INSECTICIDE RESISTANCE IN COWPEA APHID, *Aphis craccivora* (KOCH) AND ITS MANAGEMENT

by

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THESIS

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DECLARATION

I, hereby declare that this thesis entitled **"INSECTICIDE RESISTANCE IN COWPEA APHID, *Aphis craccivora* (KOCH) AND ITS MANAGEMENT"** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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CERTIFICATE

Certified that this thesis entitled **"INSECTICIDE RESISTANCE IN COWPEA APHID, *Aphis craccivora* (KOCH) AND ITS MANAGEMENT"** is a record of research work done independently by Mr. Jangam Hampaiah under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to her.

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(Jangam Hampaiah)

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LIST OF ABBREVIATIONS

%	Per cent
@	At the rate of
a.i.	Active ingredient
BQL	Below quantification level
C.D.	Critical Difference
DAT	Days after treatment
DAS	Days after spraying
EC	Emulsifiable concentrate
et al	And others
g	Gram
ha^{-1}	per hectare
kg	Kilogram
L	Litre
mL	Millilitre
ppm	Parts per million
RSD	Relative standard deviation
SC	Suspension concentrate
SL	Soluble Liquid
SP	Soluble Powder
SD	Standard deviation
sp	Species
viz.,	Namely
WG	Wettable granules
ZC	Zeon capsules

Introduction

1. INTRODUCTION

Resistance to any xenobiotic molecule is a basic biological phenomenon and a major obstacle to the management of agricultural pests. It is a heritable change in the sensitivity of a pest population that is reflected in the repeated failure of a product to achieve the expected level of control when used according to the label recommendation for that pest species (IRAC, 2018). Resistance frequently leads to the increased use, overuse, and even misuse of pesticides, which poses a risk to the environment and public health.

Cowpea, *Vigna unguiculata* subsp. *sesquipedalis* (L.) Verdc. a multipurpose grain legume is extensively cultivated in arid and semi arid regions of Asia and Africa. Infestation due to cowpea aphid, *Aphis craccivora* (Koch) is one of the major constraints in increasing the production and productivity of cowpea in the tropics. Aphid management strategy heavily depends on the use of synthetic insecticides such as organophosphates, carbamates, pyrethroids and neonicotinoids. Intensive and repeated use of insecticides results in the development of resistance in aphids, forcing farmers to use higher dose of insecticides with lesser application frequency. Several control failures have been reported from many fields and the status of insecticidal resistance in *A.craccivora* seems quite serious nowadays. Globally, insecticide resistance has been documented in over 20 aphid species in different crops (Georghiou, 1990).

The difficulties in the management of aphids in cowpea indicate an urgent need to establish an efficient resistance management strategy based on information available about the extent and nature of resistance in cowpea. Effective Insecticide Resistance Management (IRM) is an important element in maintaining the efficacy of valuable insecticides to prevent or delay the evolution of resistance to insecticides, or to help regain susceptibility in insect pest populations in which resistance has already arisen (IRAC, 2018). Study conducted at College of Agriculture, Vellayani on insecticide resistance in spotted pod borer, *Maruca vitrata* (Fabricius) on vegetable cowpea (Sreelakshmi and Paul, 2016) revealed a resistance ratio of 2.28 to 7.94 for chlorpyriphos and lambda cyhalothrin respectively in resistant population of pod borer. This study forms a maiden attempt in assessing the extent of insecticide resistance development in the populations of cowpea pod borer, *M. vitrata* in Kerala. However, no study on insecticidal resistance in *A. craccivora* has been carried out in Kerala. Hence this investigation is proposed to assess the extent of insecticide resistance in *A. craccivora* and to suggest measures for the management of insecticide resistance.

In the above perspective, the present study "Insecticide resistance in cowpea aphid, *Aphis craccivora* (Koch) and its management" was undertaken with following objectives:

- To assess the insecticide resistance in field population of cowpea aphid, *A. craccivora*
- To evaluate the efficacy of new generation insecticides against resistant population of *A. craccivora*
- To determine the persistence and dissipation rate of new generation insecticides in cowpea.

