00 ppm, 100.00 ppm, 120.00 ppm and 140.00 ppm to test in 12th selected generation. The linear mortality percentage among the tested aphids from lower to higher were 7.24, 32.73, 50.92, 63.04, 74.58, 79.65 and 83.42 per cent for those tested concentrations with control mortality of 3.33 per cent (Table 4.7). The LC_{50} was found to be 48.76 ppm with fiducial limits 38.24 ppm (lower) and 59.87 ppm (upper). The Chi-square (X^2) value for this bioassay was 1.43 (P = 0.05) with 5 degrees of freedom. The regression coefficient or the slope value was 2.12 ± 0.30 (Figure 4.18) with the regression equation Y = 2.12X + 1.46.

4.1.8 Bioassay results of the selected adult *A. craccivora* in 14th generation (F_{14})

After repetitive diagnostic bioassay different concentrations of thiamethoxam were used for testing the response of adult *A. craccivora* in 14th generation. Different concentrations selected for bioassay were 30.00 ppm, 50.00 ppm, 70.00 ppm, 100.00 ppm, 120.00 ppm, 140.00 ppm and 160.00 ppm. The linear mortality percentage among the tested aphids corresponding to the different concentrations were 14.73, 31.87, 46.35, 62.21, 69.73, 75.51 and 79.99 per cent with control mortality of 3.33 per cent

(Table 4.8). The total number of tested adults in this bioassay were 240. The LC_{50} value was found to be 75.92 ppm with fiducial limits 63.05 ppm (lower) and 89.28 ppm (upper). The Chi-square (X^2) value for this bioassay was 3.30 (P = 0.05) with 5 degrees of freedom. The slope value determined was 2.59 ± 0.41 (Figure 4.19). The regression equation was Y= 2.59X + 0.19 for this bioassay test.

4.1.9 Bioassay results of the selected adult *A. craccivora* in 16th generation (F_{16})

In 16th selected generation various concentrations of thiamethoxam were chosen after repetitive tests against the adult apterous *A. craccivora viz.*, 100.00 ppm, 130.00 ppm, 160.00 ppm, 200.00 ppm, 230.00 ppm, 250.00 ppm and 270.00 ppm to test. The linear mortality percentage among the tested aphids corresponding to the above concentrations were 21.15, 32.66, 43.22, 55.10, 62.38, 66.54 and 70.22 per cent, respectively. The control mortality was 3.33 per cent (Table 4.9). The LC_{50} was found to be 181.73 ppm with fiducial limits 157.13 ppm (lower) and 209.40 ppm (upper). The Chi-square (X^2) value for this bioassay was 4.28 (P = 0.05) with 5 degrees of freedom. The slope value was 3.08 ± 0.63 (Figure 4.20) with regression equation Y = 3.08X -1.83.

4.1.10 Bioassay results of the selected adult *A. craccivora* in 18th generation (F_{18})

After repetitive tests, different concentrations of thiamethoxam were used for testing the response of adult *A. craccivora* for 18th generation. Different concentrations chosen for this bioassay were 130.00 ppm, 160.00 ppm, 200.00 ppm, 230.00 ppm, 250.00 ppm, 270.00 ppm and 300.00 ppm. The linear mortality percentage among the tested aphids were 22.60, 35.40, 51.25, 61.23, 66.90, 71.81 and 77.89 per cent, respectively with 3.33 per cent control mortality (Table 4.10). The total number of tested adults in this bioassay were 240. The LC_{50} value was found to be 196.58 ppm with fiducial limits 174.03 ppm (lower) and 216.86 ppm (upper). The Chi-square (X^2) value for this bioassay was 5.11 (P = 0.05) with 5 degrees of freedom. The regression coefficient or slope value was 4.18 ± 0.78 (Figure 4.21). The regression equation was Y = 4.18X -4.63.

Table 4.1 Baseline toxicity of thiamethoxam to adult apterous *A. craccivora* Koch. in parental generation (F_0) (Log concentrations and linear probits)

Conc. (ppm)	Conc.×10	Log (Conc.×10)	No. of insects treated	Response (%)	Corrected response (%)	Linear response (%)	Linear probits
0.50	5.00	0.70	30	20.00	17.24	12.37	3.84
1.00	10.00	1.00	29	31.03	28.66	25.03	4.33
3.00	30.00	1.48	30	43.33	41.38	53.70	5.09
5.00	50.00	1.70	30	53.33	51.72	67.35	5.45
7.00	70.00	1.85	30	73.33	72.41	75.31	5.68
9.00	90.00	1.96	30	90.00	89.66	80.49	5.86
10.00	100.00	2.00	30	93.33	93.10	82.46	5.93
control	0.00	-	30	3.33	0.00	-	-

Conclusion:

X^2 value = 10.13, not heterogeneous at P = 0.05

Regression coefficient/ slope= 1.60±0.22

Regression equation, Y= 1.60X+4.32

LC_{50} = 2.62 ppm,

Fiducial limits = 1.91- 3.43 ppm

Table 4.2 Bioassay results of the selected Adult *A. craccivora* in second generation (F_2)

Conc. (ppm)	Conc.× 10	Log (Conc.× 10)	No. of insects treated	Response (%)	Corrected response (%)	Linear response (%)	Linear probits
1.00	10.00	1.00	30	26.66	24.14	17.82	4.08
3.00	30.00	1.48	30	43.33	41.38	44.68	4.87
5.00	50.00	1.70	29	51.72	50.06	59.21	5.23
7.00	70.00	1.85	30	63.33	62.07	68.24	5.47
9.00	90.00	1.95	30	73.33	72.41	74.37	5.66
10.00	100.00	2.00	30	80.00	79.31	76.74	5.73
14.00	140.00	2.14	30	93.33	93.10	83.44	5.97
Control	0.00	-	30	3.33	0.00	-	-

Conclusion:

X^2 value = 4.69, not heterogeneous at P = 0.05

Regression coefficient/ slope= 1.65 ± 0.28

Regression equation, Y= 1.65X +4.07

LC_{50} = 3.61 ppm,

Fiducial limits = 2.53 - 4.68 ppm

Table 4.3 Bioassay results of the selected adult *A. craccivora* in fourth generation (F_4)

Conc. (ppm)	Conc.× 10	Log (Conc.× 10)	No. of insects treated	Response (%)	Corrected response (%)	Linear response (%)	Linear probits
3.00	30.00	1.48	30	23.33	20.69	22.17	4.23
5.00	50.00	1.70	30	36.67	34.48	37.71	4.69
7.00	70.00	1.85	30	46.67	44.83	49.42	4.99
10.00	100.00	2.00	30	76.67	75.86	61.86	5.30
20.00	200.00	2.30	30	83.33	82.76	82.03	5.92
30.00	300.00	2.48	30	86.67	86.21	89.91	6.28
40.00	400.00	2.60	30	93.33	93.10	93.71	6.53
Control	0.00	-	30	3.33	0.00	-	-

Conclusion:

X^2 value = 3.33, not heterogeneous at $P = 0.05$

Regression coefficient/ slope= 2.04 ± 0.27

Regression equation, Y = 2.04X + 3.35

LC_{50} = 7.11 ppm,

Fiducial limits = 5.48 - 8.82 ppm

Table 4.4 Bioassay results of the selected Adult *A. craccivora* in sixth generation (F_6)

Conc. (ppm)	Conc.× 10	Log (Conc.× 10)	No. of insects treated	Response (%)	Corrected response (%)	Linear response (%)	Linear probits
7.00	70.00	1.85	30	20.00	17.24	12.45	3.84
9.00	90.00	1.95	30	23.33	20.69	18.04	4.08
20.00	200.00	2.30	30	36.67	34.48	43.90	4.85
30.00	300.00	2.48	30	46.67	44.83	59.20	5.23
40.00	400.00	2.60	30	73.33	72.41	69.38	5.51
50.00	500.00	2.70	30	83.33	82.76	76.39	5.72
60.00	600.00	2.78	30	86.67	86.21	81.40	5.89
Control	0.00	-	30	3.33	0.00	-	-

Conclusion:

X^2 value = 5.69, not heterogeneous at $P = 0.05$

Regression coefficient/ slope= 2.19 ± 0.30

Regression equation, Y = 2.19X + 2.09

LC_{50} = 23.49 ppm,

Fiducial limits = 19.03 - 28.65 ppm

Table 4.5 Bioassay results of the selected Adult *A. craccivora* in eighth generation (F_8)

Conc. (ppm)	Log (Conc.)	No. of insects treated	Response (%)	Corrected response (%)	Linear response (%)	Linear probits
10.00	1.00	30	23.33	20.69	14.39	3.94
20.00	1.30	29	37.93	35.79	33.81	4.58
40.00	1.60	30	50.00	48.27	59.02	5.23
60.00	1.78	30	56.67	55.17	72.76	5.61
80.00	1.90	30	86.67	86.20	80.88	5.87
100.00	2.00	30	93.33	93.10	86.02	6.08
140.00	2.15	30	96.67	96.55	91.84	6.39
Control	-	30	3.33	0.00	-	-

Conclusion:

X^2 value = 3.72, not heterogeneous at $P = 0.05$

Regression coefficient/ slope= 2.14 ± 0.28

Regression equation, Y = 2.14X + 1.85

LC_{50} =31.32 ppm,

Fiducial limits = 24.10-38.72 ppm

Table 4.6 Bioassay results of the selected Adult *A. craccivora* in tenth generation (F_{10})

Conc. (ppm)	Log (Conc.)	No. of insects treated	Response (%)	Corrected response (%)	Linear response (%)	Linear probits
10.00	1.00	30	13.33	10.35	5.76	3.42
30.00	1.48	30	36.67	34.48	32.33	4.54
50.00	1.70	30	46.67	44.83	52.43	5.06
70.00	1.85	30	50.00	48.28	65.66	5.40
100.00	2.00	30	76.67	75.86	77.82	5.77
120.00	2.08	30	90.00	89.66	82.93	5.95
140.00	2.15	30	96.67	96.56	86.61	6.11
Control	-	30	3.33	0.00	-	-

Conclusion:

X^2 value = 4.54, not heterogeneous at P = 0.05

Regression coefficient/ slope= 2.34 ± 0.32

Regression equation, Y = 2.34X + 1.25

LC_{50} =47.08 ppm,

Fiducial limits = 37.42- 56.97 ppm

Table 4.7 Bioassay results of the selected Adult *A. craccivora* in twelfth generation (F_{12})

Conc. (ppm)	Log (Conc.)	No. of insects treated	Response (%)	Corrected response (%)	Linear response (%)	Linear probits
10.00	1.00	30	10.00	6.89	7.24	3.54
30.00	1.48	30	30.00	27.59	32.73	4.55
50.00	1.70	30	60.00	58.62	50.92	5.02
70.00	1.85	30	66.67	65.52	63.04	5.33
100.00	2.00	29	75.86	75.03	74.58	5.66
120.00	2.08	30	76.67	75.86	79.65	5.83
140.00	2.15	30	83.33	82.76	83.42	5.97
Control	-	30	3.33	0.00	-	-

Conclusion:

X^2 value = 1.43, not heterogeneous at P = 0.05

Regression coefficient/ slope= 2.12 ± 0.30

Regression equation, Y = 2.12X + 1.46

LC_{50} = 48.76 ppm, Fiducial limits = 38.24-59.87 ppm

Table 4.8 Bioassay results of the selected Adult *A. craccivora* in fourteenth generation (F_{14})

Conc. (ppm)	Log (Conc.)	No. of insects treated	Response (%)	Corrected response (%)	Linear response (%)	Linear probits
30.00	1.48	30	20.00	17.24	14.73	3.95
50.00	1.70	30	36.67	34.48	31.87	4.53
70.00	1.85	30	43.33	41.38	46.35	4.91
100.00	2.00	30	60.00	58.62	62.21	5.31
120.00	2.08	30	63.33	62.07	69.73	5.52
140.00	2.15	30	76.67	75.86	75.51	5.69
160.00	2.20	30	90.00	89.66	79.99	5.84
Control	-	30	3.33	0.00	-	-

Conclusion:

X^2 value = 3.30, not heterogeneous at P = 0.05

Regression coefficient/ slope= 2.59 ± 0.41

Regression equation, Y = 2.59X + 0.19

LC_{50} = 75.92 ppm,

Fiducial limits = 63.05-89.28

Table 4.9 Bioassay results of the selected Adult *A. craccivora* in sixteenth generation (F_{16})

Conc. (ppm)	Log (Conc.)	No. of insects treated	Response (%)	Corrected response (%)	Linear response (%)	Linear probits
100.00	2.00	30	30.00	27.58	21.15	4.19
130.00	2.11	30	33.33	31.03	32.66	4.55
160.00	2.20	30	40.00	37.93	43.22	4.82
200.00	2.30	30	50.00	48.27	55.10	5.12
230.00	2.36	29	58.62	57.19	62.38	5.31
250.00	2.39	30	66.67	65.51	66.54	5.42
270.00	2.43	30	83.33	82.75	70.22	5.53
0.00	-	30	3.33	0.00	-	-

Conclusion:

X^2 value = 4.28, not heterogeneous at $P = 0.05$

Regression coefficient/ slope= 3.08 ± 0.63

Regression equation, Y = 3.08X -1.83

LC_{50} = 181.73 ppm,

Fiducial limits = 157.13-209.40 ppm

Table 4.10 Bioassay results of the selected Adult *A. craccivora* in eighteenth generation (F_{18})

Conc. (ppm)	Log (Conc.)	No. of insects treated	Response (%)	Corrected response (%)	Linear response (%)	Linear probits
130.00	2.11	30	26.66	24.14	22.60	4.25
160.00	2.20	30	43.33	41.38	35.40	4.63
200.00	2.30	30	50.00	48.28	51.25	5.03
230.00	2.36	30	53.33	51.72	61.23	5.29
250.00	2.40	30	60.00	58.62	66.90	5.44
270.00	2.43	30	73.33	72.41	71.81	5.58
300.00	2.48	30	90.00	89.66	77.89	5.77
0.00	-	30	3.33	0.00	-	-

Conclusion:

X^2 value = 5.11, not heterogeneous at P = 0.05

Regression coefficient/ slope= 4.18 ± 0.78

Regression equation, Y = 4.18X -4.63

LC_{50} = 196.58 ppm,

Fiducial limits = 174.03-216.86 ppm

Table 4.11 Bioassay results of the selected Adult *A. craccivora* in twentieth generation (F_{20})

Conc. (ppm)	Log (Conc.)	No. of insects treated	Response (%)	Corrected response (%)	Linear response (%)	Linear probits
130.00	2.11	30	26.67	24.14	22.00	4.23
160.00	2.20	30	40.00	37.93	34.52	4.60
200.00	2.30	30	50.00	48.28	50.14	5.00
230.00	2.36	30	53.33	51.72	60.08	5.26
250.00	2.40	30	60.00	58.62	65.75	5.41
270.00	2.43	30	73.33	72.41	70.70	5.54
300.00	2.48	30	86.66	86.21	76.86	5.73
0.00	-	30	3.33	0.00	-	-

Conclusion:

X^2 value = 3.34, not heterogeneous at P = 0.05

Regression coefficient/ slope= 4.14±0.78

Regression equation Y = 4.14X -4.41,

LC_{50} = 199.57 ppm,

Fiducial limits = 177.081- 220.45 ppm

Table 4.12 Bioassay results of the selected Adult *A. craccivora* in 22nd generation (F_{22})

Conc. (ppm)	Log (Conc.)	No. of insects treated	Response (%)	Corrected response (%)	Linear response (%)	Linear probits
140.00	2.15	30	26.67	24.14	19.75	4.15
160.00	2.20	30	30.00	27.59	28.74	4.44
180.00	2.26	30	40.00	37.93	38.01	4.69
230.00	2.36	30	50.00	48.28	58.95	5.23
250.00	2.40	29	62.06	60.76	65.79	5.40
270.00	2.43	30	80.00	79.31	71.71	5.57
300.00	2.48	30	83.33	82.76	78.89	5.80
0.00	-	30	3.33	0.00	-	-

Conclusion:

X^2 value = 3.25, not heterogeneous at $P = 0.05$

Regression coefficient/ slope= 4.99 ± 0.83

Regression equation, Y = 4.99X -6.24

LC_{50} = 207.21 ppm,

Fiducial limits = 189.23- 225.75 ppm

Table 4.13 Bioassay results of the selected Adult *A. craccivora* in 24th generation (F_{24})

Conc. (ppm)	Log (Conc.)	No. of insects treated	Response (%)	Corrected response (%)	Linear response (%)	Linear probits
160.00	2.20	30	26.67	26.67	21.78	4.22
200.00	2.30	30	33.33	33.33	39.17	4.73
230.00	2.36	30	53.33	53.33	51.64	5.04
250.00	2.40	30	56.67	56.67	59.09	5.23
270.00	2.43	30	60.00	60.00	65.70	5.40
300.00	2.48	29	75.86	75.86	73.97	5.64
320.00	2.50	30	83.33	83.33	78.47	5.79
0.00	-	30	0.00	0.00	-	-

Conclusion:

X^2 value = 1.86, not heterogeneous at P = 0.05

Regression coefficient/ slope= 5.20 ± 0.98

Regression equation, Y = 5.20X-7.36

LC_{50} = 225.83 ppm,

Fiducial limits = 204.45-244.40 ppm

Table 4.14 Development of resistance to thiamethoxam in cowpea aphid (*A. craccivora*) under laboratory conditions selected for 24 generations

Selected generations (F_n)	LC_{50}(ppm)	Fiducial limits (ppm)		Regression coefficient (Slope) b ± SE	Regression Equation	Resistance ratio (RR)
		Lower limit	Upper limit			
F_0	2.62	1.92	3.44	1.60 ± 0.22	Y= 1.60X + 4.32	-
F_2	3.61	2.54	4.69	1.65 ± 0.28	Y= 1.65X +4.07	1.37
F_4	7.11	5.49	8.83	2.04 ± 0.27	Y = 2.04X + 3.35	2.71
F_6	23.49	19.03	28.66	2.19 ± 0.30	Y = 2.19X + 2.09	8.96
F_8	31.32	24.10	38.72	2.14 ± 0.28	Y = 2.14X + 1.85	11.95
F_{10}	47.08	37.43	56.97	2.34 ± 0.32	Y = 2.34X + 1.25	17.96
F_{12}	48.76	38.24	59.87	2.12 ± 0.30	Y = 2.12X + 1.46	18.61
F_{14}	75.92	63.05	89.28	2.59 ± 0.41	Y = 2.59X + 0.19	28.97
F_{16}	181.73	157.13	209.41	3.08 ± 0.63	Y = 3.08X -1.83	69.36
F_{18}	196.58	174.04	216.87	4.18 ± 0.78	Y = 4.18X -4.63	75.02
F_{20}	199.57	177.08	220.45	4.14 ± 0.78	Y = 4.14X -4.41	76.17
F_{22}	207.21	189.24	225.75	4.99 ± 0.83	Y = 4.99X -6.24	79.08
F_{24}	225.83	204.45	244.41	5.20 ± 0.98	Y = 5.20X-7.36	86.19

n= number of generations selected

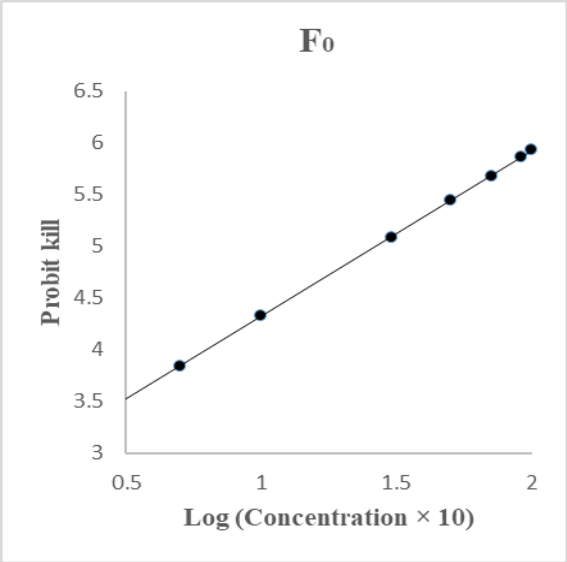

Figure 4.12 Log concentration-Probit mortality regression line graph for the parent generation (F_0)

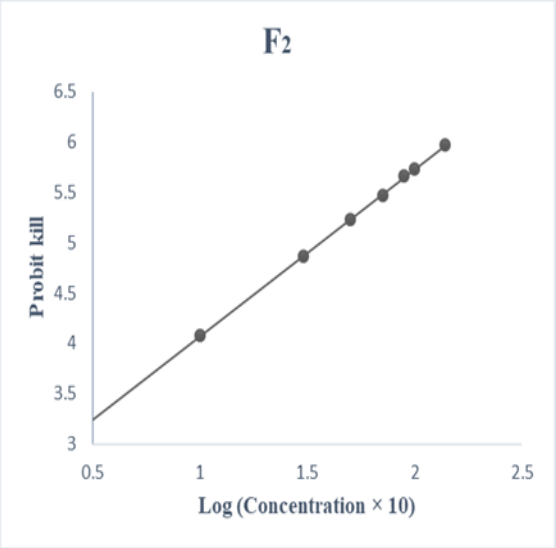

Figure 4.13 Log concentration-Probit mortality regression line graph for the selected second generation (F_2)