Review of literature

2. REVIEW OF LITERATURE

Cowpea aphid, *A. craccivora* is the most important polyphagous insect-pest that inhabits a wide range of plant hosts with preference to legumes. In addition to cause direct damage to the host by sucking the sap from different plant parts, they reduce the yield, quality and marketability of crops by transferring plant viruses which brings yellowing, stunting and premature plant death and the production of excess quantity of honey dew on which colonizes sooty mold.

Farmers usually adopt frequent sprays of chemical insecticides for aphid management in the field. Aphid management strategy mainly involved the use of synthetic insecticides *viz.* organophosphates, carbamates, pyrethroids, and neonicotinoids. The extensive and repeated use of these insecticides has resulted in the development of insecticide resistance (Jackal and Daoust, 1986; Hollingsworth *et al.*, 1994; Han and Li, 2004; Tang *et al.*, 2013) and observations made in recent years showed that the pest has acquired reduced susceptibility to insecticides that already have been effective. The literature regarding the biology of *A. craccivora*, occurrence of insecticide resistance, management of *A. craccivora* and occurrence of pesticide residues were reviewed and expressed here.

2.1. BIOLOGY AND NATURE OF DAMAGE OF *A. craccivora*

2.1.1 Biology

According to Ofuya (1997), adult aphid, *A. craccivora* is primarily shiny black or dark brown with size ranging from 1.5 to 2 mm long and nymphs are wingless, dark or dusty brown with wax coated and somewhat rounded in shape. Before obtaining adulthood, the insect passes through four nymphal instars. Parthenogenetic apterous adult females were successively produced, in case of abundant good quality food and under favorable climate conditions.

2.1.1.1 *Life stages of A. craccivora*

Yadav *et al.* (1991) reported that nymphal period for red and pale green morphs of *Myzus persicae* (Sulzer) ranged from 6 to 7 and 7 to 8 days respectively. While the nymphal period of *A. craccivora* from first to fourth instar was 2.45 to 2.25, 2.70 to 2.50, 2.73 to 2.50 and 2.95 to 2.65 days respectively (Singh and Kumar, 1999). However, Angayarkanni and Nadarajan (2008) reported that nymphal period of *A. craccivora* was 7.6 days.

Bakhetia and Sidhu (1976) reported that the pre reproductive, reproductive and post reproductive period were 4.20 to 20.80, 4.40 to 29.20 and 0.70 and 3.70 days respectively in different seasons. Again for the red and pale green morphs of *M. persicae*, the pre-reproductive, reproductive and post reproductive stages were reported by Yadav *et al.* (1991) *viz.,* 4.0, 13.8, 3.2 days and 3.0, 8.67, 2.67 days respectively. Whereas, Angayarkanni and Nadarajan (2008) reported that the pre reproductive, reproductive and post reproductive period of *A. craccivora* as 4.53, 6.57 and 2.03 days respectively.

Studies revealed that the adult longevity of *A. craccivora* was 10 to 12 days (Behura, 1956) and 9.6 to 68.2 days (Bakhetia and Sidhu, 1976). The longevity of aphid was 15.8 to 17.1 days which was less in flowering stage than in vegetative stage i.e. 18.9 to 20.3 days (Joshi *et al.* 1998). Whereas, Angayarkanni and Nadarajan (2008) recorded 13.39 days.

Based on report made by Joshi *et al.* (1998), fecundity of *A. craccivora* in cowpea at vegetative and flowering stage was 65.2 and 66.1 nymphs respectively and reproductive rate at vegetative and flowering stage was 4.85 and 5.76 nymphs per day respectively. In 2008, Angayarkanni and Nadarajan reported that fecundity and rate of reproduction were 53.02 and 8.07 nymphs per day respectively.

2.1.2 Hosts of *A. craccivora*

Hosts of *A. craccivora* were represented in Table. 1

Table 1. Hosts of *A. craccivora*

Host plants	Place	Reference
Cowpea	Africa, Asia and Latin America	Singh and Jackai, 1985
Groundnut	India	Jena and Kuila, 1997
Faba bean	Egypt	El-Defrawi *et al*., 1998
faba bean, cowpea and pea	Egypt	El-Ghareeb *et al*., 2002
Som plant	North-eastern India	Mathur and Upadhyay, 2002.
Groundnut	Uganda	Okello *et al*., 2010

2.1.3. Nature of Damage and Extent of Loss

Cowpea aphid, *A. craccivora* was a sporadic pest of pulses usually causing serious damage to the crop. Adults and nymphs of *A. craccivora* together affect the plant growth severely and feed on leaves, branches, flower buds and pods of cowpea by sucking the sap. Singh and Van Emden (1979) reported that the direct damage caused to the host plant was mainly due to the removal of sap by the depletion of assimilates jointly with a rise in respiration rate in the plant, where as high populations cause distorted leaves and stunted plants along with tiny poorly nodulated root system, reduction in yield and in severe cases lead to death of the plants.

Singh and Allen (1980) reported that the cowpea aphid, *A. craccivora* causes 20-40 per cent yield loss. Instead of causing infestation, *A. craccivora* also act as a vector of many plant virus. Based on the studies conducted by Mansour *et al*. (2000) and Larsson (2005) indicated that heavy aphid infestation causes more yield loss which was up to 2.60 t ha^{-1} in bean, corn and barley.

In groundnut cultivated regions of India, *A. craccivora* causes serious pod loss up to 40 per cent (Jena and Kuila, 1997). According to the experiment done by Klingler *et al*. (2001), high fecundity and short generation time of the aphid causes huge reproductive potential during the growing season.

Different viruses transmitted by *A. craccivora* were reviewed in Table 2.

Table 2. Viruses transmitted by *A. craccivora*

Virus	Geographical distribution	References
Peanut mottle	Worldwide	Kuhn, 1965
Peanut stunt	USA	Miller and Troutman, 1966
Groundnut rosette assistor	Africa	Hull and Adams, 1968
Groundnut eyespot	Ivory Coast	Dubern and Dollet, 1980
Peanut stripe	China, India, Indonesia, Japan, Philippines, Thailand, USA	Demski *et al*., 1984
Bean yellow mosaic	USA	Bays and Demski, 1986

2.2 DEVELOPMENT OF INSECTICIDE RESISTANCE

The intensity of pesticide use is linked to selection pressure, and this genetic selection pressure operating at the population level has repeatedly resulted in resistance and control failure across all the commonly used insecticides globally (Whalon *et al*., 2008). There are more than 7747 cases of resistance with more than 331 insecticide compounds involved. About 553 species are reported with resistance to insecticides (Mokbel, 2013).

2.2.1 Resistance against Organophosphates and Carbamates

The studies on the resistance of aphid against organophosphates and carbamates are presented in Table 3.

Table 3. Resistance against organophosphates and carbamates

Sl. no	Name of the pest	Crop	Country /Location	Against insecticide	Intensity of resistance	References
1	*A. gossypii*	Cotton	China	Parathion Demeton Phosphamidon Carbaryl	23 fold 89 fold 11.3 fold 9.3 fold	Tang and Huang, 1982
2	*M. persicae*	Tobacco	Central Virginia	Acephate	6-13 fold	Koziol and Semtner, 1984
3	*A. gossypii*	Cotton	Alabama and Texas	Methamidophos	5 fold	Kerns and Gaylor, 1992
4	*A. fabae*	Sugarbeet	Greece	Methamidophos Pirimicarb	50 fold 8 fold	Ioannidis, 2000
5	*A. gossypii*	Cotton	Egypt	Fenitrothion Primiphos-methyl Carbosulfan Aldicarb	6.11-8.64 fold 8.84-14.4 fold 10.05-27.7 fold 8.57-18.3 fold	El-Kady, 2007