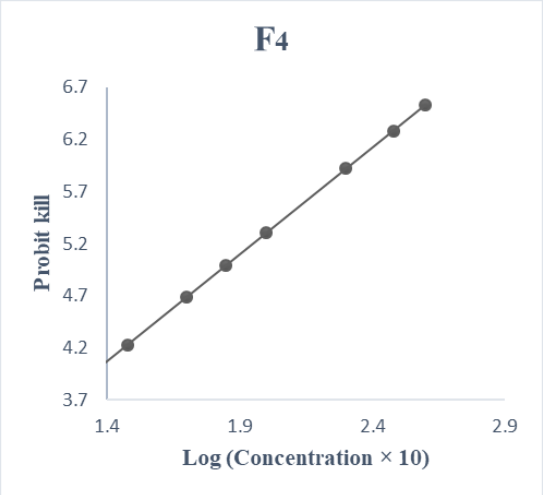

Figure 4.14 Log concentration-Probit mortality regression line graph for the selected fourth generation (F_4)

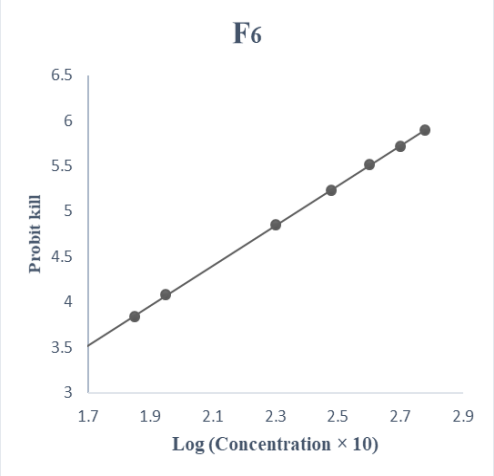

Figure 4.15 Log concentration-Probit mortality regression line graph for the selected sixth generation (F_6)

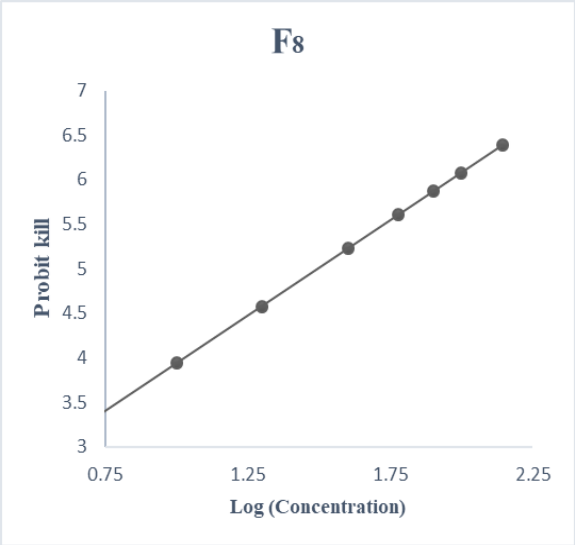

Figure 4.16 Log concentration-Probit mortality regression line graph for the selected eighth generation (F_8)

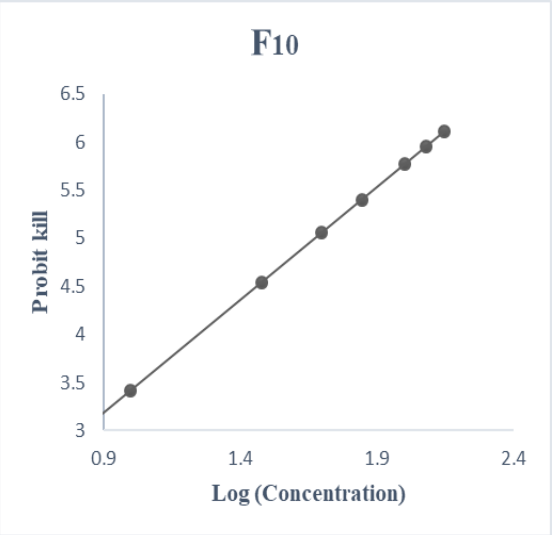

Figure 4.17 Log concentration-Probit mortality regression line graph for the selected tenth generation (F_{10})

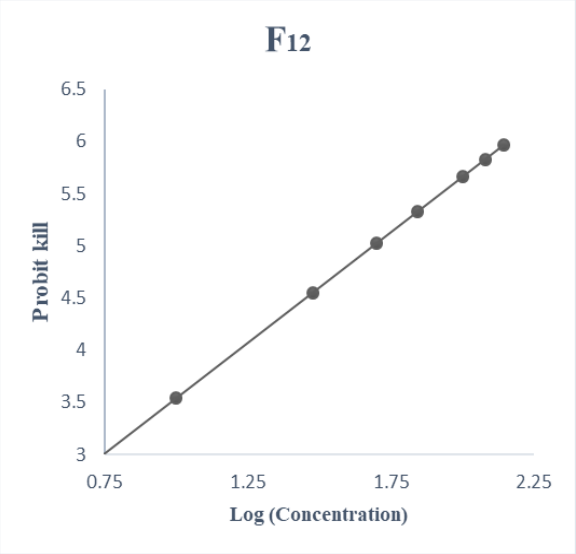

Figure 4.18 Log concentration-Probit mortality regression line graph for the selected twelfth generation (F_{12})

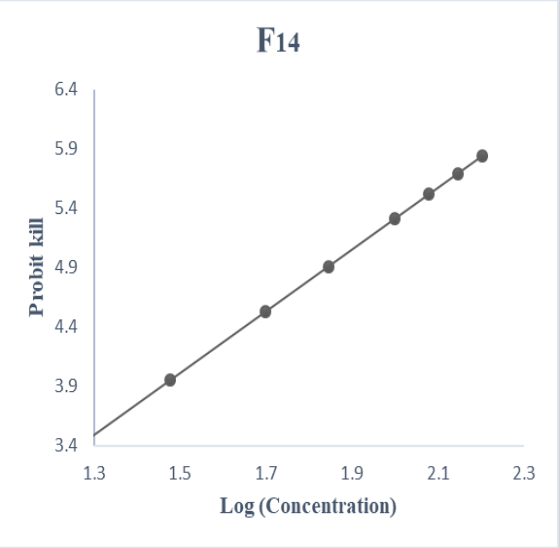

Figure 4.19 Log concentration-Probit mortality regression line graph for the selected 14th generation (F_{14})

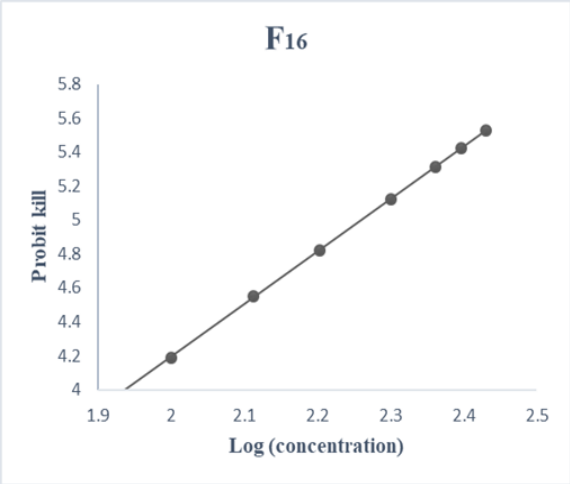

Figure 4.20 Log concentration-Probit mortality regression line graph for the selected 16th generation (F_{16})

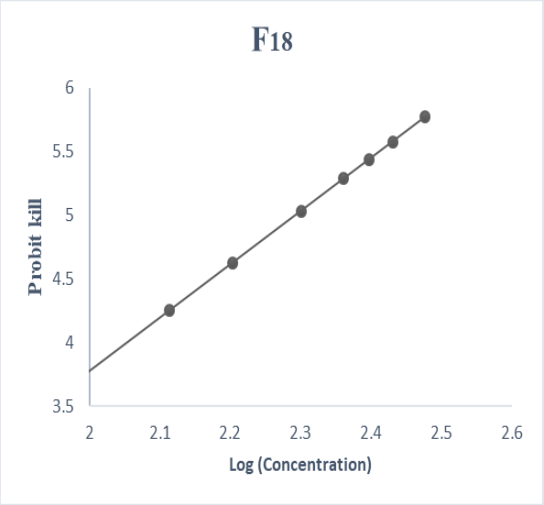

Figure 4.21 Log concentration-Probit mortality regression line graph for the selected 18th generation (F_{18})

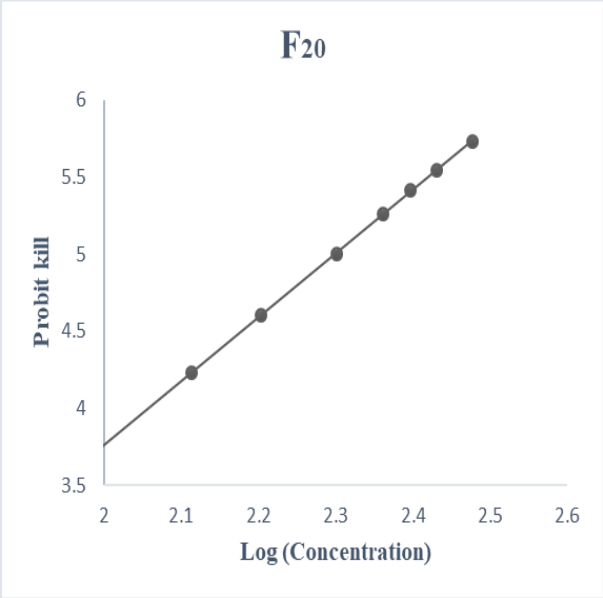

Figure 4.22 Log concentration-Probit mortality regression line graph for the selected 20th generation (F_{20})

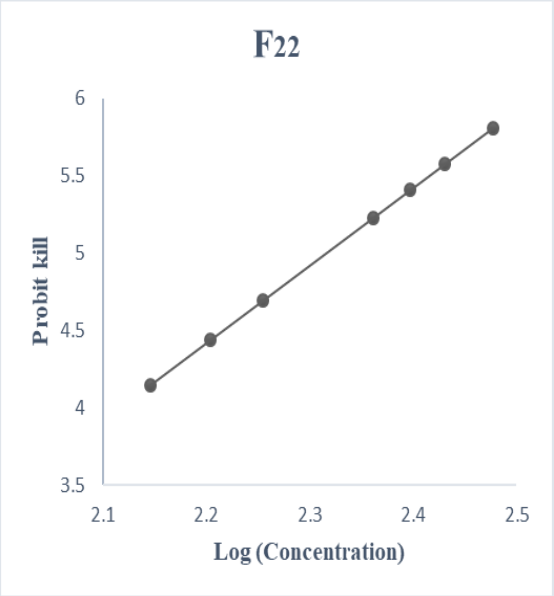

Figure 4.23 Log concentration-Probit mortality regression line graph for the selected 22nd generation (F_{22})

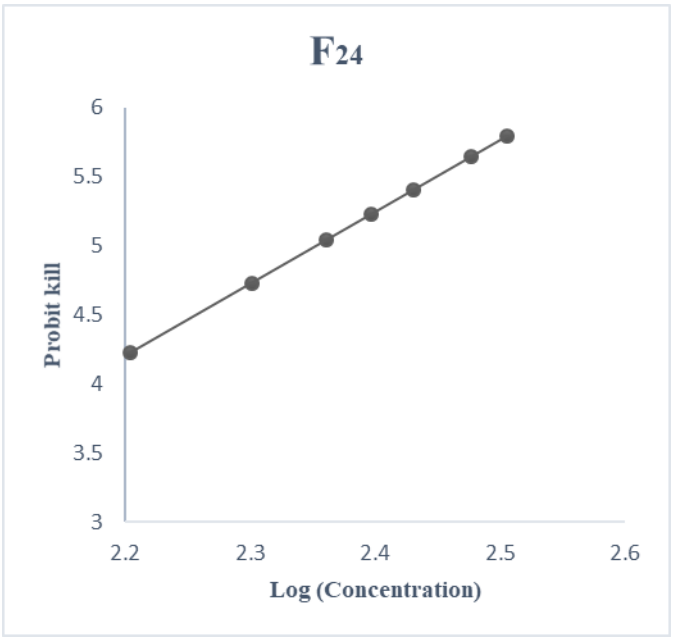

Figure 4.24 Log concentration-Probit mortality regression line graph for the selected 24th generation (F_{24})

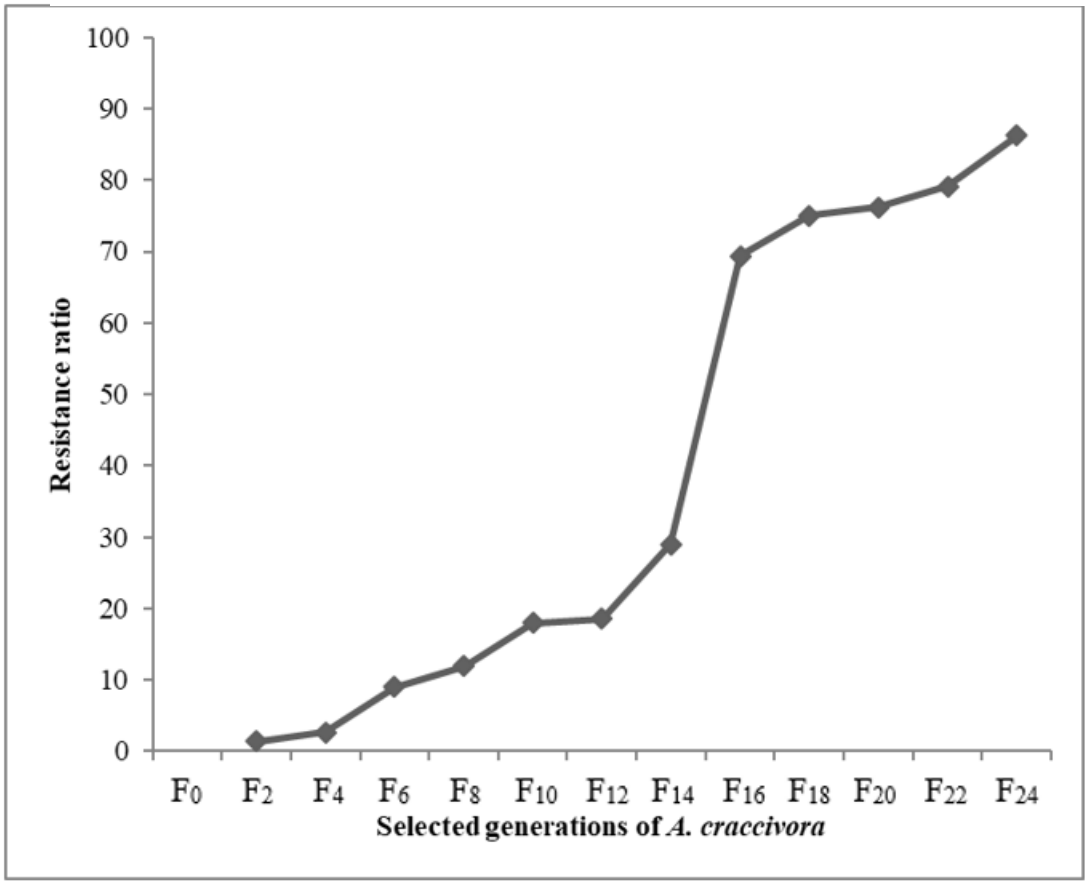

Figure 4.25 Resistance ratio in different selected generations of *A. craccivora*

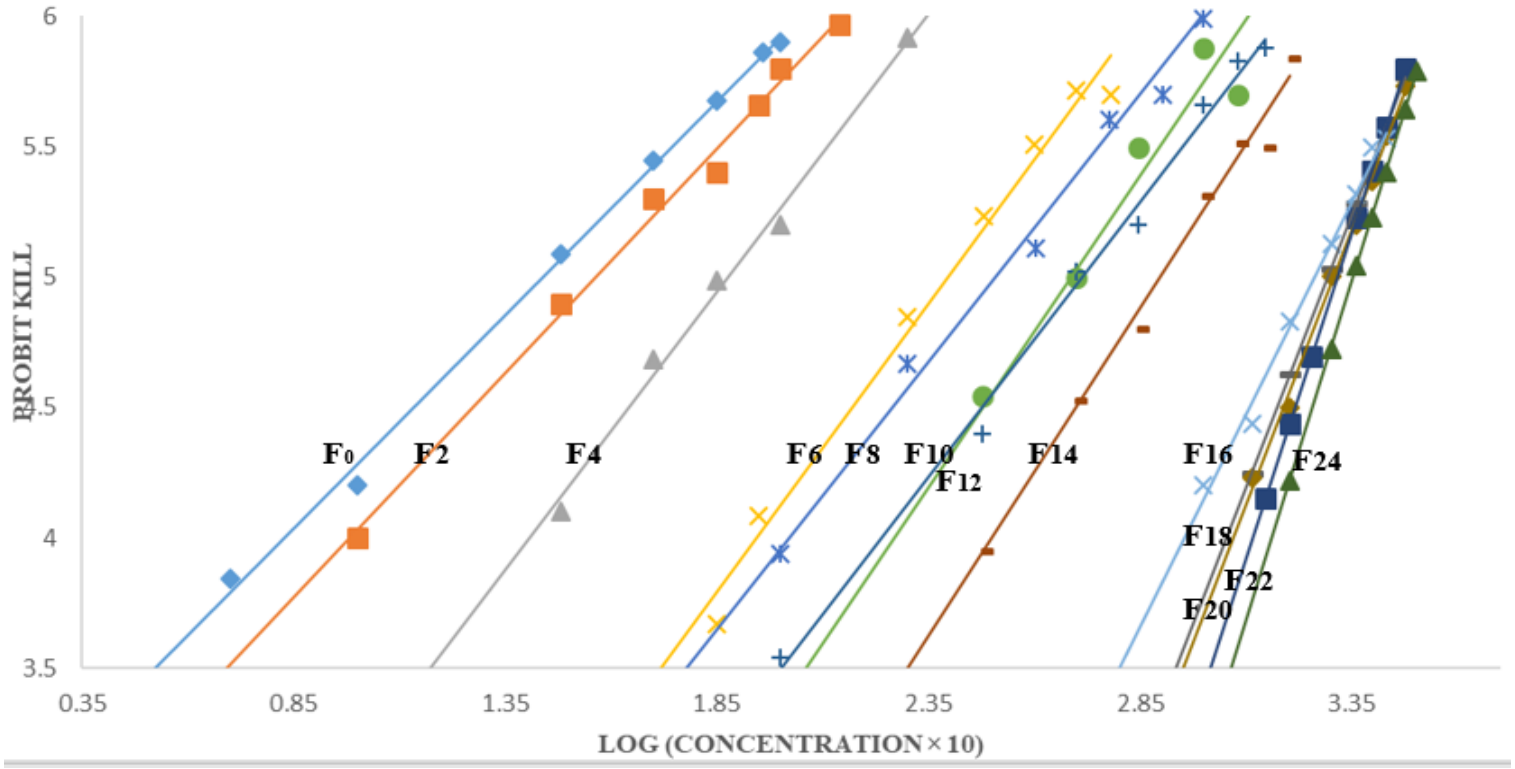

Figure 4.26 Log Concentration-probit mortality regression lines for thiamethoxam to the adults of *A. craccivora* for parental and successive generations of selection of resistant strains

4.1.11 Bioassay results of the selected adult *A. craccivora* in 20th generation (F_{20})

In 20th generation, the various thiamethoxam concentrations chosen for bioassay against the adult apterous *A. craccivora* were 130.00 ppm, 160.00 ppm, 200.00 ppm, 230.00 ppm, 250.00 ppm, 270.00 ppm and 300.00 ppm. The linear mortality percentage among the tested aphids were 22.00, 34.52, 50.14, 60.08, 65.75, 70.70 and 76.86 per cent, respectively for those tested concentrations with 3.33 per cent control mortality (Table 4.11). The LC_{50} value was found to be 199.57 ppm with fiducial limits 177.08 ppm (lower) and 220.45 ppm (upper). The Chi-square (X^2) value for this bioassay was 3.34 (P = 0.05) with 5 degrees of freedom. The slope value was determined to be 4.14 ± 0.78 (Figure 4.22) with regression equation Y = 4.14X -4.41.

4.1.12 Bioassay results of the selected adult *A. craccivora* in 22nd generation (F_{22})

After repetitive diagnostic tests different concentrations of thiamethoxam were used for testing the response of adult *A. craccivora* for 22nd generation. Different concentrations taken for this bioassay were 140.00 ppm, 160.00 ppm, 180.00 ppm, 230.00 ppm, 250.00 ppm, 270.00 ppm and 300.00 ppm. The linear mortality percentage among the tested aphids were 19.75, 28.74, 38.01, 58.94, 65.79, 71.71 and 78.89 per cent, respectively. The control mortality was 3.33 per cent (Table 4.12). The total number of tested adults in this bioassay were 240. The LC_{50} value was found to be 207.21 ppm with fiducial limits 189.23 ppm (lower) and 225.75 ppm (upper). The Chi-square (X^2) value for this bioassay was 3.25 (P = 0.05) with 5 degrees of freedom. The slope value was 4.99 ± 0.83 (Figure 4.23). The regression equation was found to be Y = 4.99X -6.24.