6	*A. craccivora*	Faba bean	Egypt	Malathion Pirimicarb Carbosulfan	4.74 fold 1.16 fold 1.35 fold	Mokbel, 2013
7	*B. brassicae*	Rape seed	Pakistan	Deltamethrin	456 fold	Ahmad and Akhtar, 2013
8	*M. persicae*	Tobacco	China	Carbosulfan	14.47-28.69 fold	Li *et al.*, 2016
9	*A. craccivora*	Faba bean	Egypt	Pirimicarb Chlorpyriphos-methyl Malathion	78 % 55.6 % 50 %	Fouad *et al.,* 2016
10	*A. craccivora*	Faba bean	Egypt	Carbosulfan Malathion Chlorpyriphos-methyl	16.8 fold 14.24 fold 13.45 fold	Kandil *et al.*, 2017

2.2.2 Resistance of Aphids against Synthetic Pyrethroids

Kerns and Gaylor (1992) reported that field populations of the cotton aphid *A. gossypii* showed resistance to bifenthrin and cypermethrin in the range of 20 to 50 fold. Ahmad *et al.*, 2003 revealed a very high resistance in the field populations of *A. gossypii* collected from Multan, against seven pyrethroid insecticides *viz.* cypermethrin, alpha cypermethrin, zeta cypermethrin, cyfluthrin, fenpropathrin, bifenthrin, and lambda cyhalothrin. But, lower resistance was observed against deltamethrin.

Qaliobia and Damytta strains of *A. gossypii* recorded 14.6 and 3.11-fold resistance to lambda-cyhalothrin whereas Dakahlia and Damytta strains recorded 11.49 and 5.71-fold resistance to deltamethrin respectively in Egypt (El-Kady, 2007). Mokbel (2013) investigated resistance in *A. craccivora* and reported an apparent resistance to lambda cyhalothrin (21.4 fold) and vigor tolerance level was observed against fenvalerate (8.46 fold). In Pakistan, study of Ahmad and Akhtar (2013) revealed that population of *B. brassicae* showed the highest resistance to deltamethrin (456 fold). In China, Nanchuan, Shizhu, Qianjiang, Wushan, Wulong populations of *M. persicae* have developed medium resistance (10.36 to 41.28 fold) against cyhalothrin (Li *et al.*, 2016).

In Kerala, Sreelakshmi and Paul (2016) reported that resistant population of spotted pod borer, *Maruca vitrata* (Fabricius) developed a resistance of 7.94 fold for lambda cyhalothrin in cowpea. According to study conducted by Sreelakshmi (2017), Kovilnada population of *S. litura* showed a resistance of 6.48, 1.79 and 8.50 fold when compared with Kanjikuzhi population (field check) and 2566, 916 and 826 fold when compared with baseline susceptibility check (NBAIR strain)for cypermethrin, fenvalerate and lambda-cyhalothrin.

2.2.3. Resistance of Aphids against New Generation Insecticides

The studies on the resistance of aphid against new generation insecticides are presented in Table 4.

Table 4. Resistance of aphids against new generation insecticides

Sl. No.	Name of the pest	crop	Country /Location	Against insecticide	Intensity of resistance	Reference
1	*A. fabae*	Sugarbeet	Greece	Imidacloprid	7 fold	Ioannidis, 2000
2	*A. gossypii*	Cotton	Texas	Imidacloprid	8 fold	Wang *et al.*, 2002