4.1.13 Bioassay results of the selected adult *A. craccivora* in 24th generation (F_{24})

Various concentrations of thiamethoxam was evaluated against the adult apterous *A. craccivora viz*., 160.00 ppm, 200.00 ppm, 230.00 ppm, 250.00 ppm, 270.00 ppm, 300.00 ppm and 320 ppm to test in 24th selected generation. The linear mortality percentage among the tested aphids were 21.78, 39.17, 51.64, 59.09, 65.70, 73.97 and 78.47 per cent, respectively for those tested concentrations with 0.00% control mortality (Table 4.13). The LC_{50} was found to be 225.83 ppm with fiducial limits 204.45 ppm (lower) and 244.40 ppm (upper). The Chi-square (X^2) value for this bioassay was 1.86 (P

= 0.05) with 5 degrees of freedom. The regression coefficient or, slope value was 5.20 ± 0.98 (Figure 4.24) with regression equation Y = 5.20X-7.36.

4.1.14 Development of resistance in different generations

It was envisaged from Table 4.14 that LC_{50} value increased through various generations of selection. Initially, in parental generation (susceptible) the LC_{50} value was 2.62 ppm which increased during subsequent generations, to 3.61 ppm in F_2 and 7.11 ppm in F_4, 23.49 ppm in F_6, 31.32 ppm, 47.08 ppm and 48.76 ppm, respectively in F_8, F_{10} and F_{12} generation of selection. In F_{14} the value increased to 75.92 ppm. During further generation of selection, the LC_{50} values were 181.73 ppm, 196.58 ppm, 199.57 ppm, 207.21 ppm and 225.83 ppm, respectively in F_{16}, F_{18}, F_{20}, F_{22} and F_{24} generations of selection.

When the resistance ratio was calculated, it was found increasing in various generations of selection (Figure 4.25). In 2^{nd} (F_2) and 4^{th} (F_4) generations of selection the resistance ratio were 1.37 and 2.71, respectively. Further in F_6 and F_8 the resistance ratio was found to be 8.96 and 11.95. In F_{10}, F_{12} and F_{14} the resistance ratio values were 17.96, 18.61 and 28.97, respectively. There was a marked increase of resistance ratio from F_{14} to F_{16} (28.97 to 69.36). In F_{18}, F_{20}, F_{22} and F_{24} the resistance ratio calculated were 75.02, 76.17, 79.08 and 86.16, respectively.

The regression coefficient or the slope value also increased through various generations of selection to thiamethoxam. In parental generation the slope value was 1.60 ± 0.22. Further, in different generations the value increased. In F_2, F_4, F_6, F_8, F_{10}, F_{12}, F_{14}, F_{16}, F_{18}, F_{20}, F_{22} and F_{24} generations the slope values were found to be 1.65 ± 0.28, 2.04 ± 0.27, 2.19 ± 0.30, 2.14 ± 0.28, 2.34 ± 0.32, 2.12 ± 0.30, 2.59 ± 0.41, 3.08 ± 0.63, 4.18 ± 0.78, 4.14 ± 0.78, 4.99 ± 0.83 and 5.20 ± 0.98, respectively. When log concentration-probit mortality regression lines were compared, it was found that the lines for thiamethoxam resistant strains gradually shifted to right in different selected generations and were distinctly apart from that of the parental (susceptible) strain (Figure 4.26).

4.2 Inheritance of resistance

The inheritance pattern of resistance (realised heritability) were calculated and presented in Table 4.15. The data pertaining to the projected rate of resistance evolution are showed in Table 4.16 and 4.17.

4.2.1 Estimation of realized heritability (h^2)

LC_{50} values of thiamethoxam increased from 2.62 ppm to 48.76 ppm after 12 generations of selection. During further selection until 24th generation (F_{24}) the LC_{50} value increased to 225.83 ppm (Table 4.14). The realized heritability of resistance (h^2) estimated over the first 12 generations of selection showed comparatively high value (0.28) decreasing to (0.19) from 12 to 24 generations of selection. Response to selection (R) was higher in the first half (0.10) *i.e.*, up to 12th generation of selection than that in the second half (0.038) *i.e.*, up to 24th generation of selection. Therefore, the estimated h^2 of resistance to thiamethoxam was higher for the first half than that for second half of selection process in the present study. The higher variance for resistance to thiamethoxam was lower and additive genetic variance was higher during the first half (Table 4.15). In general, results of 24 generations of selection elucidated in Table 4.15 revealed that realized heritability (h^2) showed a moderate value (0.30).

4.2.2 Projected rate of resistance evolution

The projected rate of resistance development is proportional to h^2 and intensity of selection (Figure 4.27). For example, if we assume slope = 3.06 (average mean slope for 24 generations) and h^2= 0.30, then 13 to 5 generations are needed for the 10-fold increase in the LC_{50} value of thiamethoxam as selection intensities increased from 50 to 95 per cent (Table 4.16). However, at the similar slope and h^2= 0.28, around 16 to 6 generations are needed for the 10-fold increase of LC_{50} values of thiamethoxam at 50 to 95 per cent selection intensities. Likewise, the same would occur in 20 to 7 generations in 50 to 95 per cent selection intensity if h^2= 0.19.

Table 4.15 Estimation realized heritability (h^2) of resistance to thiamethoxam in adults of *A. craccivora* Koch.

Selected generations	No of selected generations (n)	Estimate of mean response per generation		R	Estimate of mean selection differential per generation			$^{\delta}p$	S	h^2
		Log initial LC_{50}	Log final LC_{50}		p	i	Mean slope			
F_1 to F_{12}	12	0.55	1.68	0.100	50.0	0.798	2.08	0.48	0.383	0.28
F_{12} to F_{24}	12	1.88	2.35	0.038	50.0	0.798	4.03	0.25	0.200	0.19
F_1 to F_{24}	24	0.55	2.35	0.075	50.0	0.798	3.06	0.32	0.255	0.30

n, number of generations selected

R, response to selection; R= (log final LC_{50} - log initial LC_{50})/ n)

p is the average percentage of surviving rate of thiamethoxam Selected strain

i, intensity of selection (Falconer, 1989) was estimated from p, which is the percentage of the population with values above the selection threshold (*i.e*. the percentage surviving selection) using Appendix of Falconer (1989), which is based on the properties of normal distribution

it was estimated as $i = 1.583 – 0.0193336p + 0.0000428\ p^2 + 3.65194/p$

$^{\delta}p$, the phenotypic standard deviation ($^{\delta}p = [1/2(\text{initial slope} + \text{final slope})]^{-1}$

S, selection differential ($S=i.\ ^{\delta}p$)

h^2, realized heritability ($h^2=R/S$).

Table 4.16 Effect of realized heritability (h^2) on the number of generations of *A. craccivora* required for a tenfold increase in LC_{50} of thiamethoxam (slope = 3.06) at different selection intensities

Different selection intensities (mortality)	No of generations required for a tenfold increase		
	h^2= 0.30	h^2= 0.28	h^2= 0.19
50	13.00	16.00	20.00
60	11.00	12.00	16.00
70	9.00	11.00	13.00
80	7.00	9.00	11.00
90	6.00	7.00	9.00
95	5.00	6.00	7.00

Table 4.17 Effect of slope on the number of generations of *A. craccivora* required for a tenfold increase in LC_{50} of thiamethoxam (h^2 = 0.30) at different selection intensities

Different selection intensities (mortality)	No of generations required for a tenfold increase		
	Slope 3.06	Slope 4.03	Slope 2.08
50	13.00	17.00	9.00
60	11.00	14.00	7.00
70	9.00	12.00	6.00
80	7.00	10.00	5.00
90	6.00	8.00	4.00
95	5.00	6.00	3.00

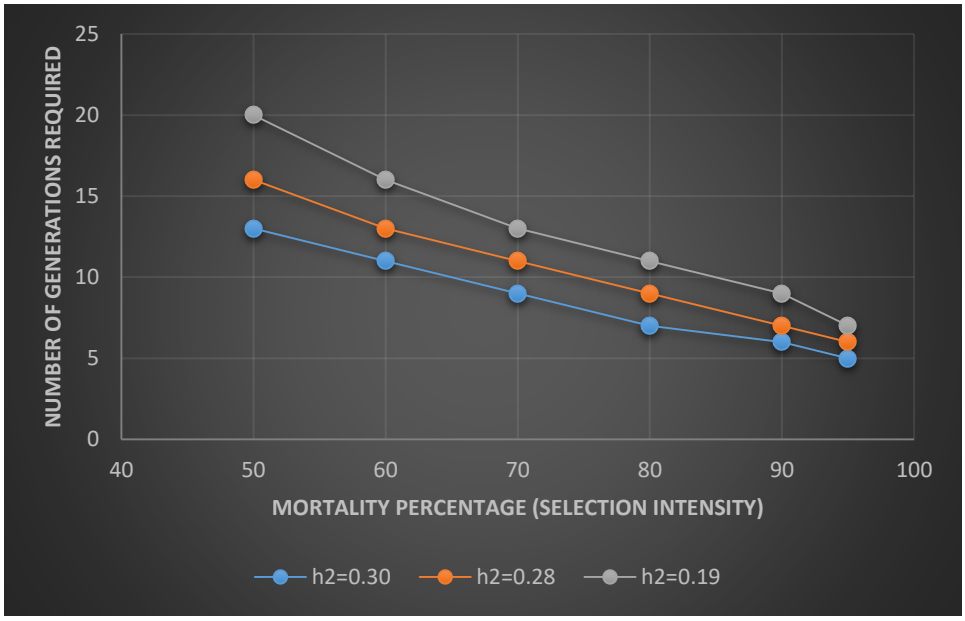

Figure 4.27 Effect of realized heritability (h^2) on the number of generations of *A. craccivora* required for a tenfold increase in LC_{50} of thiamethoxam (slope = 3.06) at different selection intensities

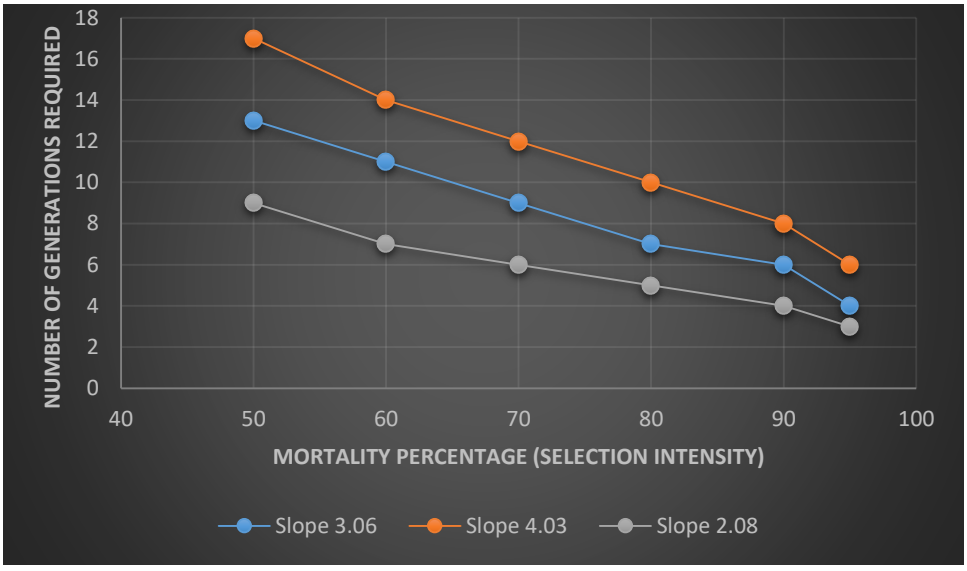

Figure 4.28 Effect of slope on the number of generations of *A. craccivora* required for a tenfold increase in LC_{50} of thiamethoxam (h^2 = 0.30) at different selection intensities

The projected rate of resistance development is inversely proportional to the slope. For example, if we assume that h^2 = 0.30 (heritability of thiamethoxam resistance estimated in the present study), and slope = 3.06 (the value of mean slope for the present work), then 13–5 generations are required for ten-fold increase in LC_{50} at 50 to 95% selection intensity. However, at the same h^2 if slope = 4.03, then 17 to 6 generations are required for ten-fold increase in LC_{50} at 50 to 95% selection intensity, respectively (Table 4.17). Similarly, if slope = 2.08, then the same would happen in 9 to 3 generations at 50 to 95% selection intensity, respectively (Figure 4.28).

4.3 Reversion of resistance in resistant populations of *A. craccivora*

The results regarding the reversion of resistance in resistant populations of *A. craccivora* in various generations have been presented in Table 4.18 to 4.26. During 26th to 42nd generations of selections, there was no selection pressure with thiamethoxam. The results are elaborated as follows.

4.3.1 Bioassay results of the 26th generation (F_{26}) of *A. craccivora*

Various concentrations of thiamethoxam were chosen and tested in 26th generation after repetitive diagnostic bioassays. The concentrations chosen were 140.00 ppm, 160.00 ppm, 170.00 ppm, 220.00 ppm, 230.00 ppm, 250.00 ppm and 290.00 ppm. In the bioassay testing the linear mortality percentage from lower to higher order were 24.68, 34.89, 39.98, 62.49, 66.17, 72.65 and 82.42 per cent with 0.00 per cent control mortality (Table 4.18). The LC_{50} value was 190.58 ppm with fiducial limits 173.34-206.74 ppm. The Chi-square (X^2) value for this bioassay was 2.06 (P= 0.05) with 5 degrees of freedom. The regression coefficient or slope was 5.11±0.88 (Figure 4.29) and the regression equation was Y = 5.11X- 6.78.

4.3.2 Bioassay results of the 28th generation (F_{28}) of *A. craccivora*

After repetitive diagnostic bioassay different concentrations of thiamethoxam were used for testing the response of adult *A. craccivora* in 28th generation. Different concentrations were 130.00 ppm, 150.00 ppm, 160.00 ppm, 210.00 ppm, 220.00 ppm, 240.00 ppm and 280.00 ppm. The linear mortality percentage among the tested aphids from lower to higher concentrations of thiamethoxam were 22.51, 31.66, 36.24, 56.97, 60.49, 66.85 and 76.87 per cent with control mortality of 3.33 per cent (Table 4.19).

Table 4.18 Bioassay results of the adults of *A. craccivora* in 26th generation

Conc. (ppm)	Log (Conc.)	No. of insects treated	Response (%)	Corrected response (%)	Linear response (%)	Linear probits
140.00	2.15	30	23.33	23.33	24.68	4.32
160.00	2.20	30	30.00	30.00	34.89	4.61
170.00	2.23	30	50.00	50.00	39.98	4.75
220.00	2.34	30	56.66	56.66	62.49	5.32
230.00	2.36	30	66.66	66.66	66.17	5.42
250.00	2.40	30	73.33	73.33	72.65	5.60
290.00	2.46	30	83.33	83.33	82.42	5.93
Control	-	30	0.00	0.00	-	-

Conclusion:

X^2 value = 2.06, not heterogeneous at P = 0.05

Regression coefficient/ slope= 5.11±0.88

Regression equation, Y = 5.11X- 6.78

LC_{50} =190.58 ppm,

Fiducial limits = 173.34- 206.74 ppm

Table 4.19 Bioassay results of the adults of *A. craccivora* in 28th generation

Conc. (ppm)	Log (Conc.)	No. of insects treated	Response (%)	Corrected response (%)	Linear response (%)	Linear probits
130.00	2.11	30	23.33	20.69	22.51	4.25
150.00	2.17	30	30.00	27.58	31.66	4.52
160.00	2.20	30	46.66	44.82	36.24	4.65
210.00	2.32	30	53.33	51.72	56.97	5.18
220.00	2.34	30	63.33	62.06	60.49	5.27
240.00	2.38	30	70.00	68.96	66.85	5.44
280.00	2.44	30	76.66	75.86	76.87	5.74
Control	-	30	3.33	0.00	-	-

Conclusion:

X^2 value = 1.52, not heterogeneous at P = 0.05

Regression coefficient/ slope= 4.30±0.82

Regression equation, Y = 4.29X- 4.83

LC_{50} =187.05 ppm,

Fiducial limits = 168.06- 206.44 ppm

Table 4.20 Bioassay results of the adults of *A. craccivora* in 30th generation

Conc. (ppm)	Log (Conc.)	No. of insects treated	Response (%)	Corrected response (%)	Linear response (%)	Linear probits
120.00	2.08	30	23.33	23.33	22.49	4.25
150.00	2.18	30	30.00	30.00	35.75	4.64
160.00	2.20	30	46.66	46.66	40.04	4.75
210.00	2.32	30	53.33	53.33	58.85	5.22
220.00	2.34	30	63.33	63.33	61.98	5.31
240.00	2.38	30	70.00	70.00	67.63	5.46
280.00	2.45	30	76.66	76.66	76.65	5.73
Control	-	30	0.00	0.00	-	-

Conclusion:

X^2 value = 1.47, not heterogeneous at P = 0.05

Regression coefficient/ slope= 4.03±0.77

Regression equation, Y = 4.03X- 4.16

LC_{50} = 184.80 ppm,

Fiducial limits = 164.75- 205.26 ppm

Table 4.21 Bioassay results of the adults of *A. craccivora* in 32th generation

Conc. (ppm)	Log (Conc.)	No. of insects treated	Response (%)	Corrected response (%)	Linear response (%)	Linear probits
110.00	2.04	30	23.33	20.69	20.41	4.17
130.00	2.11	30	30.00	27.58	29.68	4.46
150.00	2.17	30	46.67	44.82	38.91	4.71
200.00	2.30	30	50.00	48.27	58.87	5.22
210.00	2.32	30	63.33	62.06	62.17	5.31
220.00	2.34	30	70.00	68.96	65.24	5.39
250.00	2.39	30	76.67	75.86	73.13	5.61
Control	-	30	3.33	0.00	-	-

Conclusion:

X^2 value = 2.08, not heterogeneous at P = 0.05

Regression coefficient/ slope= 3.88±0.75

Regression equation, Y = 3.88X- 3.73

LC_{50} = 171.18 ppm,

Fiducial limits = 152.09- 191.01 ppm

Table 4.22 Bioassay results of the adults of *A. craccivora* in 34th generation

Conc. (ppm)	Log (Conc.)	No. of insects treated	Response (%)	Corrected response (%)	Linear response (%)	Linear probits
10.00	1.00	30	26.67	24.14	17.04	4.05
20.00	1.30	30	30.00	27.59	32.27	4.54
40.00	1.60	30	50.00	48.28	51.30	5.03
60.00	1.78	30	53.33	51.72	62.58	5.32
80.00	1.90	30	70.00	68.97	70.03	5.53
100.00	2.00	30	76.67	75.87	75.29	5.68
120.00	2.08	30	90.00	89.66	79.20	5.81
Control	-	30	3.33	0.00	-	-

Conclusion:

X^2 value = 5.02, not heterogeneous at $P = 0.05$

Regression coefficient/ slope= 1.63±0.26

Regression equation, Y = 1.63X+ 2.44

LC_{50} = 38.20 ppm,

Fiducial limits = 28.19- 49.31ppm

Table 4.23 Bioassay results of the adults of *A. craccivora* in 36th generation

Conc. (ppm)	Conc.×10	Log (Conc.×10)	No. of insects treated	Response (%)	Corrected response (%)	Linear response (%)	Linear probits
5.00	50.00	1.70	30	16.67	13.79	20.29	4.16
7.00	70.00	1.85	30	30.00	27.58	27.32	4.39
10.00	100.00	2.00	30	50.00	48.27	35.90	4.64
20.00	200.00	2.30	30	53.33	51.72	54.36	5.11
40.00	400.00	2.60	30	70.00	68.96	71.91	5.58
60.00	600.00	2.78	30	76.66	75.86	80.38	5.86
80.00	800.00	2.90	30	90.00	89.65	85.32	6.05
Control	0.00	-	30	3.33	0.00	-	-

Conclusion:

X^2 value = 3.83, not heterogeneous at P = 0.05

Regression coefficient/ slope= 1.56±0.22

Regression equation, Y = 1.56X+ 3.03

LC_{50} = 17.02 ppm,

Fiducial limits = 12.67- 22.42 ppm

Table 4.24 Bioassay results of the adults of *A. craccivora* in 38th generation

Conc. (ppm)	Conc.×10	Log (Conc.×10)	No. of insects treated	Response (%)	Corrected response (%)	Linear response (%)	Linear probits
1.00	10.00	1.00	30	16.67	13.69	11.41	3.79
3.00	30.00	1.48	30	30.00	27.50	32.16	4.53
5.00	50.00	1.69	30	50.00	48.21	45.31	4.88
7.00	70.00	1.85	30	53.33	51.66	54.35	5.10
10.00	100.00	2.00	30	66.67	65.47	63.69	5.35
20.00	200.00	2.30	30	76.67	75.83	79.35	5.81
30.00	300.00	2.48	30	90.00	89.64	86.27	6.09
Control	0.00	-	30	3.33	0.00	-	-

Conclusion:

X^2 value = 1.20, not heterogeneous at P = 0.05

Regression coefficient/ slope= 1.55±0.22

Regression equation, Y = 1.55X+ 3.80

LC_{50} = 5.95 ppm,

Fiducial limits = 4.40- 7.85 ppm

Table 4.25 Bioassay results of the adults of *A. craccivora* in 40th generation

Conc. (ppm)	Conc.×10	Log (Conc.×10)	No. of insects treated	Response (%)	Corrected response (%)	Linear response (%)	Linear probits
0.50	5.00	0.70	30	16.67	13.79	10.66	3.76
1.00	10.00	1.00	29	31.03	28.66	23.07	4.26
3.00	30.00	1.48	30	43.33	41.38	52.77	5.07
5.00	50.00	1.70	30	53.33	51.72	67.17	5.44
7.00	70.00	1.85	30	73.33	72.41	75.53	5.69
9.00	90.00	1.95	30	90.00	89.66	80.94	5.88
10.00	100.00	2.00	30	93.33	93.10	82.97	5.95
Control	0.00	-	30	3.33	0.00	-	-

Conclusion:

X^2 value = 4.56, not heterogeneous at P = 0.05

Regression coefficient/ slope= 1.68±0.23

Regression equation, Y = 1.68X+ 4.26

LC_{50} = 2.72 ppm,

Fiducial limits = 2.02- 3.53 ppm

Table 4.26 Bioassay results of the adults of *A. craccivora* in 42nd generation

Conc. (ppm)	Conc.×10	Log (Conc.×10)	No. of insects treated	Response (%)	Corrected response (%)	Linear response (%)	Linear probits
0.50	5.00	0.70	29	17.24	14.39	11.97	3.82
1.00	10.00	1.00	29	31.03	28.66	25.33	4.33
3.00	30.00	1.48	30	53.33	51.72	55.88	5.15
5.00	50.00	1.70	30	60.00	58.62	70.04	5.55
7.00	70.00	1.85	29	68.96	67.90	78.06	5.77
9.00	90.00	1.95	30	90.00	89.66	83.14	5.96
10.00	100.00	2.00	30	96.66	96.56	85.03	6.03
Control	0.00	-	30	3.33	0.00	-	-

Conclusion:

X^2 value = 5.21, not heterogeneous at P = 0.05

Regression coefficient/ slope= 1.70±0.23

Regression equation, Y = 1.70X+ 4.31

LC_{50} = 2.45 ppm,

Fiducial limits = 1.79- 3.18 ppm

Table 4.27 Reversion of resistance in *A. craccivora* without selection pressure

Selected generations (F_n)	LC_{50} (ppm)	Fiducial limits (ppm)		Regression coefficient (slope) b±SE	Regression equation	Resistance ratio (RR)
		Lower limit	Upper limit			
F_{24}	225.83	204.45	244.41	5.20±0.98	Y = 5.20X-7.36	86.19
F_{26}	190.58	173.33	206.74	5.11±0.88	Y = 5.11X- 6.78	72.74
F_{28}	187.04	168.06	206.44	4.30±0.82	Y = 4.29X- 4.83	71.39
F_{30}	184.80	164.75	205.26	4.03±0.77	Y = 4.03X- 4.16	70.53
F_{32}	171.18	152.09	191.01	3.88±0.75	Y = 3.88X- 3.73	65.33
F_{34}	38.20	28.19	49.31	1.63±0.26	Y = 1.63X+ 2.44	14.58
F_{36}	17.02	12.67	22.42	1.56±0.22	Y = 1.56X+ 3.03	6.49
F_{38}	5.95	4.40	7.85	1.55±0.22	Y = 1.55X+ 3.80	2.27
F_{40}	2.72	2.02	3.53	1.68±0.23	Y = 1.68X+ 4.26	1.03
F_{42}	2.45	1.79	3.18	1.70±0.23	Y = 1.70X+ 4.31	0.93

n= number of generations selected

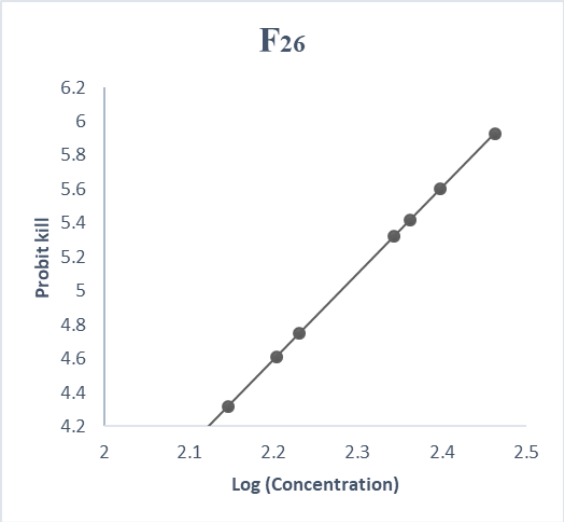

Figure 4.29 Log concentration-Probit mortality regression line graph for the 26th generation (F_{26})

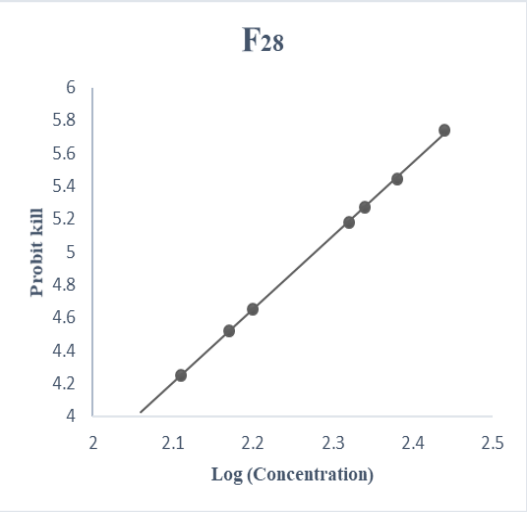

Figure 4.30 Log concentration-Probit mortality regression line graph for the 28th generation (F_{28})

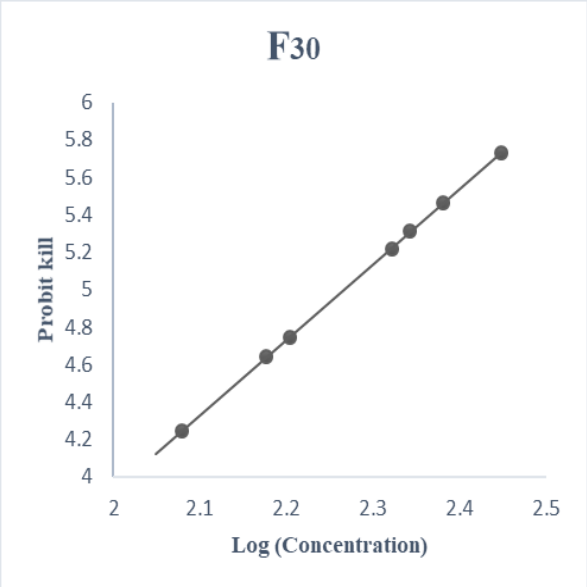

Figure 4.31 Log concentration-Probit mortality regression line graph for the 30th generation (F_{30})

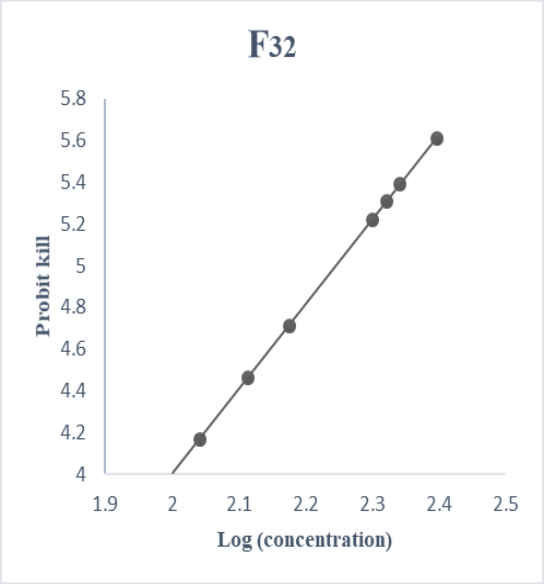

Figure 4.32 Log concentration-Probit mortality regression line graph for the 32nd generation (F_{32})

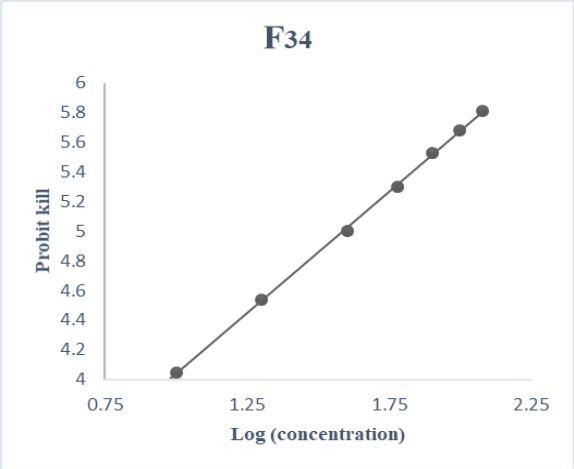

Figure 4.33 Log concentration-Probit mortality regression line graph for the 34th generation (F_{34})

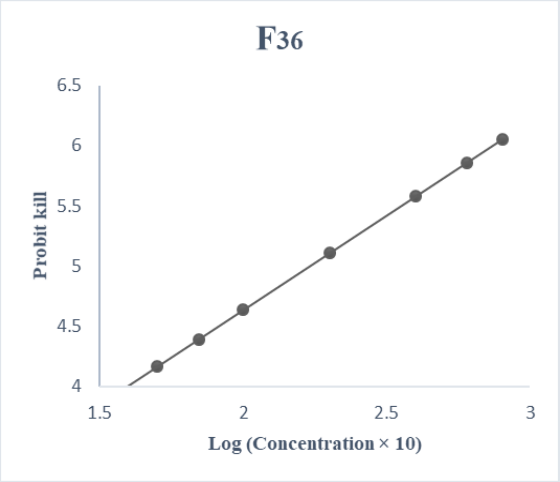

Figure 4.34 Log concentration-Probit mortality regression line graph for the 36th generation (F_{36})

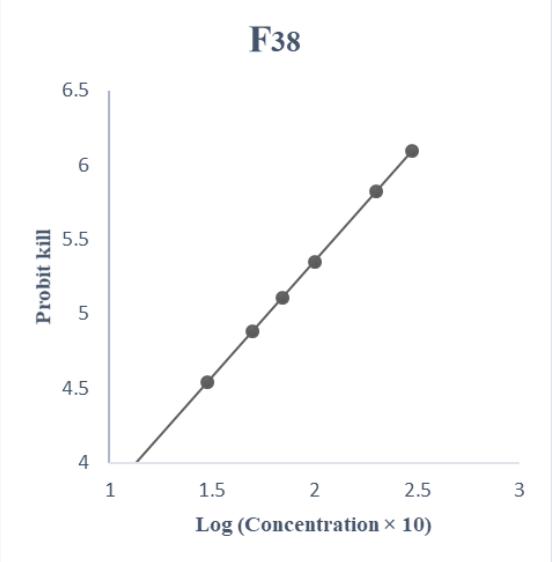

Figure 4.35 Log concentration-Probit mortality regression line graph for the 38th generation (F_{38})

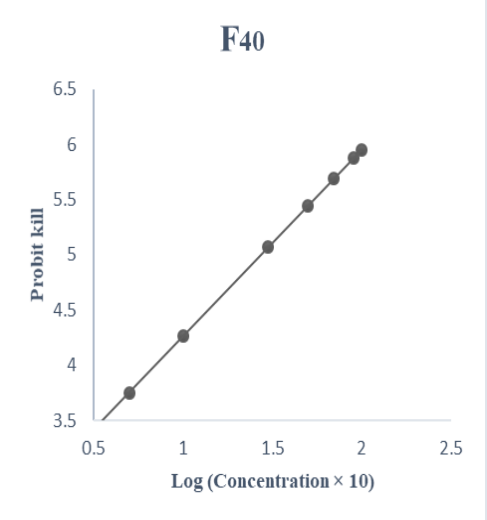

Figure 4.36 Log concentration-Probit mortality regression line graph for the 40th generation (F_{40})

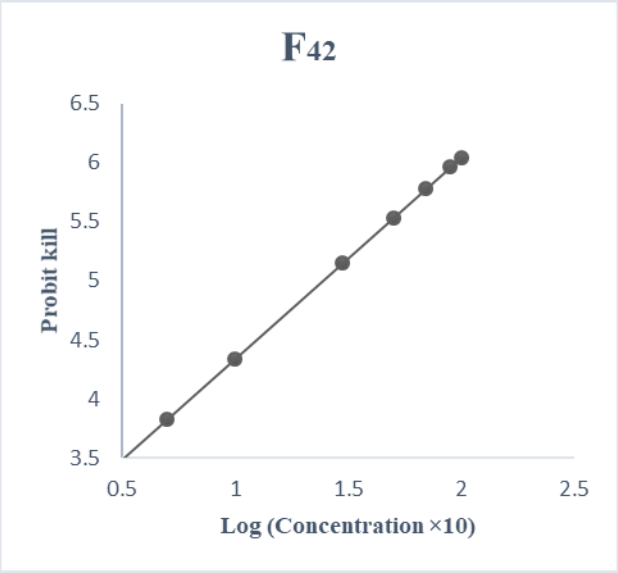

Figure 4.37 Log concentration-Probit mortality regression line graph for the 42nd generation (F_{42})

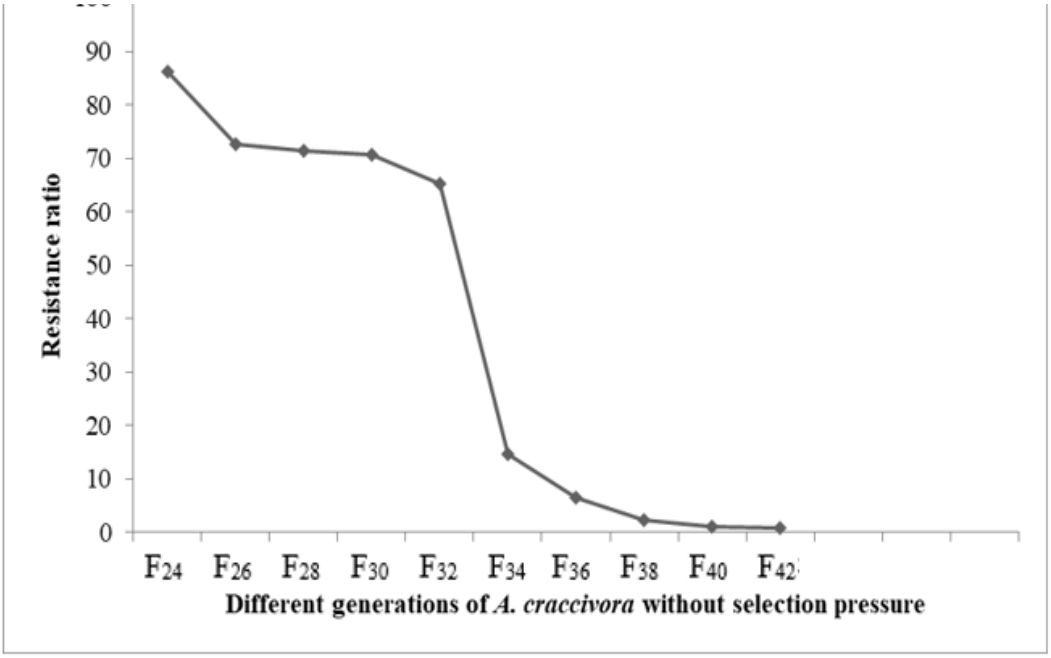

Figure 4.38 Resistance ratio (Resistance folds) in different generations of *A. craccivora* during reversion of resistance

The total number of tested adults in this bioassay were 240. The LC_{50} value was found to be 187.05 ppm with fiducial limits 168.06 ppm (lower) and 206.44 ppm (upper). The Chi-square (X^2) value for this bioassay was 1.52 (P = 0.05) with 5 degrees of freedom. The slope value 4.30±0.82 (Figure 4.30). The regression equation was Y = 4.29X- 4.83 for this bioassay test.

4.3.3 Bioassay results of the 30th generation (F_{30}) of *A. craccivora*

Various concentrations of thiamethoxam were chosen and tested in 30th generation after repetitive diagnostic bioassays. The concentrations finally selected for bioassay were 120.00 ppm, 150.00 ppm, 160.00 ppm, 210.00 ppm, 220.00 ppm, 240.00 ppm and 280.00 ppm. In the bioassay testing, the linear mortality percentage from lower to higher order were 22.49, 35.75, 40.04, 58.85, 61.98, 67.63 and 76.65 per cent with 0.00 per cent control mortality (Table 4.20). The LC_{50} value was 184.80 ppm with fiducial limits 164.75-205.26 ppm. The Chi-square (X^2) value for this bioassay was 1.47 (P = 0.05) with 5 degrees of freedom. The regression coefficient or slope was 4.03±0.77 (Figure 4.31) and the regression equation was Y = 4.03X- 4.16.

4.3.4 Bioassay results of the 32nd generation (F_{32}) of *A. craccivora*

After repetitive diagnostic bioassay, different concentrations of thiamethoxam were used for testing the response of adult *A. craccivora* in 32nd generation. Different concentrations chosen were 110.00 ppm, 130.00 ppm, 150.00 ppm, 200.00 ppm, 210.00 ppm, 220.00 ppm and 250.00 ppm. The linear mortality percentage among the tested aphids from lower to higher were 20.41, 29.68, 38.91, 58.87, 62.17, 65.24 and 73.13 per cent with control mortality of 3.33 per cent (Table 4.21). The total number of tested adults in this bioassay were 240. The LC_{50} value was found to be 171.18 ppm with fiducial limits 152.09 ppm (lower) and 191.01 ppm (upper). The Chi-square (X^2) value for this bioassay was 2.08 (P = 0.05) with 5 degrees of freedom. The slope value was found to be 3.88±0.75 (Figure 4.32). The regression equation was Y = 3.88X- 3.73 for this bioassay test.

4.3.5 Bioassay results of the 34th generation (F_{34}) of *A. craccivora*

For the 34th generation there was marked changes in the ranges of various concentrations of thiamethoxam. Various concentrations were chosen and tested in 34th generation after repetitive diagnostic bioassays. The concentrations selected were 10 ppm, 20 ppm, 40 ppm, 60 ppm, 80 ppm, 100 ppm and 120 ppm. In the bioassay testing the linear mortality percentage from lower to higher order were 17.04, 32.27, 51.30, 62.58, 70.03, 75.29 and 79.20 per cent with 3.33 per cent control mortality (Table 4.22). The LC_{50} value found was 38.20 ppm with fiducial limits 28.19 to 49.31 ppm. The Chi-square (X^2) value for this bioassay was 5.02 (P = 0.05) with 5 degrees of freedom. The regression coefficient or slope was determined to be 1.63±0.26 (Figure 4.33) and the regression equation was Y = 1.63X+ 2.44.

4.3.6 Bioassay results of the 36th generation (F_{36}) of *A. craccivora*

After repetitive diagnostic bioassay, different concentrations of thiamethoxam were used for testing the response of adult *A. craccivora* in 36th generation. Different concentrations chosen were 5.00 ppm, 7.00 ppm, 10.00 ppm, 20.00 ppm, 40.00 ppm, 60.00 ppm and 80.00 ppm. The linear mortality percentage among the tested aphids from lower to higher concentration of thiamethoxam were 20.29, 27.32, 35.90, 54.36, 71.91, 80.38 and 85.32 per cent with control mortality of 3.33 per cent (Table 4.23). The total number of tested adults in this bioassay were 240. The LC_{50} value was found to be 17.02 ppm with fiducial limits 12.67 ppm (lower) and 22.42 ppm (upper). The Chi-square (X^2) value for this bioassay was 3.83 (P = 0.05) with 5 degrees of freedom. The slope value was found to be 1.56±0.22 (Figure 4.34). The regression equation was Y = 1.56X+ 3.03 for this bioassay test.

4.3.7 Bioassay results of the 38th generation (F_{38}) of *A. craccivora*

Various concentrations were chosen and tested in 38th generation after repetitive diagnostic bioassays. The concentrations selected were 1.00 ppm, 3.00 ppm, 5.00 ppm, 7.00 ppm, 10.00 ppm, 20.00 ppm and 30.00 ppm. In the bioassay testing the linear mortality percentage from lower to higher order were 11.41, 32.16, 45.31, 54.35, 63.69, 79.35 and 86.27 per cent with 3.33 per cent control mortality (Table 4.24). The LC_{50}

value was 5.95 ppm with fiducial limits 4.40 to 7.85 ppm. The Chi-square (X^2) value for this bioassay was 1.20 (P = 0.05) with 5 degrees of freedom. The regression coefficient or slope was 1.55±0.22 (Figure 4.35) and the regression equation was Y = 1.55X + 3.80.

4.3.8 Bioassay results of the 40th generation (F_{40}) of *A. craccivora*

After repetitive diagnostic bioassays different concentrations of thiamethoxam were used for testing the response of adult *A. craccivora* in 40th generation. Different concentrations chosen for this bioassay were 0.50 ppm, 1.00 ppm, 3.00 ppm, 5.00 ppm, 7.00 ppm, 9.00 ppm and 10.00 ppm. The linear mortality percentage among the tested aphids from lower to higher were 10.66, 23.07, 52.77, 67.17, 75.53, 80.94 and 82.97 per cent respectively with control mortality of 3.33 per cent (Table 4.25). The total number of tested adults in this bioassay were 239. The LC_{50} value was found to be 2.72 ppm with fiducial limits 2.02 ppm (lower) and 3.53 ppm (upper). The Chi-square (X^2) value for this bioassay was 4.56 (P = 0.05) with 5 degrees of freedom. The slope value 1.68±0.23 (Figure 4.36). The regression equation was Y = 1.68X+ 4.26 for this bioassay test.

4.3.9 Bioassay results of the 42th generation (F_{42}) of *A. craccivora*

Various concentrations were chosen and tested in 42nd generation after repetitive diagnostic bioassays. The concentrations finally selected were 0.50 ppm, 1.00 ppm, 3.00 ppm, 5.00 ppm, 7.00 ppm, 9.00 ppm and 10.00 ppm. In the bioassay testing the linear mortality percentage from lower to higher order were 11.97, 25.33, 55.88, 70.04, 78.06, 83.14 and 85.03 per cent, respectively with 3.33 per cent control mortality (Table 4.26). The LC_{50} value was 2.45 ppm with fiducial limits 1.79 to 3.18 ppm. The Chi-square (X^2) value for this bioassay was 5.21 (P = 0.05) with 5 degrees of freedom. The regression coefficient or slope was 1.70±0.23 (Figure 4.37) and the regression equation was Y = 1.70X + 4.31.

4.3.10 Reversion of resistance in different generations

The trends of reversion of resistance in *A. craccivora* were summarized in Table 4.27. It was clear that without any selection pressure with thiamethoxam there was marked changes in the resistance values in different generations (Table 4.27). The LC_{50}

values were changed through various generations. In F_{24} the LC_{50} value was 225.83 ppm. Without selection pressure this value was decreased to 190.58 ppm in F_{26}, 187.04 ppm in F_{28}, 184.80 ppm in F_{30}, 171.18 ppm, 38.20 ppm and 17.02 ppm in F_{32}, F_{34} and F_{36} respectively. Further, the value decreased in F_{38}, F_{40} and F_{42} with values 5.95 ppm, 2.72 ppm and 2.45 ppm, respectively.

Resistance ratios (RR) when calculated for various generations, it was clear and evident that it followed a decreasing trend in different generations. In $F_{24,}$ RR was 86.19. Further in F_{26}, F_{28} and F_{30} the ratios were 72.74, 71.39 and 70.53, respectively. In between F_{32} and F_{34} there was marked changes in resistance ratios. The value changed from 65.33 (in F_{32}) to 14.58 (in F_{34}). Further, the LC_{50} values in F_{36}, F_{38}, F_{40} and F_{42} generations were 6.49, 2.27, 1.03 and 0.93, respectively (Figure 4.38).

The regression coefficient or the slope value also decreased through various generations without any selection pressure of thiamethoxam. After attaining highest level of resistance in F_{24} the slope was 5.20±0.98. Further in different generations during the reversal of resistance the value decreased. In F_{26}, F_{28}, F_{30}, F_{32}, F_{34}, F_{36}, F_{38}, F_{40} and F_{42} the slope values were found to be 5.11±0.88, 4.30±0.82, 4.03±0.77, 3.88±0.75, 1.63±0.26, 1.56±0.22, 1.55±0.22, 1.68±0.23 and 1.70±0.23, respectively.

4.4 Persistent toxicity of different dosages of thiamethoxam to *A. craccivora* in cowpea

4.4.1 After 24 hours of exposure

The details of the insecticides chosen, for persistence toxicity studies, their doses, trade names and manufacturer have been listed in Table 3.1. After 24 hours of exposure, 100% mortality was observed in thiamethoxam 25g a.i./ha, thiamethoxam 30g a.i./ha, thiamethoxam 35g a.i./ha and dimethoate 300g a.i./ha. Thiamethoxam @ 35g a.i./ha persisted as long as 23 days followed by imidacloprid @ 25g a.i./ha (21 days), thiamethoxam @ 25g a.i./ha and @ 30g a.i./ha (each 15 days), dimethoate @ 300g a.i./ha (13 days) and thiamethoxam 20g a.i./ha (11 days) (Table 4.28) (Figure 4.39, 4.40 and 4.41).

Average residual toxicity (T) was highest in thiamethoxam @ 30g a.i./ha (55.82), followed by dimethoate @ 300g a.i./ha (53.9) and thiamethoxam @ 20g a.i./ha (47.55). The order of insecticides based on persistence toxicity values were found to be thiamethoxam @ 35g a.i./ha (1009.7) > imidacloprid @ 25g a.i./ha (927.99) > thiamethoxam @ 30g a.i./ha (837.3) > thiamethoxam @ 25g a.i./ha (713.1) > dimethoate @ 300g a.i./ha (700.7) > thiamethoxam @ 20g a.i./ha (523.05).

The order of relative efficacy among all treatments were thiamethoxam @ 35g a.i./ha > imidacloprid @ 25g a.i./ha > thiamethoxam @ 30g a.i./ha > thiamethoxam @ 25g a.i./ha > dimethoate @ 300g a.i./ha > thiamethoxam @ 20g a.i./ha when mortalities were studied after 24 hours of exposure.

The median lethal time (LT_{50}) was least in thiamethoxam @ 20g a.i./ha (4.74 days) followed by dimethoate @ 300g a.i./ha (6.69 days), thiamethoxam @ 25g a.i./ha (6.79 days), imidacloprid @ 25g a.i./ha (7.83 days), thiamethoxam @ 30g a.i./ha (8.12 days) and thiamethoxam @ 35g a.i./ha (9.02 days).

4.4.2 After 48 hours of exposure

After 48 hours of exposure all the treatments gave cent per cent mortality. Thiamethoxam @ 35g a.i./ha persisted for longest period (23 days) followed by imidacloprid @ 25g a.i./ha (21 days), thiamethoxam @ 25g a.i./ha and thiamethoxam @ 30g a.i./ha (both 15 days), thiamethoxam @ 20g a.i./ha and dimethoate @ 300g a.i./ha (both 13 days) (Table 4.29).

Average residual toxicity (T) was highest in case of thiamethoxam @ 35g a.i./ha (59.89) followed by dimethoate @ 300g a.i./ha (57.37), thiamethoxam @ 25g a.i./ha (52.45), imidacloprid @ 25g a.i./ha (50.01), thiamethoxam @ 35g a.i./ha (48.5) and thiamethoxam @ 20g a.i./ha (45.31).

The order of insecticides based on persistent toxicity values are thiamethoxam @ 35g a.i./ha (1164) > imidacloprid @ 25g a.i./ha (1100.22) > thiamethoxam @ 30g a.i./ha (958.24) > thiamethoxam @ 25g a.i./ha (839.2) > dimethoate @ 300g a.i./ha (803.18) > thiamethoxam @ 20g a.i./ha (634.34).

Table 4.28 Corrected mortality (%) after 24hr of the release of adult *A. craccivora*

Treatment	1 DAT	3DAT	5DAT	7DAT	9DAT	11DAT	13DAT	15DAT	17DAT	19DAT	21DAT	23DAT	25DAT
T_1 (Thiamethoxam 20g a.i./ha)	96.66	73.33	44.82	31.03	22.22	17.24	0.00	0.00	0.00	0.00	0.00	0.00	0.00
T_2 (Thiamethoxam 25g a.i./ha)	100.00	90.00	82.75	48.27	32.14	13.78	10.00	3.44	0.00	0.00	0.00	0.00	0.00
T_3 (Thiamethoxam 30g a.i./ha)	100.00	93.33	79.31	65.51	53.33	27.58	20.68	6.89	0.00	0.00	0.00	0.00	0.00
T_4 (Thiamethoxam 35g a.i./ha)	100.00	93.33	82.75	68.96	63.33	28.65	25.67	20.68	16.66	13.33	10.00	3.44	0.00
T_5 (Imidacloprid 25g a.i./ha)	96.66	90.00	79.31	48.27	46.66	34.47	23.33	24.13	20.00	13.33	10.00	0.00	0.00
T_6 (Dimethoate 300g a.i./ha)	100.00	83.33	65.51	48.27	36.66	29.8	13.79	0.00	0.00	0.00	0.00	0.00	0.00

Table 4.28 continued.

Treatment	P	T	PT	RP	ORE	LT_{50} (days)	LL	UL	Slope
T_1 (Thiamethoxam 20g a.i./ha)	11	47.55	523.05	1.00	6	4.74	4.27	5.23	-2.75
T_2 (Thiamethoxam 25g a.i./ha)	15	47.54	713.10	1.36	4	6.79	6.40	7.18	-4.53
T_3 (Thiamethoxam 30g a.i./ha)	15	55.82	837.30	1.60	3	8.12	7.13	9.16	-4.04
T_4 (Thiamethoxam 35g a.i./ha)	23	43.90	1009.70	1.93	1	9.02	8.48	9.56	-3.65
T_5 (Imidacloprid 25g a.i./ha)	21	44.19	927.99	1.77	2	7.83	6.84	8.80	-2.71
T_6 (Dimethoate 300g a.i./ha)	13	53.90	700.70	1.34	5	6.69	6.15	7.25	-3.06

P=Period of toxicity; T= Average residual toxicity; PT= Persistent toxicity; RP= Relative Persistence; O.R.E. = Order of relative efficacy LL= Lower limit; UL= Upper limit

Table 4.29 Corrected mortality (%) after 48hr of the release of adult *A. craccivora*

Treatment	1 DAT	3DAT	5DAT	7DAT	9DAT	11DAT	13DAT	15DAT	17DAT	19DAT	21DAT	23DAT	25DAT
T_1 (Thiamethoxam 20g a.i./ha)	100.00	82.14	53.56	32.14	24.61	21.42	3.33	0.00	0.00	0.00	0.00	0.00	0.00
T_2 (Thiamethoxam 25g a.i./ha)	100.00	96.42	85.70	53.56	42.30	21.42	13.33	6.89	0.00	0.00	0.00	0.00	0.00
T_3 (Thiamethoxam 30g a.i./ha)	100.00	96.42	85.70	71.42	59.14	28.57	27.58	10.34	0.00	0.00	0.00	0.00	0.00
T_4 (Thiamethoxam 35g a.i./ha)	100.00	96.42	85.70	71.42	64.09	29.80	43.33	31.03	20.00	20.00	13.33	6.89	0.00
T_5 (Imidacloprid 25g a.i./ha)	100.00	92.85	85.70	53.56	53.32	42.59	35.71	28.65	23.00	21.42	13.33	0.00	0.00
T_6 (Dimethoate 300g a.i./ha)	100.00	89.28	67.85	53.56	42.55	31.12	17.24	0.00	0.00	0.00	0.00	0.00	0.00

Table 4.29 Continued.

Treatment	P	T	PT	RP	ORE	LT_{50} (days)	LL	UL	Slope
T_1 (Thiamethoxam 20g a.i./ha)	14	45.31	634.34	1.00	6	6.52	5.42	7.43	-4.09
T_2 (Thiamethoxam 25g a.i./ha)	16	52.45	839.20	1.32	4	8.78	8.36	9.19	-5.57
T_3 (Thiamethoxam 30g a.i./ha)	16	59.89	958.24	1.51	3	10.03	9.54	10.54	-5.02
T_4 (Thiamethoxam 35g a.i./ha)	24	48.50	1164.00	1.83	1	11.34	10.77	11.91	-3.89
T_5 (Imidacloprid 25g a.i./ha)	22	50.01	1100.22	1.73	2	10.66	10.05	11.28	-3.34
T_6 (Dimethoate 300g a.i./ha)	14	57.37	803.18	1.26	5	8.47	7.94	9.05	-3.84

P=Period of toxicity; T= Average residual toxicity; PT= Persistent toxicity; RP= Relative Persistence; O.R.E. = Order of relative efficacy, LL= Lower limit; UL= Upper limit

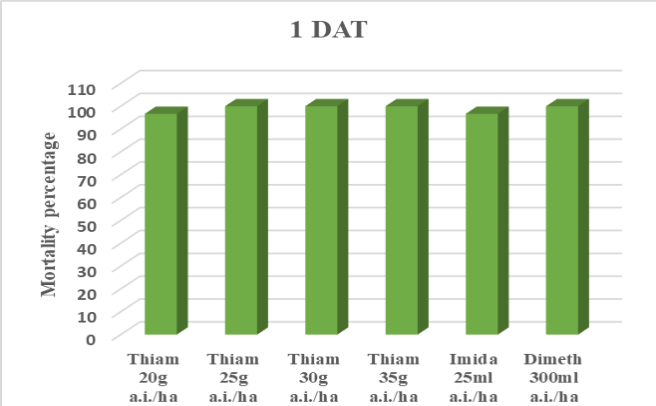

Figure 4.39 Efficacy of various dosages of thiamethoxam including other insecticides and morality percentage (after 24 hour) of adult *A. craccivora* at 1 DAT (one day after treatment)

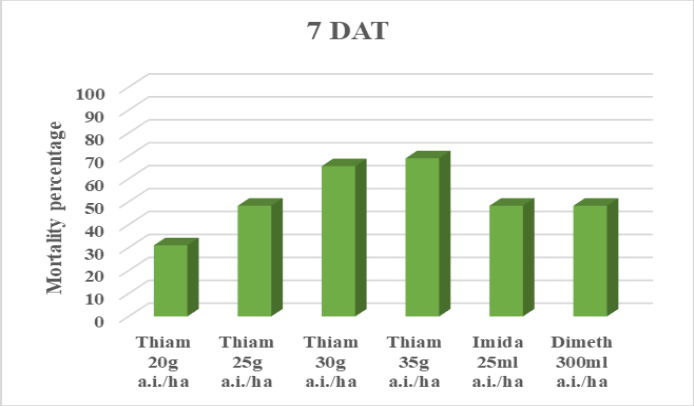

Figure 4.40 Efficacy of various dosages of thiamethoxam including other insecticides and morality percentage (after 24 hour) of adult *A. craccivora* at 7 DAT (seven days after treatment)

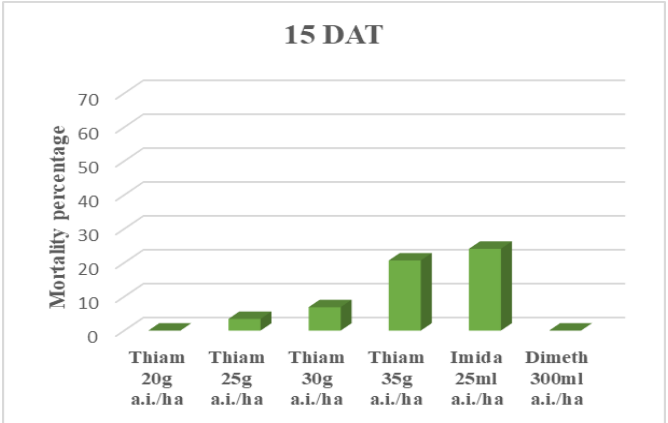

Figure 4.41 Efficacy of various dosages of thiamethoxam including other insecticides and morality percentage (after 24 hour) of adult *A. craccivora* at 15 DAT (fifteen days after treatment)

The order of relative efficacy among all treatments were thiamethoxam @ 35g a.i./ha > imidacloprid @ 25g a.i./ha > thiamethoxam @ 30g a.i./ha > thiamethoxam @ 25g a.i./ha > dimethoate @ 300g a.i./ha > thiamethoxam @ 20g a.i./ha when mortalities were studied after 48 hr of exposure.

The median lethal time (LT_{50}) was least in thiamethoxam @ 20g a.i./ha (6.52 days) followed by dimethoate @ 300g a.i./ha (8.47 days), thiamethoxam @ 25g a.i./ha (8.78 days), thiamethoxam @ 30g a.i./ha (10.03 days), imidacloprid @ 25g a.i./ha (10.66 days) and thiamethoxam @35g a.i./ha (11.34 days).

4.5 Activity of detoxification enzymes (Cytochrome P-450 monoxygenases (MFO), Esterases and Glutathione-S-transferease (GST)) and target-site insensitivity (Acetylcholinesterase (AChE)) in resistant and susceptible population of *A. craccivora*

4.5.1 Total body protein

The results pertaining to the total body protein (mg/g body weight) in both the resistant (R) and susceptible (S) strains are presented in Table 4.30. The total body protein in susceptible strain varied from 0.10 mg/g bw to 0.31 mg/ g bw with a mean value of 0.21 mg/g body weight. In resistant strains the total body protein varied from 0.26 mg/g bw to 0.72 mg/g bw with a mean value of 0.41 mg/ g body weight (Figure 4.42).

4.5.2 Activities of carboxylesterases, MFOs an GSTs

The results regarding various detoxifying enzymes (metabolic resistance) were tabulated in Table 4.31. The mean activity of Mixed Function Oxidase (MFO) was 4.20±1.76 n mol. min^{-1}. mg^{-1}. protein in susceptible stains of *A. craccivora* whereas the same value was 29.61 ± 6.49 n mol. min^{-1}. mg^{-1}. protein (significantly different at P= 0.05) in resistant strains (Figure 4.43). The activities of carboxylesterase enzyme (CbE) differ clearly in both susceptible and resistant strains. In susceptible strains the value was 0.03 ± 0.021 n mol. min^{-1}. mg^{-1}. protein which was 1.20±0.60 n mol. min^{-1}. mg^{-1}. protein (significantly different at P=0.01) in R strain (Figure 4.44). The activities of glutathione-

Table 4.30 Total body protein (mg/g bw) in susceptible (S) and resistant strains (R) of *A. craccivora*

Susceptible strain		Resistant starin	
Sample no(s)	**Protein (susc.) (mg/g bw)**	**Sample no(s)**	**Protein (res) (mg/g bw)**
1	0.20	1	0.27
2	0.31	2	0.34
3	0.10	3	0.26
4	0.23	4	0.31
5	0.21	5	0.56
6	0.25	6	0.72
7	0.23	7	0.40
Mean	0.21	Mean	0.41
SD	0.06	SD	0.17

Table 4.31 Activities of detoxifying enzymes (metabolic resistance) in both the resistant and susceptible strains of *A. craccivora*

		Mean ± SE		
Sl. No	**Detoxification enzymes**	**S strain**	**R strain**	**Activity ratio**
1	Mixed function oxidase (MFO) (n mol. min^{-1}. mg^{-1}. Protein)	4.20 ± 1.76	29.61 ± 6.49*	7.05
2	Carboxylesterase (CbE) (n mol. min^{-1}. mg^{-1}. Protein)	0.03 ± 0.02	1.20 ± 0.60**	42.02
3	Glutathione-S-Transferase (GST) (n mol. min^{-1}. mg^{-1}. Protein)	6.24 ± 3.01	18.80 ± 5.87	3.01

Specific activity of fifteen replicates (expressed as the means±SE)
*Significantly different at P = 0.05
**Significantly different at P =0.01
Activity ratio = Activity in R strain / Activity in S-strain

Table 4.32 Activity of acetylcholinesterase (AChE) in the thiamethoxam susceptible (S) and resistant (R) strains of *A. craccivora*

Strain	Specific activity [µmoles. min^{-1}. mg^{-1}]	Activity ratio
Susceptible strain (S)	8.27 ± 1.98	1
Resistant starin (R)	8.52 ± 1.08	1.03

Specific activity of fifteen replicates (expressed as the means±SE)

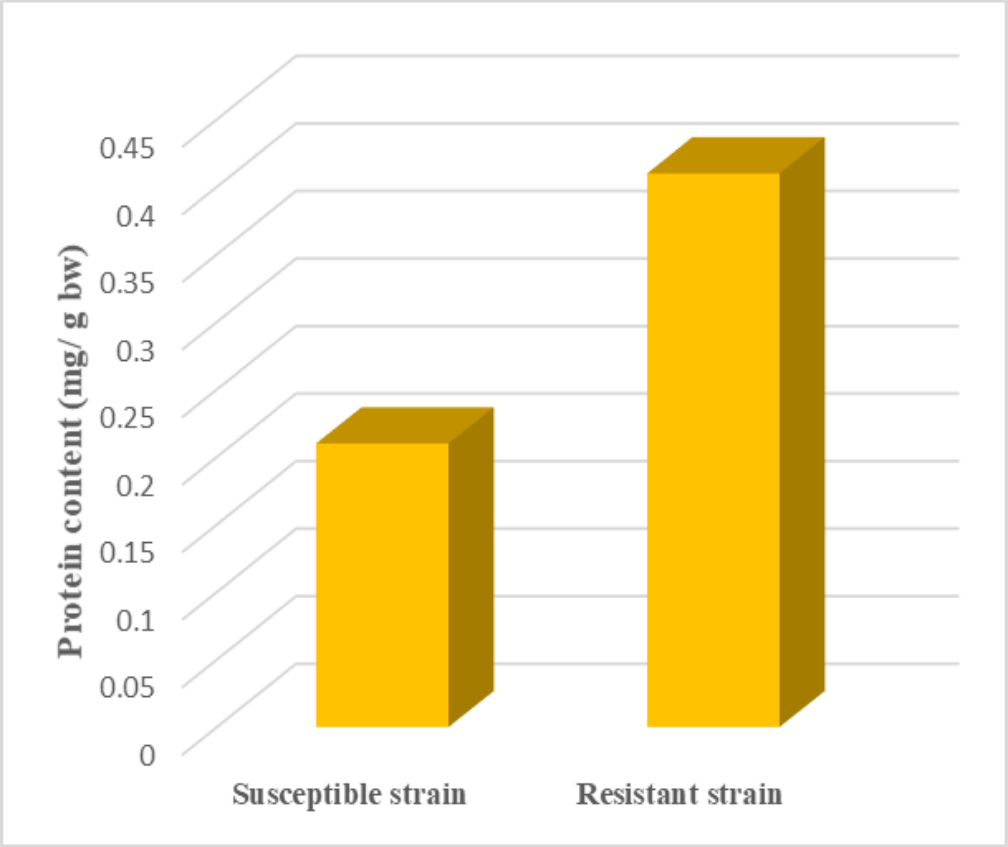

Figure 4.42 Total body protein (mg/g bw) in susceptible and resistant strains of *A. craccivora*

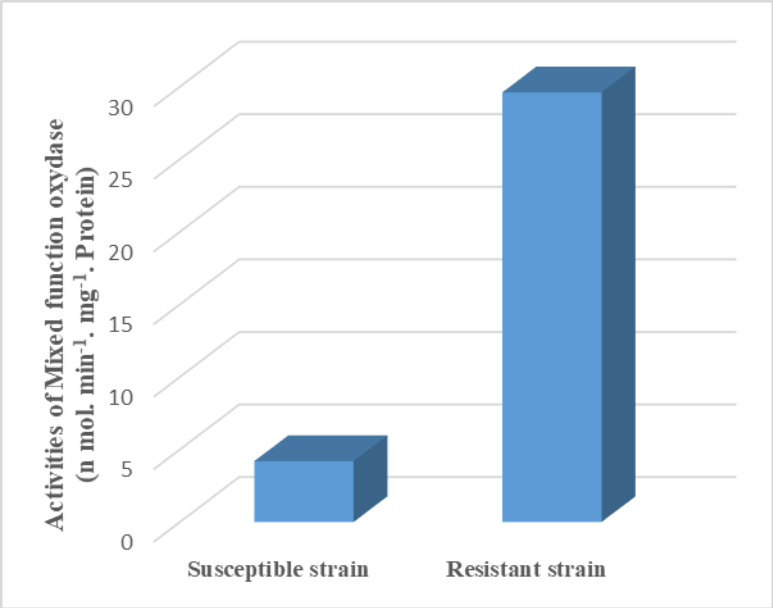

Figure 4.43 Activities of Mixed function oxidase (MFO) in susceptible and resistant strains of *A. craccivora*

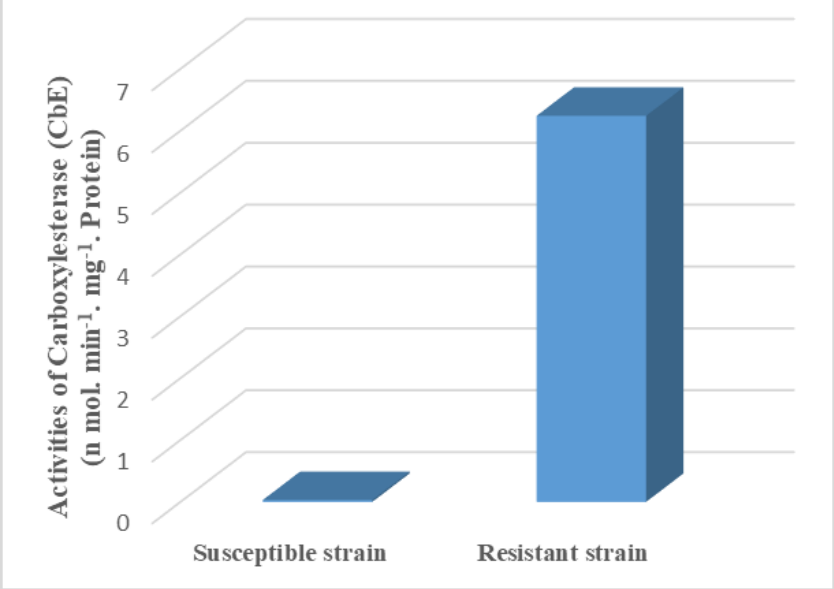

Figure 4.44 Activities of Carboxylesterase (CbE) in susceptible and resistant strains of *A. craccivora*

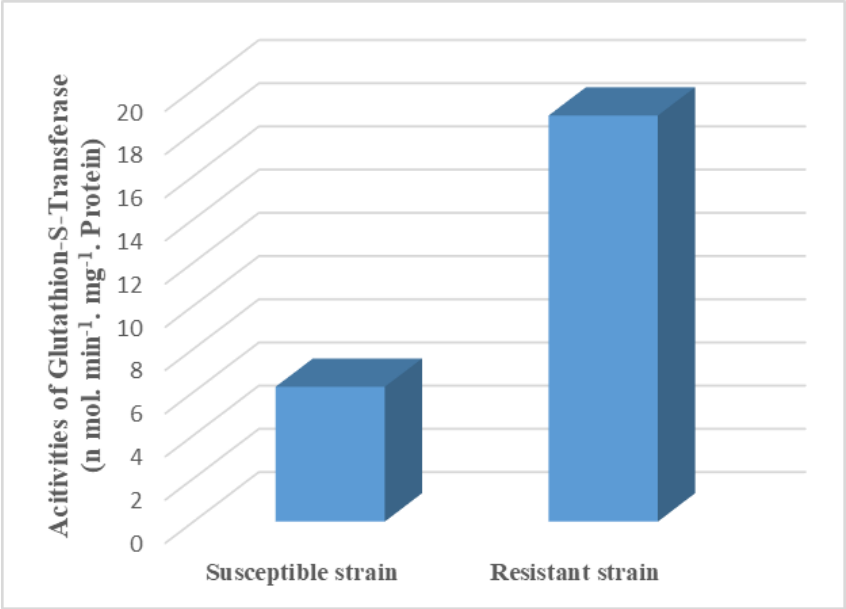

Figure 4.45 Activities of glutathione-S-transferase (GST) in susceptible and resistant strains of *A. craccivora*

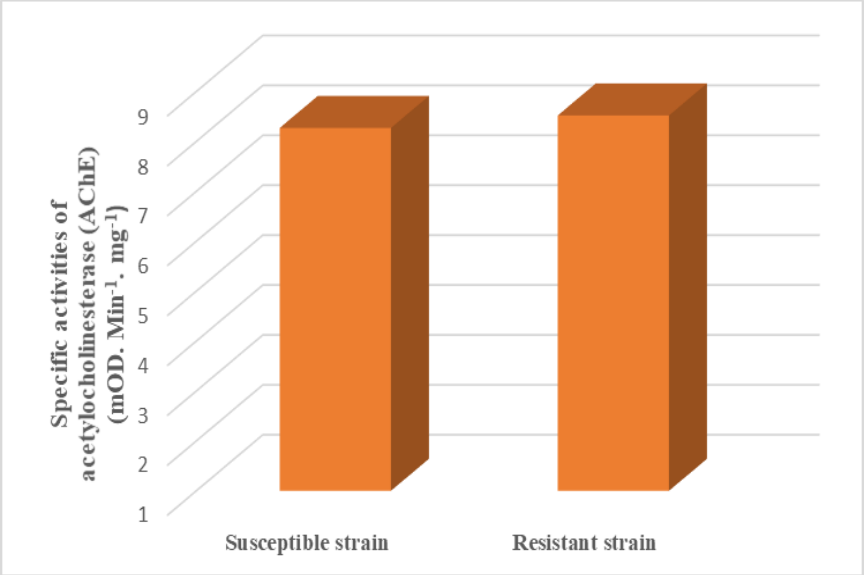

Figure 4.46 Activity of acetylcholinesterase (AChE) in susceptible and resistant strains of *A. craccivora*

S-transferase (GST) was 6.24 ± 3.01 n mol. min^{-1}. mg^{-1}. protein. in susceptible strain, whereas in resistant strain the value was found to be 18.80 ± 5.87 n mol. min^{-1}. mg^{-1}. protein (Table 4.31) (Figure 4.45).

Comparing the activity ratio of all the above detoxifying enzymes, highest activity was seen by carboxylesterases (42.02 times) in resistant strains followed by mixed function oxidase (7.05 times) and glutathione-S-transferase (3.01 times).

4.5.3 Activities of Acetylcholinesterase

The specific activity of Acetylcholinesterase was 8.27±1.98 µ moles. min^{-1}. mg^{-1} protein in susceptible strain whereas the same value was 8.52±1.08 µ moles. min^{-1}. mg^{-1} protein in resistant strain with an activity ratio of 1.03 (not significantly different at p = 0.01) (Table 4.32) (Figure 4.46).

4.6 Fitness cost of the thiamethoxam resistance in *A. craccivora*

The data regarding nymphal durations and nymphal developmental periods are summarized in Table 4.33 and the data regarding adult longevity and fecundity of different selected generations are presented in Table 4.34.

4.6.1 Influence of thiamethoxam resistance on the developmental characteristics

The total nymphal duration increased through the resistance acquisition in different selected generations of aphids (Table 4.33). In the susceptible strain the nymphal duration was 4.35 ±1.02 days which did not vary up to F_6 generation (4.49±1.15 days) and the same value at F_{24} was 7.90 ±0.57 days (significantly different). From F_{12} to F_{24}, although the total nymphal duration significantly increased in comparision to F_0 to F_6 but, did not vary among themselves (7.14±0.69 to 7.90±0.57 days). The duration of 1st instar nymph (varied from 1.21±0.26 to 1.51 ±0.37 days) did not differ significantly among different generations. The duration of 2nd instar in F_{14}- F_{24} was 1.42±0.24 to 1.59 ±0.28 days which was on par with each other but was significantly different from that of the F_0 to F_8 (1.00 ±0.40 days to 1.21±0.39). The mean duration for 3rd instar nymphal instar up to F_{10} (1.42±0.44 days) did not vary from that of F_0 (1.07±0.44 days) but was 1.83 ± 0.44 days in F_{24} which was found to be significantly different from that of susceptible generation (F_0) (1.07 ±0.44 days). From F_{12} (1.57±0.44 days) to F_{24} the 3rd nymphal duration was same. The duration of 4th instar nymph of F_{24} also differed

Table 4.33 Nymphal durations of different selected generations of *A. craccivora* for resistance to thiamethoxam

Generations	I instar (days)	II instar (days)	III instar (days)	IV instar (days)	Nymph duration (days)
F_0	1.28^{a} ±0.26	1.00^{a} ±0.40	1.07^{a} ±0.44	1.00^{a} ±0.40	4.35^{a} ±1.02
F_2	1.21^{a} ±0.26	1.07^{a} ±0.34	1.00^{a} ±0.40	1.00^{a} ±0.28	4.28^{a} ±0.95
F_4	1.28^{a} ±0.26	1.07^{a} ±0.34	1.00^{a} ±0.40	1.00^{a} ±0.28	4.35^{a} ±0.95
F_6	1.28^{a} ±0.26	1.14^{a} ±0.47	1.00^{a} ±0.40	1.07^{a} ±0.44	4.49^{a} ±1.15
F_8	1.35^{a} ±0.24	1.21^{a} ±0.39	1.07^{a} ±0.18	1.85^{ab} ±0.55	5.48^{ab} ±1.00
F_{10}	1.35^{a} ±0.24	1.28^{ab} ±0.26	1.42^{a} ±0.44	2.78^{c} ±0.26	6.83^{bc} ±0.62
F_{12}	1.42^{a} ±0.18	1.35^{ab} ±0.24	1.57^{b} ±0.44	2.80^{c} ±0.34	7.14^{c} ±0.69
F_{14}	1.42^{a} ±0.34	1.42^{b} ±0.24	1.67^{b} ±0.37	2.85^{c} ±0.34	7.36^{c} ±0.74
F_{16}	1.49^{a} ±0.18	1.49^{b} ±0.34	1.69^{b} ±0.26	2.86^{c} ±0.26	7.53^{c} ±0.24
F_{18}	1.49^{a} ±0.34	1.52^{b} ±0.26	1.71^{b} ±0.34	2.89^{c} ±0.55	7.61^{c} ±0.93
F_{20}	1.50^{a} ±0.23	1.57^{b} ±0.26	1.75^{b} ± 0.25	2.89^{c} ±0.50	7.71^{c} ±0.74
F_{22}	1.51^{a} ±0.37	1.59^{b} ±0.24	1.82^{b} ± 0.34	2.90^{c} ±0.34	7.82^{c} ±0.95
F_{24}	1.50^{a} ±0.34	1.59^{b} ±0.28	1.83^{b} ± 0.44	2.98^{c} ±0.26	7.90^{c} ±0.57
SE(m)±	0.117	0.124	0.113	0.325	0.527
CD (P= 0.05)	0.35	0.37	0.35	0.98	1.58

Means within a column followed by the same letters are not significantly different.

Table 4.34 Adult longevity and fecundity of different selected generations of *A. craccivora* for resistance to thiamethoxam

Generations	Pre oviposition (days)	Oviposition (days)	Post oviposition (days)	Adult longevity (days)	Fecundity
F_0	0.57^{a} ±0.18	7.78^{a} ±1.40	1.14^{a} ±0.24	9.50^{a} ±1.22	54.71^{a} ±7.63
F_2	0.57^{a} ±0.18	7.85^{a} ±1.28	1.21^{a} ±0.26	9.64^{a} ±1.21	53.57^{a} ±8.22
F_4	0.57^{a} ±0.18	7.85^{a} ±1.28	1.21^{a} ±0.26	9.71^{a} ±1.21	52.00^{a} ±8.22
F_6	0.64^{ab} ±0.24	7.92^{a} ±1.30	1.21^{a} ±0.26	9.85^{a} ±1.14	45.14^{a} ±9.59
F_8	0.64^{ab} ±0.24	8.28^{a} ±1.28	1.21^{a} ±0.26	10.14^{a} ±1.28	38.85^{b} ±5.69
F_{10}	0.64^{ab} ±0.24	8.35^{a} ±1.24	1.28^{a} ±0.26	10.28^{a} ±1.4	22.71^{c} ±7.04
F_{12}	0.85^{ab} ±1.17	8.57^{a} ±1.17	1.42^{a} ±0.44	10.85^{ab} ±1.57	19.71^{c} ±5.70
F_{14}	1.28^{c} ±0.56	8.71^{ab} ±1.07	1.92^{b} ±0.34	11.92^{b} ±1.20	18.85^{c} ±5.66
F_{16}	1.34^{c} ±0.56	8.80^{b} ±1.23	1.95^{b} ±0.44	12.09^{b} ±1.30	18.50^{c} ±5.89
F_{18}	1.40^{c} ±0.24	8.90^{b} ±1.30	1.97^{b} ±0.34	12.27^{bc} ±1.43	17.40^{c} ±5.38
F_{20}	1.50^{c} ± 0.24	8.80^{b} ±1.27	1.97^{b} ±0.35	12.27^{bc} ±1.45	17.20^{c} ±5.76
F_{22}	1.50^{cd} ± 0.56	8.90^{b} ±1.28	2.00^{b} ±0.36	12.40^{c} ±1.43	16.90^{c} ±5.89
F_{24}	1.60^{d} ±0.34	8.89^{b} ±1.27	2.00^{b} ±0.37	12.49^{c} ±1.44	16.00^{c} ±5.34
SE(m)±	0.085	0.327	0.109	0.488	3.474
CD (P=0.05)	0.26	0.98	0.32	1.50	10.20

Means within a column followed by the same letters are not significantly different.

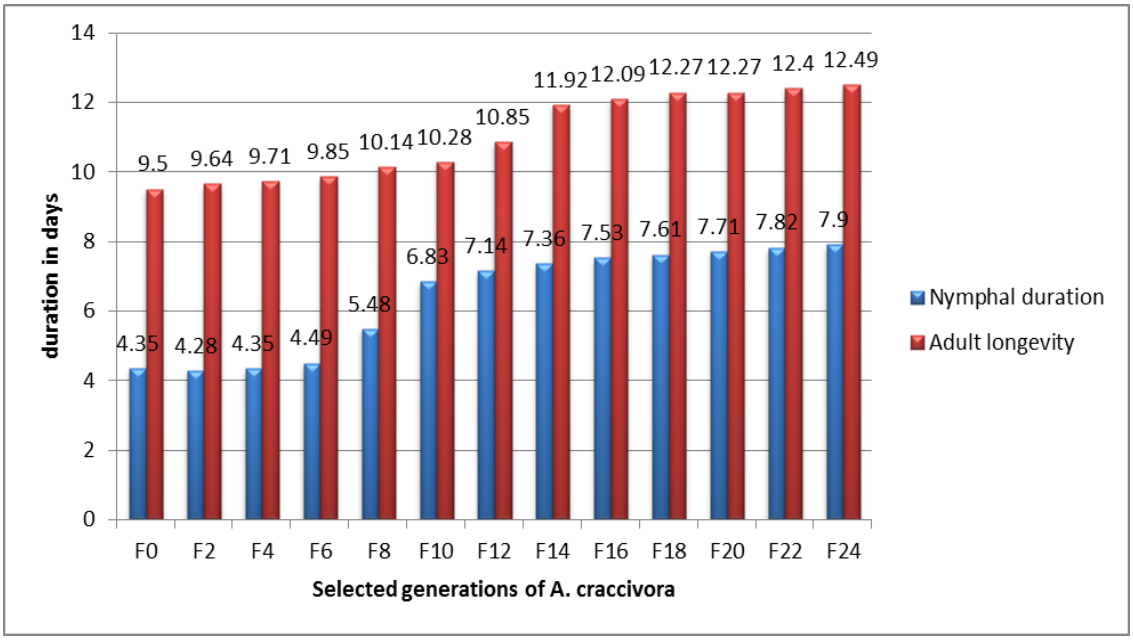

Figure 4.47 Nymphal duration and adult longevity of *A. craccivora* in different selected generations

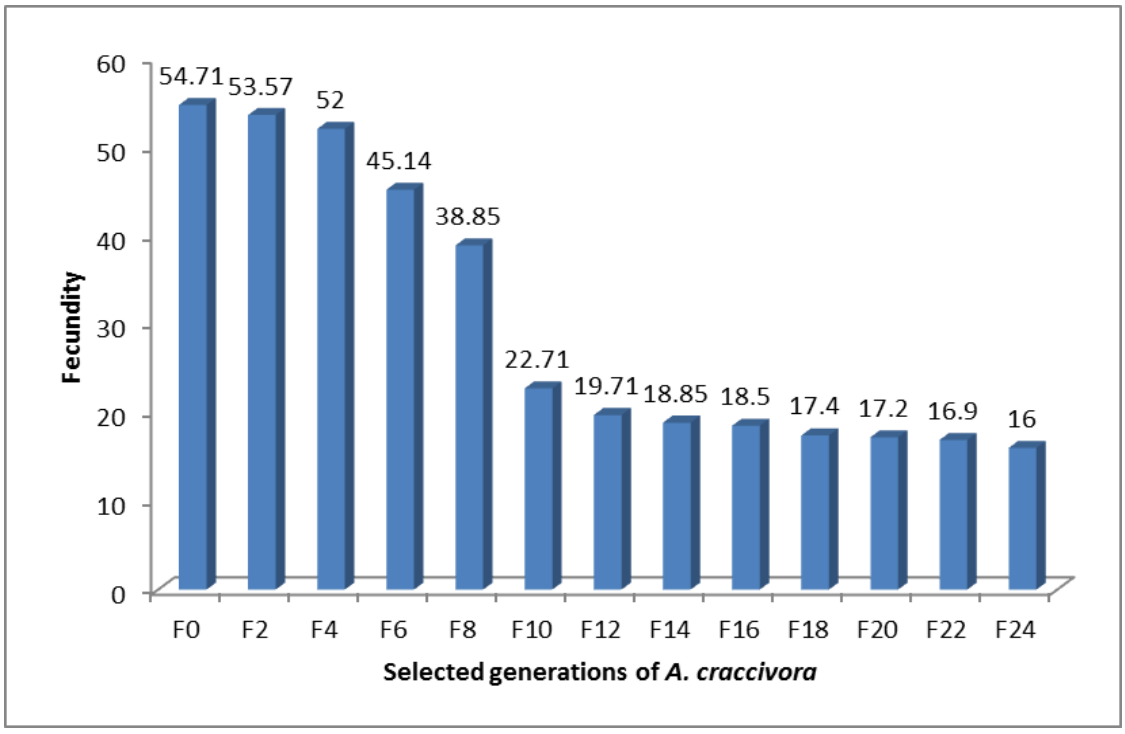

Figure 4.48 Mean fecundity values of *A. craccivora* in different selected generations

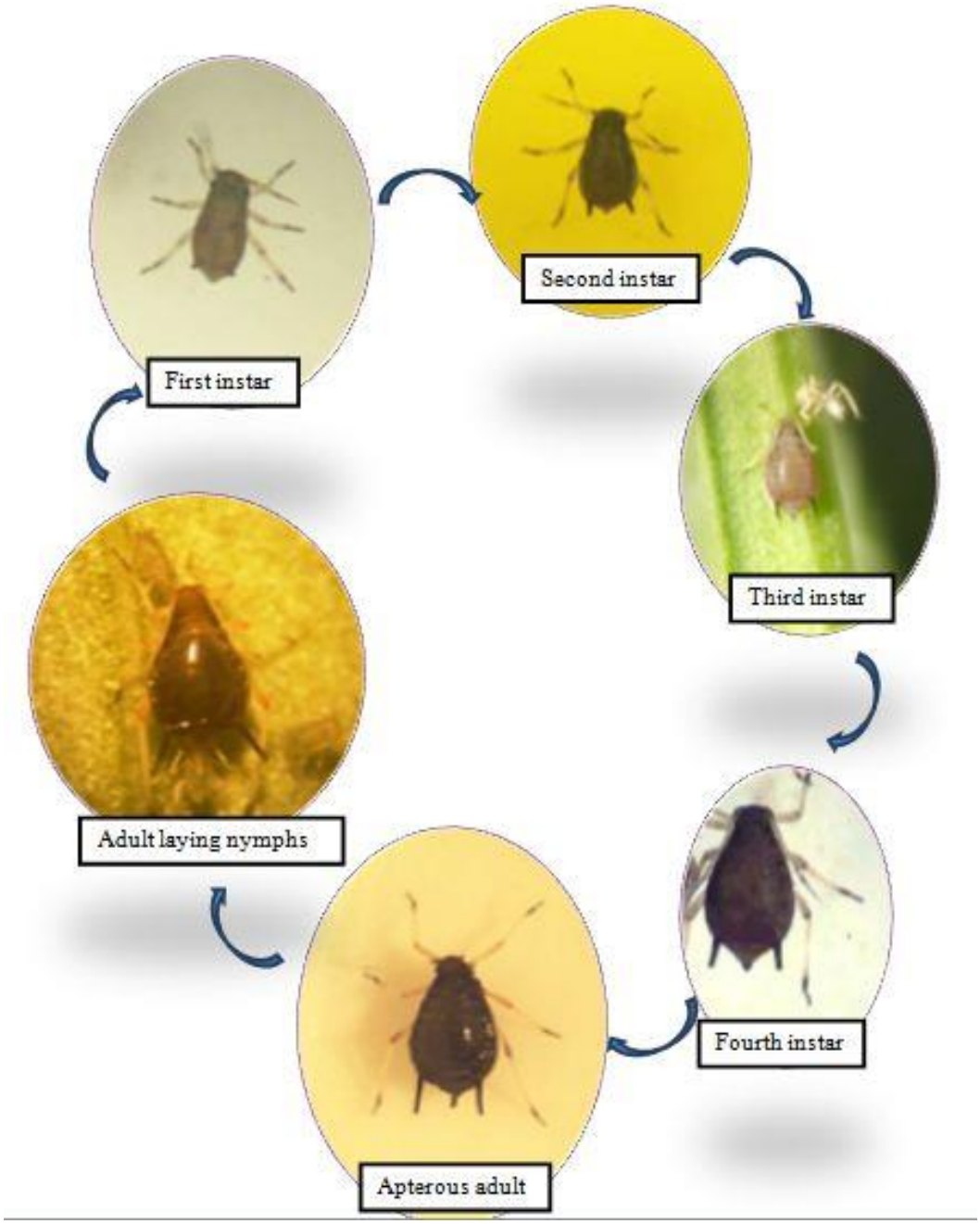

Figure 4.49 Various instars and nymph laying by adult *A. craccivora* during biological parameter studies

significantly (2.98 ±0.26 days) from the susceptible generation (1.00 ±0.40 days) (Figure 4.47). But, the duration of 4th nymphal instar from F_0 to F_6 (1.07±0.44 days) was on par. Similarly, it did not vary significantly from F_{10} (2.78±0.26 days) till F_{24} (2.98±0.26 days) in 4th instar.

4.6.2 Influence of resistance on the reproductive characteristics

Thiamethoxam resistance clearly affected the oviposition period, adult longevity and fecundity of aphids (Table 4.34). Both pre-oviposition and post-oviposition periods increased as the resistance to thiamethoxam was acquired.

The pre-oviposition period in F_0 (0.57±0.18 days) did not vary up to F_4 (0.57±0.18 days). However, significant increase in pre-oviposition period was observed from F_{14} (1.28±0.56 days) till F_{20} (1.50±0.24 days) which were on par. But, in F_{24} (1.60±0.34 days) significantly longer oviposition period was observed.

The oviposition period from F_2 to F_{12} (7.85±1.28 to 8.57±1.17 days) did not vary from that of F_0 (7.78±1.40 days). But, from F_{14} it increased and from F_{16} (8.80 ±1.23 days) to F_{24} (8.89±1.27 days) marked increase was observed being at par with each other.

The post-oviposition period did not show significant increase upto F_{12} (1.42±0.44 days) which compared to F_0 (1.14±0.24 days) generation. However, significantly longer post-oviposition period was observed from F_{14} (1.92±0.34 days) to F_{24} (2.00±0.37 days) which was at par with each other.

The adult longevity also has shown an increasing trend a selection process continued and resistance progressed. It did not show marked increase till 10th generation of selection F_{10} (10.28±1.40 days) being at par with parental generation F_0 (9.50±1.22 days). But, from F_{12} it started increasing visibly (10.85±1.87 days) and significantly longer adult longevity was evidenced from F_{22} (12.40±1.43 days) being on par with F_{24} (12.49±1.44 days) generation of selection.

Contrary to the above findings, the fecundity of *A. craccivora* was found decreasing as the selection process progressed for resistance to thiamethoxam. The fecundity did not show down ward trend till 6th generation (F_6) of selection (45.14±9.59)

being at par with parental generation F_0 (54.71±7.63). Significant reduction in fecundity was observed in F_8 (38.85±5.69). Later, from F_{10} (22.71±7.04) the fecundity showed further reduction compared to F_8 and F_0 to F_6 but, was on par with F_{12}, F_{14}, F_{16}, F_{18}, F_{20}, F_{22} and F_{24} (16.00±5.34) indicating no further reduction in fecundity even if the resistance to thiamethoxam progressed in an upward manner (Figure 4.48).

DISCUSSION

The results under various objectives described in the previous chapter are discussed below.

5.1 Development and build-up of resistance in *A. craccivora* to thiamethoxam in the laboratory

5.1.1 Determination of baseline toxicity of thiamethoxam to adult *A. craccivora*

Most insecticides usually give stable mortality after 48 or 72 hour after treatment (Huang *et al.*, 2006). But, in the present study, considering the characteristics of aphids, and control mortality, 24 hours' exposure to score mortality was chosen. During the baseline toxicity studies at present the LC_{50} value was found to be 2.62 ppm with 1.91 ppm as fiducial lower limit and 3.43 ppm as fiducial upper limit for the adult *A. craccivora*. Earlier, several workers have reported different LC_{50} values of thiamethoxam for the same species and related species *i.e.*, *A. gossypii* using leaf-dip bioassay method and other methods. Abdallah *et al*., (2016) found the LC_{50} value of the susceptible population of *A. craccivora* to thiamethoxam by leaf dip method as 0.079 ppm. According to Mokbel *et al*. (2017), the baseline toxicity of *A. craccivora* to thiamethoxam was 0.44 ppm using leaf-dip bioassay. Using the same method, Koo *et al*., (2014) found the baseline toxicity of a related species of aphid, the cotton aphid, *A. gossypii* to thiamethoxam as 0.14 ppm. Pan *et al.*, (2018) reported the baseline toxicity of cotton aphid to thiamethoxam as 2.85 ppm. But, utilizing a different modified dipping method Chen *et al*. (2015) determined the baseline toxicity of *A. gossypii* to thiamethoxam as 1.88 ppm.

The basic susceptible baseline toxicity data is required for insecticide resistance monitoring. It also provides guide for formulating resistance management strategies in Integrated Pest Management programmes. In above reports, different values in LC_{50} values may be because of the differential responses of susceptible populations due to environmental influences and variability among them. Besides bioassay, exposure time to insecticide might be another important factor affecting bioassay against aphids (Huang *et al.*, 2006; Lu *et al.*, 2009).

5.1.2 Development and buildup of resistance

In the present study it was found that thiamethoxam has greater potentiality to impart resistance in *A. craccivora.* In only 24 generations of selection of *A. craccivora* with the neonicotinoid insecticide thiamethoxam, 86.19-folds resistance could be developed. Earlier workers have reported similar findings. Other neonicotinoid insecticides are reported effective against the black legume aphids and related aphids particularly *A. gossypii* in several crops. The popularity of neonicotinoids has exerted mounting selection pressure for resistance development in aphids. About 48.01 folds of resistance under laboratory conditions was obtained when *A. craccivora* population was selected under thiamethoxam pressure for 12 generations (Abdallah *et al.,* 2016). According to Mokbel (2007), resistance to the neonicotinoid insecticide dinotefuran in *A. craccivora* was slow and reached 4.13 fold in relation to parent strain after 10 generations of intense selection. Mokbel and Mohamed (2009) reported 148.8-fold resistance in *A. craccivora* to related neonicotinoid dinotefuran after 20 generations of selection. In related aphid species, *A. gossypii* Wang *et al*., (2002) reported 8.1 folds of resistance to imidacloprid (a related neonicotinoid insecticide of chloronicotinyl group having similar mode of action as the thionicotinyl insecticide thiamethoxam) after being selected for 13 generations. Koo *et al.,* (2014) found that the field populations of *A. gossypii*, demonstrated extremely high resistance ratio to neonicotinoids *viz.*, acetamiprid, clothianidin, thiacloprid and imidacloprid. A resistant strain of *A. gossypii* with 13.79 folds of resistacne to thiamethoxam was also reported by Wei *et al.,* (2017). Pan *et al*., (2018) observed 19.35-folds of resistance in a thiamethoxam resistant strain of cotton aphid, due to presence of multiple UDP-Glucuronosyltransferases in them.

Various authors have used differences in slope to make inferences about the progression of resistance. The slope of the concentration-mortality line is an indicator of phenotypic variation, which includes environmental as well as genetic variation (Hoskins 1960, Finney, 1971, Falconer, 1989, Tabashnik and Cushing, 1989).

Initially scientists expressed that the slope of the concentration – mortality line from pesticide bioassays was greatest both at low and high levels of resistance because genetic variation was lowest at these two extremes (Hoskins and Gordon, 1956). They

opined that an unselected susceptible population is relatively homogeneous and, thus, has a concentration-mortality line with a steep slope. In the initial stages of resistance development, the population includes a heterogeneous mixture of susceptible, heterozygous, and resistant individuals. Such variation among individual response is reflected in a shallow slope. As continued selection eliminates susceptible and moderately resistant individuals, heterogeneity is reduced and slope increases. Rosenheinm and Hoy (1986) also hypothesized that slope increases as resistance increases and genetic variation decreases. It has also been proposed that the slope of dose mortality curve represents the phenotypic variations in susceptibility in a population comprising both environmental and genetic components (Hoskins, 1960). Later, Chilcut and Tabashnik (1995) tested the hypothesis on an exhaustive scale and opined that slope was not a good indicator of the genetic variation of susceptibility and for most of the pests and pesticide combinations; slopes were not highest at the two extremes. According to them slope does not generally change in a consistent way as resistance progresses. Hence, with 'b' values obtained in the present investigations, no inference on the progression of resistance in relation to genetic variability could be drawn during selection of the thiamethoxam resistant strain of *A. craccivora*.

The slope of the dose mortality curve in the parental population (F_0) in the present study was low suggesting the presence of genetic variation in the field collected population. Alizadeh *et al.* (2011) stated that the slope of a susceptible population is comparatively lower than that of a resistant population because of the higher number of heterozygotes in the susceptible population. After a gradual selection pressure over 24 generations, the slope value increased to a stable value in F_{24} (5.20 ± 0.98). Hence, under continual selection pressure, *A. craccivora* is more liable to develop resistance to thiamethoxam. Both LC_{50} and slope values are the common parameters for studying insecticide resistance. The higher values of LC_{50} in subsequent selected generations indicate reduced susceptibility or more homozygous individuals in a population (Chilcut and Tabashnik, 1995). In the present study the LC_{50} values increased over selection and marked increase in the value was observed between F_{12} and F_{14} (48.76 ppm to 75.92 ppm). When selected further the LC_{50} values were stabilized giving more or less similar range of values showing a homozygous population with reduced susceptibility towards

thiamethoxam. The intercept values decreased over generations and the probit graphs became steeper towards the end indicating the thiamethoxam resistant population became more homogeneous compared to the earlier population.

5.2 Inheritance of resistance

To formulate an efficient resistance management strategy assessment of resistance risk to an insecticide provides useful information (Sayyed *et al*., 2004). Further, the study of genetic variation is essential in insecticide resistance studies to correlate how such variation changes over time in progress of its magnitude over time. To evaluate the phenotypic variation caused by genetic variation an investigator can use quantitative genetic techniques such as sibling analysis or estimation of realized heritability (Falconer 1989, Tabashnik *et al*., 1987). The estimation of realized heritability (h^2) represents an effective tool to estimate the rate of resistance development and it can also predict the further rate of resistance development.

In the current studies the selection pressure of thiamethoxam over 24 generations in *A. craccivora* resulted in increasing frequencies of resistant individuals and consequently resistance evolution occurred. Laboratory selection experiments provide essential information to assess the resistance risk in an insect species to a particular insecticide. Moreover, selection experiments data is analyzed by the quantitative genetic tools to obtain additive genetic variance and realized heritability of resistance (Jutsum *et al*., 1998; Firkoi and Hayes, 1990).

Realized heritability (h^2) has a great importance to predict the rate of development of insecticide resistance among the pest populations as well as the ability of a pest to develop resistance to insecticides (Johnson and Tabashnik, 1999; Roush and Daly, 1990). The realized heritability value can be used to predict future resistance status to any pesticide in certain pests (Abbas and Shad, 2015). According to Moulton *et al.*, (2002) the estimated h^2 values provide evidences for the potential of resistance development.

On the other hand, realized heritability (h^2) is an important indicator for evaluating the sustainability of a chemical on a pest population (Sayyed *et al.*, 2005). Realized heritability (h^2) is the proportion of phenotypic variation accounted for by

additive genetic variation. Low realized heritability (less than 0.1) occurs when the offspring of the selected parents differ little from the original population, in spite of a big difference between the population as a whole and the selected parents. A high h^2 (greater than 0.6) occurs when the offspring of the selected parents differ from the original population almost as much as the selected parents do. Phenotypic variation is composed of genetic variation and environment variation (Yang, 2000). Under laboratory conditions, higher phenotypic variation may be observed from selection stress and gene mutation, but in field conditions, the same may be seen due to pest migration, alternation of insecticides, selection pressure and environmental factors (Tabashnik, 1992). Phenotypic variation is increased due to additive genetic variation and environmental variance (Abbas *et al.*, 2014). Realized heritability (h^2) may decrease either due to the decrease in genetic variance or increase in environmental variance (Falconer *et al.*, 1996).

In the current study, comparatively higher h^2 (0.28) in the first 12 generations of the selection (F_2-F_{12}) indicated comparatively higher chance of resistance development to thiamethoxam. In contrast, the second half of selection (F_{12}- F_{24}) showed lower h^2 (0.19) indicating less chance for resistance development to thiamethoxam. Higher h^2 in the first round compared with second round of selection resulted from increased response to selection. Higher heritability in initial generations selected with thiamethoxam and low heritability with the later ones, suggested that by the 12^{th} generation the population had approximately stabilized. The calculated h^2 due to the laboratory selection tests might be higher than in field because of decreased ecological differences (Zhang *et al.*, 2008). Though laboratory trials do not absolutely indicate field circumstances, the approximated h^2 value provides proof for the prospective of further improvement in the level of resistance (Tabashnik, 1992).

Estimation of h^2 in conjunction with estimates of selection intensity can be used to project rates of resistance development. Prediction based on h^2 must be interpreted cautiously because h^2 of resistance to a particular insecticide can vary between conspecific populations as well as within populations as a result to allele frequencies and environmental variation over time. So, the predictions made from quantitative genetic theory on the basis of $G=R^{-1}$ gives valuable information to develop strategies for

managing pesticide resistance (Tabashnik, 1992). Estimating h^2 from laboratory selection experiments for resistance is necessary to assess the risk of insecticide resistance in pests (Lai and Su, 2011). The present results of the selection experiment for resistance to thiamethoxam showed that *A. craccivora* populations have the ability to develop resistance to this insecticide in the field.

If the laboratory estimates of h^2 is applied to the field strains and 95% mortality occurred in each generation, then the *A. craccivora* strain can be expected to increase ten-fold resistance after only 5 generations (h^2 = 0.30). Similarly, at a constant h^2=0.19 and slope =3.06, about 7 generations will be required for the 10-fold increase in the resistance if 95% mortality occurs in each generation.

Furthermore, the projected rate of resistance evolution is inversely proportional to the slope of the probit line. For example, assuming that h^2 = 0.30 (the heritability of thiamethoxam resistance observed in this study) and selection mortality = 95%, a ten-fold increase in LC_{50} would occur in only 3 generations at a slope of 2.08, whereas, it would take 5 generations for the same to happen at a slope of 3.06.

Some scientists working with several other insect pests exhibited the similar trends. The estimated realized heritability (h^2) of chloropyrifos-methyl resistance in a field strain of *A. craccivora* (after 24 generations of selection) was found to be 0.35 (Mokbel, 2015). Shah *et al*. (2015) found that the h^2 value in the housefly, *Musca domestica* to methoxyfenozide was 0.17. Realized heritability (h^2) calculated from resistance development studies to lambda-cyhalothrin, bifenthrin, methomyl, imidacloprid, and fipronil was 0.07, 0.05, 0.01, 0.08 and 0.08, respectively (Abbas and Shad, 2015). Realized heritability values indicate lower additive genetic variations for a related neonicotinoid insecticide acetamiprid resistance in *A. gossypii*. Response to selection was declined as the selection pressure of acetamiprid continued, causing lower h^2 in the second round than in the first round of selection.

Similarly, Mokbel (2018) studied resistance risk in *A. gossypii*, to acetamiprid. After 16 generations of selection, there was a 22.55-fold increase in LC_{50} and the realized heritability (h^2) of resistance was 0.17.

The realized heritability (h^2) of insecticide resistance was very high in imidacloprid selected strains of *B. tabaci* compared to bifenthrin and fenvalerate selected strains of those insects. The results indicated high level of risk in the field populations for the development of resistance to imidacloprid (Sethi *et al.*, 2008).

In the present study, the estimated realized heritability was affected mainly by the response to the selection by *A. craccivora*. The h^2 was higher in the first round than in the second round of selection. This indicated the reasonable presence of a resistance alleles in the collected field population and that due to the continuous selection the heritability declined.

5.3 Reversion of resistance

Since a stable resistance prevents the successful re-use of an insecticide for pest management, a study on the reversion of insecticide resistance assumes prime importance. In the current study, the resistant population took 18 generations (F_{24} to F_{42}) to revert to the susceptibility without any insecticide selection pressure by thiamethoxam.

In the first half of the reversion process the LC_{50} values remained more or less similar till eight generations (225.83 ppm to 171.18 ppm). That indicated that the stability in resistance didn't vary from F_{24} to F_{32}. In F_{34} there was marked drop in LC_{50} value (38.20 ppm). Further, the value decreased to 17.01 ppm, 5.95 ppm, 2.72 ppm and 2.45 ppm in F_{36}, F_{38}, F_{40} and F_{42}, respectively (nearly equal to the level of pre-selection population).

The reversion process after attaining the insecticide resistance is a major pre-requisite for the successful resistance management in the field (Tabashnik, 1990). Reversion could be attributed to the inability of the resistant individuals to compete effectively with the susceptible ones in terms of reproductive potential and other biotic factors (Georghiou, 1963; Ninsin and Tanaka, 2005).

The decreased slope (5.20±0.98 to 1.70±0.23) value also indicated that the population reverted to the susceptibility. Many other investigators also found similar kind of results while studying reversion of resistance. Mokbel (2015) found that the resistance level in chloropyriphos-methyl resistant *A. craccivora* decreased steadily from 91-fold to

2.9- fold over nine generations without any selection pressure. Gutiérrez-Olivares *et al.* (2007) opined that the resistance in *B. tabaci* to thiamethoxam (F_2 to F_6) and imidacloprid (F_3 to F_6) was unstable. With respect to imidacloprid, resistance ratio (RR) of LC_{85} in F_3 was 8.1-fold and descended to 4.8-fold in the following generation; from F_5 to F_6, RR decreased from 3.5 to 2.4-fold. RR to thiamethoxam was 6.9-fold in F_2 and descended to 2.8-fold in F_6. Sawicki *et al.* (1980) while studying the OP and carbamate resistance in *M. persicae* reported that the high esterase activity regulating resistance was not stable in the population. In another study, in the absence of selection pressure, acetamiprid resistance in *A. gossypii* was unstable and resistance reversed in five generations without exposure (Mokbel, 2018).

In the current study, the decrease in resistance ratio (86.19 to 0.93) over 18 generations and increase in susceptibility of the resistant individuals in *A. craccivora* showed that thiamethoxam resistance was unstable in the same. In resistance management strategies, reduction or temporary stop of insecticides are helpful to promote the buildup of refuge, which can protect the susceptible nature in insect diluting the recessive resistant individuals (Crowder and Carrie`re (2009); Lu *et al.,* 2012). No exposure to insecticides could provide the opportunity to combat resistance (Wilson *et al.,* 2007). Temporary stop or rotation of insecticide application was one of alternatives that were put into use to cope with pest resistance (Yang *et al.,* 2014).

5.4 Persistent toxicity of thiamethoxam to *A. craccivora* in cowpea

Toxicity of different dosages of thiamethoxam (@ 20g a.i./ha, @ 25g a.i./ha, @ 30g a.i./ha and @ 35g a.i./ha) as foliar spray on cowpea persisted for 11 to 23 days (24 hr of exposure) and 14 to 24 days (48 hr of exposure). According to Preetha *et al*. (2009) thiamethoxam @ 25g a.i./ha persisted for 25 days against *A. gossypii* on bhendi crop when studied using clip-on-cage method. However, thiamethoxam when applied as foliar spray @ 15g a.i./ha, the toxicity persisted for 11 and 19 days (24 hr and 48 hr of exposure, respectively) when tested against *A. craccivora* on cowpea (Patil, 2015). Thus, the present finding is almost in line with the findings of Patil (2015).

In the present investigation, the median lethal time (LT_{50}) of thiamethoxam @ 25g a.i./ha was 6.79 days and 8.78 days (after 24 and 48 hrs of exposure, respectively).

According to Abd-Ella (2014), higher LT_{50} values of 6.95 and 7.85 days, of thiamethoxam at 50 gm/100 L were observed after 24 and 48 hours of exposure, respectively against cowpea aphids, *A. craccivora*. Thus the present result is in agreement with the findings of Abd-Ella (2014).

In the present findings, imidacloprid @ 25g a.i./ha persisted for 21 and 22 days with LT_{50} values 7.83 and 10.66 days (24hr and 48 hr of exposure, respectively) as foliar spray. Similar findings were reported by Preetha *et al*. (2009), who reported that imidacloprid @ 25 g a.i./ha persisted for 25 days. According to Patil and Lingappa (2001), imidacloprid @ 40gm a.i/ha when applied as plant hole treatment (PTH), persisted for more than 25 days against aphids *M. nicotianae* on tabacco.

Diamethoate @ 300ml a.i./ha had median lethal time (LT_{50}) 6.69 and 8.47 days in the present study. According to Gaikwad *et al*. (2015), dimethoate at 0.03 per cent showed highest PT values of 909.16 and 861.7 on leaves and shoots of safflower and LT_{50} values estimated to be 7.68 and 7.07 days, respectively against nymphs of *Uroleucon compositae* (Theoblad). Patil (2015) reported that LT_{50} values of diamethoate was 5.76 and 10.54 days (24 hr and 48 hr of exposure, respectively) when tested against *A. craccivora*.

Summarising the above results it may be stated that thiamethoxam in cowpea persisted for 11, 15, 15 and 23 days when applied at dosages of 20g a.i., 25g a.i., 30g a.i. and 35g a.i./ ha, respectively aginst adult apterous *A. craccivora*. Imidacloprid and dimethoate persisted for 21 and 13 days when applied at 25 ml a.i. and 300 ml a.i./ha., respectively.

5.5 Activity of various detoxifying enzymes

5.5.1 Total body protein

The overall body protein was increased in *A. craccivora* resistant strains. It has also been studied by other scientists that there is a significant relationship between the total body protein and the resistance level in an insect. The activity of the associated enzyme levels also increased along with the total body protein that aid in detoxifications of the xenobiotic. Rufingier *et al.* (1999) reported that the pirimicarb and endosulfan

resistance in lettuce aphid *Nasonovia ribisnigri* were associated with increased level of total body protein, detoxification enzyme activities and modification of the target proteins. In an alpha-methrin resistant strain of *M. persicae* significantly high level of protein content and enzyme activities were seen (Shuai and Wang, 2005).

5.5.2 Activities of carboxylesterases, MFOs and GSTs

Out of all the detoxifying enzymes conferring metabolic basis of resistance, the activity of carboxylesterases was found highest (42.02 times) in comparison to glutathione-S-Transferase (3.01 times) and Mixed function oxiadse (7.04 times). In support of the present findings, many workers have also reported the role of carboxylesterases conferring resistance to both neonicotinoid and other group of insecticides. Abdallah *et al.* (2016) reported that carboxylestersae activity was 30 times greater in the thiamethoxam resistant strain of *A. craccivora* when compared to the susceptible strain. In addition, they also found the enzyme activity of glutathione-S-transferase (GST) and mixed function oxidases (mfo) increased only in the resistant strain 3.7 and 2.7 times, respectively, in relation to the susceptible strain (the control). This indicated significant activity of the detoxifying enzymes, particularly carboxylesterase, in the resistant strain of *A. craccivora* in conferring thiamethoxam resistance corroborating the present findings. Further, Fouad *et al*. (2016) after monitoring the resistance in three field populations of *A. craccivora* to seven insecticides belonging to organophosphates, carbamates and neonicotinoids and different enzyme levels in them opined that resistance in cowpea aphid to those insecctcides may be due to the higher activity of the enzyme carboxylesterases. Cao *et al*. (2008) proposed increased carboxylesterase detoxification due to gene overexpression accounting for omethoate resistance in laboratory selected *A. gossypii*. In a thiamethoxam resistant strain of *A. gossypii*, no target site mutation was found involved, rather overexpression of P-450s and esterases were seen (Wei *et al*., 2017). In contrast Koo *et al*. (2014) found no significant difference between the susceptible and neonicotinoid resistant strain of *A. gossypii* with respect to the activities of cytochrome P-450, GST and esterase detoxification enzymes. Similarly, Shang *et al*. (2012) found that the activity of carboxylesterase showed no significant difference in omethoate resistant strain (231.2-fold) of the cotton aphid, *A. gossypii*.

5.5.3 Activities of Acetylcholinesterase

In the current studies, the specific activities of acetylocholinesterase (AChE) were 1.30 times higher in resistant strains which was not significantly different. In this line Mokbel (2013) reported that in an acetamiprid resistant strain of *A. craccivora* the activity ratio of acetylcholinesterase was 0.92 with a mean value of 5.68 x 10^6 mg substrate/ g protein / min comparing to the susceptible strain (with a mean value of 6.18 x 10^6 mg substrate/ g protein / min). Usually there is no correlation between AChE and neonicotinoids. Still there is some exceptions in the findings of some investigators. In an experiment when *A. craccivora* was selected for 12 generations with 48.01 folds of resistance to thiamethoxam the activity ratio of acetylcholinesterase was 3.68 with specific activity of 37.55±1.18 mOD. min^{-1}. mg^{-1} (Abdallah *et al*., 2016). Samson-Robert *et al.* (2015) reported that in rare cases one of the pyrethroids (deltamethrin) and the neonicotinoid insecticides can cause an increase in AChE activity.

5.6 Fitness cost of the thiamethoxam resistance in *A. craccivora*

Insecticide resistance is associated with fitness costs, such as a reduction in reproductive performance, longer development times and a reduction in body size in several insect species in agricultural fields that are sprayed with insecticides and in environments that are also free of insecticides (Berticat *et al*., 2008).

In the present study, the resistance acquisition in different generations of aphids increased the nymphal durations. It also resulted in lesser fecundity (16.0 ±5.34 nymphs per adult in F_{24}), longer adult longevity (12.49 ±1.44 days in F_{24} in comparision to 9.50 ±1.22 days in F_0) and oviposition periods (8.89 ±1.27 days in F_{24} in comparision to 7.78 ±1.40 days in F_0).

Although no reports on fitness costs, asscociated with thiamethoxam resistance in *A. craccivora* population was found while browsing literature but, similar reports involving the same insecticide with other sucking pests, and related neonicotinoid insecticide with other sucking pests and other crop pests have been found. According to Feng *et al.* (2009), a laboratory selected thiamethoxam resistant strain of *B. tabaci* (60-fold resistance in 36 generations) had obvious fitness disadvantages in their development,

reproduction and morphology. According to Gao *et al* (2014), survival percentage of 1st instar larva of thiamethoxam resistant western flower thrips, *Frankliniella occidentalis* (15.1 fold of resistance in 55 generations) was significantly lower for the resistant strain (60.2 ± 5.2%) than for the susceptible strain (72.2 ± 6.0%). The percentage of pupation (75.4 ± 2.8%) and fecundity (47.7 ± 1.8 eggs per female) of resistant strain were significantly lower than those of susceptible strain (83.9 ± 2.0% and 54.0 ± 3.2% eggs per female, respectively).

Similar findings were reported by Liu and Han (2006). In their study, a laboratory selected imidacloprid resistant strain of *Nilaparvata lugens* (250-fold resistance in 37 generations) had disadvantages in reproduction. The larval survival rate (78%), adult emergence rate (69.6%), copulation rate (64.9%), fecundity (217.9) and hatchability (57.3%) were all significantly lower in resistant population as comparing to that of the susceptible strain that is, survival rate (93.7%), adult emergence rate (92.1%), copulation rate (87.5%), fecundity (491.3) and hatchability (88.2%). Fitness costs are suggested to be a consequence of tradeoffs in energy between traits underlying insecticide resistance and fitness-related traits such as reproduction, development time and adult body size (Fenton *et al*., 2010).

According to Belinato and Martins (2016), an organophosphate and insect growth regulator (IGR) resistant population of *Aedes aegypti* had longer developmental time, lower longevity, problems with blood feeding and low reproductive traits. According to Afzal and Shad (2017) in a spinosad resistant strain of cotton mealybug, *Phenacoccus solenopsis* (282.45 resistance fold in 9 generations) there was increased male and female nymphal duration, pupal duration, emergence rates, male and female generation time with decreased fecundity. The longevity of male and female were significantly increased as compared to the susceptible populations (4.67 to 6.75 days in male and 10.77 to 23.23 days in female).

Biological characteristics evaluation of insecticide resistant populations can be very useful in formulating the insecticide resistance management strategies (Campanhola *et al.*, 1991). Often, insecticide resistance may cost significant fitness to the pest population. The biological parameters of insects, *e.g.*, reduced fecundity and enhanced

growth period, may bring changes in relative fitness. The pest survival rate decreases because of resistance, which finally leads to declining in fitness (Roush and McKenzie, 1987; Forrester *et al.*, 1993). In resistant population the reduced fecundity might be the result of less energy utilized for reproduction, *i.e.* part of the metabolic energy may be used in physiological and biochemical defences for insecticides detoxification (Ribeiro *et al.*, 2001).

Thus, from the present investigation it was understood that thiamethoxam resistance development has strong effect upon different biological parameters of *A. craccivora*. So, may be suggested that removal of selection pressure from the *A. craccivora* population may facilitate the reversion to susceptibility. Fitness costs have vital role in sustainable utilization of pesticides because they restrain the progression of resistance in agro-ecosystems.

SUMMARY AND CONCLUSION

A study on the development of insecticide resistance in Black legume aphid, *A. craccivora* Koch. to thiamethoxam was carried out under laboratory conditions in the insectary, Department of Entomology, College of Agriculture, Odisha University of Agriculture and Technology (OUAT), Bhubaneswar. The study started with collection of black legume aphids from untreated cowpea crop grown at the Central Research Station, OUAT and were reared under laboratory conditions in 15 to 20 days old cowpea seedlings planted in a staggered manner.

The baseline toxicity of thiamethoxam to adult apterous aphids were determined using leaf-dip bioassay method. The data were analysed using Ldp line software. The baseline susceptibility of the parental susceptible *A. craccivora* was determined to be 2.62 ppm with a fiducial limit of 1.91 to 3.43 ppm using above method. From a parental stock with genetic heterogeneity, a strain resistant to thiamethoxam was selected by continuous breeding in cowpea plants using a selection pressure of LC_{60} of thiamethoxam every alternate generation to the adult apterous aphids. The selection process was continued up to 24 generations. Realized heritability (h^2) was also estimated using LC_{50} values and slopes (of regression lines) of various generations. Accordingly, the number of generations required for a ten-fold increase in LC_{50} values (resistance) was estimated. Applying continuous selection pressure with thiamthoxam for 24 generations, *A. craccivora* developed 86.19-folds of resistance. Starting with an LC_{50} value of 2.62 ppm in the F_0 (parental) generation, the LC_{50} value increased to 3.61, 7.11, 23.49, 31.32, 47.08, 48.76, 75.92, 181.73, 196.58, 199.57, 207.21 and 225.83 in the F_2, F_4, F_6, F_8, F_{10}, F_{12}, F_{14}, F_{16}, F_{18}, F_{20}, F_{22} and F_{24} generations, respectively in the process of selection for resistance to thiamethoxam. The degree of resistance was found to be 1.37 (F_2), 2.71 (F_4), 8.96 (F_6), 11.95 (F_8), 17.96 (F_{10}), 18.61 (F_{12}), 28.97 (F_{14}), 69.36 (F_{16}), 75.02 (F_{18}), 76.17 (F_{20}), 79.08 (F_{22}) and 86.19 (F_{24})-folds compared to the parental one. Initially the rate of resistance development was slow till it reached F_{12}. From F_{14} to F_{16} there was marked changes in resistance folds. Towards the end of the selection process the resistance level was stabilized remaining more or less same. A highly resistant population was obtained at the end of F_{24}.

From the realized heritability (h^2) studies it was found that during the first half of the selection (first 12 generations) (F_2-F_{12}), the realized heritability was comparatively higher (0.28) indicating comparatively higher response to the selection to thiamethoxam. In contrast, the second half of selection (F_{12}- F_{24}) showed lower h^2 (0.19). Higher h^2 in first round compared with the second round of selection resulted from increased response to selection. Higher heritability in initial generations selected with thiamethoxam and low heritability with the later ones, suggested that by the 12th generation the population had approximately stabilized with the insecticidal pressure. From the realized heritability studies, the projected rate of resistance development could be predicted. For the entire selection process (*i.e.* 24 generations) it was estimated that 13 to 5 generations would be required for 10-fold increase in the resistance to thiamethoxam as the selection intensities increase from 50 to 95 per cent.

After establishing sufficient resistance in the strains of *A. craccivora*, reversion pattern of resistance was studied by rearing the resistant population without any selection pressure (spraying) with insecticide. The adult apterous aphids were collected from the resistant strains and were released on 20 days old untreated cowpea seedlings. After getting next generation from the released adults, again newly formed adults were shifted and released on new plants. The LC_{50} value of thiamethoxam was determined at an interval of each two generations by the same leaf-dip method of bioassay. The acquired resistance to thiamethoxam in *A. craccivora* was found to be unstable as the resistance ratio reverted back to susceptibility in another 18 generations of selection (F_{24} to F_{42}) without any selection pressure with thiamethoxam. The LC_{50} value decreased from 225.83 ppm (in F_{24}) to 0.93 ppm (in F_{42}). Resistance ratios (RR) when calculated for various generations, it was clear and evident that it followed a decreasing trend in different generations. In F_{24} RR was 86.19. Further in F_{26}, F_{28} and F_{30} the ratios decreased to 72.74, 71.39 and 70.53, respectively. In between F_{32} and F_{34} there was marked changes in resistance ratios. The value dropped drastically from 65.33 (in F_{32}) to 14.58 (in F_{34}). Further, the LC_{50} values in F_{36}, F_{38}, F_{40} and F_{42} generations were calculated to be 6.49, 2.27, 1.03 and 0.93, respectively. The regression coefficient or the slope value also decreased through various generations without any selection pressure of thiamethoxam. After attaining highest level of resistance in F_{24} the slope was 5.20±0.98. Further, in

different generations during the reversal of resistance the slope value decreased to 1.70±0.23 in F_{42}.

In a completely randomized design, 15 days old seedlings of cowpea were used to study the persistence of toxicity of different dosages of thiamethoxam and other conventionally used insecticides against the aphids. Various treatments were T_1 (Thiamethoxam 20g a.i./ha), T_2 (Thiamethoxam 25g a.i./ha), T_3 (Thiamethoxam 30g a.i./ha), T_4 (Thiamethoxam 35g a.i./ha), T_5 (Imidacloprid 25g a.i./ha), T_6 (Dimethoate 300g a.i./ha) and and control (distilled water). The persistence of toxicity was studied in microcage method. The observation on the mortality (24 hours and 48 hours after release in insecticide sprayed plants) were taken using visual counting method. It was found that thiamethoxam in cowpea persisted for 11, 15, 15 and 23 days when applied at dosages of 20g a.i., 25g a.i., 30g a.i. and 35g a.i./ ha, respectively aginst adult apterous *A. craccivora*. Imidacloprid and dimethoate persisted for 21 and 13 days when applied at 25g a.i. and 300 g a.i./ha., respectively.

The relative efficacy of insecticides evaluated in terms of persistency was thiamethoxam@ 35g a.i./ha> imidacloprid@ 25g a.i./ha> thiamethoxam@ 30 g a.i./ha> thiamethoxam@ 25g a.i./ha> dimethoate@ 300g a.i./ha> thiamethoxam@20 g a.i./ha when mortalities of aphids were studied after 24 hours of exposure.

The median lethal time LT_{50} was least in thiamethoxam @20g a.i./ha (4.74 days) which followed an increasing trend in diamethoate @300g a.i./ha (6.69 days), thiamethoxam @25g a.i./ha (6.79 days), imidacloprid @25g a.i./ha (7.83 days), thiamethoxam @30g a.i./ha (8.12 days) and thiamethoxam@ 35 g a.i./ha (9.02 days) when mortality observation was taken 24 hours after exposure.

The activities of various detoxifying enzymes were estimated using standard protocols in susceptible and thiamethoxam resistant aphid strains. The total body protein content was determined by following the method described by Bradford (1976), using bovine serum albumin (BSA) as the standard. The esterases activity were assayed with α-naphthyl acetate (α-NA), as substrate following the process described by Van Asperen (1962). The glutathione-S-transferase was measured as per the method described by Habing *et al*. (1974). Acetylcholinesterase activity was measured following to Ellman *et*

al. (1961) in both resistant and susceptible *A. craccivora* population. The Mixed function oxidases (MFO) activities (mono oxygenases) were analyzed according to the protocol given by Hansen and Hodgson (1971).

In resistant population, the mean total body protein was 0.41 mg/ g body weight whereas in susceptible population the value was estimated to be 0.21mg/ g body weight implying elevated levels of protein responsible for resistance to thiamethoxam. Comparing the activity ratio of all the detoxifying enzymes, highest activity was seen by carboxylesterases (42.02 times) in resistant strains followed by mixed function oxidase (7.05 times) and glutathione-S-transferase (3.01 times) when compared with the susceptible strain. The specific activity of Acetylcholinesterase was 8.27±1.98 µmoles. min^{-1}. mg^{-1} protein in susceptible strain whereas in resistant strain the value was 8.52 ± 1.08 µmoles. min^{-1}. mg^{-1} protein with an activity ratio of 1.30.

Different biological parameters *viz*., nymphal duration, pre-oviposition, oviposition, post-oviposition period, adult longevity and fecundity were studied in different generations of selection for resistance of *A. craccivora* to thiamethoxam (parental strain, F_2, F_4, F_6, F_8, F_{10}, F_{12}, F_{14}, F_{16}, F_{18}, F_{20}, F_{22} and F_{24}) under laboratory conditions (28±2ºC; 75-80%). While studying the fitness cost of the acquired resistance in *A. craccivora* it was found that thiamethoxam resistance development had strong effect upon different biological parameters of the aphids. The average nymphal duration was increased from 4.35 ±1.02 days (in F_0) to 7.9 ±0.57 days (in F_{24}). The pre-oviposition period increased from 0.57±0.18 days in F_0 to 1.60±0.34 days in F_{24}. The oviposition period shown an increasing trend from 7.78±1.40 days in parental generation (F_0) to 8.89±1.27 days in the 24^{th} generation (F_{24}) of selection. Similarly, the post-oviposition period registered an increasing trend from 1.14±0.24 days in F_0 to 2.00±0.37 days in the F_{24} generation of selection with thiamethoxam. The adult longevity was also increased over the selection pressure (9.50 ±1.22 days in F_0 to 12.49 ±1.44 days in F_{24}). Whereas, the mean fecundity through parthenogenetic reproduction was significantly decreased over generations. In F_{24}, the fecundity was 16.0 ±5.34 whereas in F_0 the same value was 54.71 ±7.63.

Thus, it may be concluded from the study that the neonicotinoid insecticide thiamethoxam if used repeatedly for the control of *A. craccivora* then there is every chance of rapid development of resistance development to *A. craccivora* to the tune of 86.19-folds in 24 generations of selection at 60% selection pressure. But, if it is used at the recommended field dosage (LC_{95}) then only 5 generations would be required for 10-folds' increase in the magnitude of resistance. However, if the selection pressure is taken off, 18 generations would be required for the thiamethoxam resistant strain of *A. craccivora* to revert back to susceptibility.

The increase in the levels of carboxylesterase and related detoxifying enzymes *e.g.* glutathione-S-transferase (GST) and mixed function oxidases (MFO) in the resistant strain of *A. craccivora* are responsible for increased level of resistance to thiamethoxam.

The persistence of toxicity of thiamethoxam is comparatively greater than the conventionally used insecticides such as imidacloprid and diamethoate requiring less frequent application.

The increase in nymphal duration and adult longevity in resistant aphids indicated more damage to the crops and are in more attention for protection. On the other hand, decreasing fecundity of resistant individuals although appears to be less in their number the field, but difficult to be controlled by thiamethoxam and other insecticides having similar mode of action.

FUTURE LINE OF RESEARCH

1. Verifying the results obtained under laboratory conditions concerning the rate of resistance development, reversion of resistance, fitness of resistant strains and persistence of toxicity of thiamethoxam under field conditions.
2. Studying the cross-resistance pattern of thiamethoxam resistant *A. craccivora*.
3. Studying the existence of resistance in field collected thiamethoxam resistant strain of *A. craccivora*, if any.
4. Studying the depletion pattern of detoxifying enzymes when the insecticide pressure is taken off from the resistant strain of aphids.

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