3	*A. gossypii*	Cotton	Egypt	Imidacloprid	1.82-32.55 fold	El-Kady, 2007
4	*A. gossypii*	Cotton	Australia	Acetamiprid Clothianidin Thiamethoxam	22 fold 10 fold 17 fold	Herron and Wilson, 2011
5	*A. craccivora*	Faba bean	Egypt	Thiamethoxam Dinotefuran Pymetrozine	4.86 fold 2.40 fold 1.94 fold	Mokbel, 2013
6	*B. brassicae*	Rape seed	Pakistan	Imidacloprid Acetamiprid Thiamethoxam	95 fold 55 fold 143 fold	Ahmad and Akhtar, 2013
7	*M. persicae*	Tobacco	China	Imidacloprid	1.06-6.51 fold	Li *et al.*, 2016
8	*A. craccivora*	Faba bean	Egypt	Thiamethoxam	66.7 %	Fouad *et al.*, 2016
9	*A. craccivora*	Faba bean	Egypt	Thiamethoxam Acetamiprid	10.23 fold 8.65 fold	Kandil *et al.*, 2017

2.3. MANAGEMENT OF *A. craccivora*

The studies on the management of *A. craccivora* in different hosts by different insecticides *viz.* conventional and new generation insecticides were reviewed and presented in Table 5 and 6 respectively.

Table 5. Management of *A. craccivora* by conventional insecticides

Crop	Insecticides	Reference
Cowpea	Malathion 0.05%, quinalphos 0.025%	Sudharma *et al.*, 1987
Cowpea	Cypermethrin @ 80 g. a.i. ha^{-1}	Opolot *et al.*, 2006.
Broad bean	Chlorpyriphos @ 0.79 mg L^{-1}, Methomyl @ 1.03 mg L^{-1}, Pirimicarb @ 3.73 mg L^{-1}, Dimethoate @ 1.81 mg L^{-1}, Profenofos @ 3.39 mg L^{-1}, Malathion @ 7.24 mg L^{-1}, Phoxin @ 4.83 mg L^{-1}, Lambda cypermethrin @ 2.02 mg L^{-1}, Beta cypermethrin @ 4.81 mg L^{-1}, Bifenthrin @ 1.11 mg L^{-1}, Deltamethrin @ 1.24 mg L^{-1}.	Tang *et al.*, 2013.
Cowpea	Acephate 75SP @ 0.075% Profenophos 50EC @ 0.1% Dimethoate 30EC @ 0.06 %	Reddy *et al.*, 2014
Cowpea	Dimethoate @ 0.03 %	Swarnalata *et al.*, 2015.
Faba bean	Pirimicarb @ 0.027 mg L^{-1}L, Carbosulfan @ 0.17 mg L^{-1}, Fenitrothion @ 0.418 mL L^{-1}, Malathion @ 0.228 mL L^{-1}, Chlorpyriphos methyl @ 0.059 mL L^{-1}	Fouad *et al.*, 2016
Faba bean	Carbosulfan @ 2.856 mg L^{-1}, Fenitrothion @ 2.448 mL L^{-1}, Malathion @ 3.258 mL L^{-1}, Chlorpyriphos methyl @ 0.794 mL L^{-1}	Kandil *et al.*,2017

Table 6. Management of *A. craccivora* by new generation insecticides

Crop	Insecticides	Reference
Cowpea	Imidacloprid @ 0.003 %	Thamilvel, 2010
Broad bean	Imidacloprid @ 6.33 mg L^{-1}, Acetamiprid @ 7.58 mg L^{-1}, Pymetrozine 13.60 @ mg L^{-1}, Abamectin @ 52.23 mg L^{-1}	Tang *et al*., 2013
Cowpea	Thiamethoxam, @ 0.60 g L^{-1}, Acetamiprid @ 0.71 mg L^{-1} and Imidacloprid @ 1.16 mL L^{-1},	Abd-Ella, 2014
Cowpea	Acetamiprid 20SP @ 0.004 %, Imidacloprid 17.8 SL @ 0.005%, Thiamethoxam 25 WG @ 0.005 %, Diafenthiuron 50WP @ 0.06 %.	Reddy *et al.,* 2014
Cowpea	Thiamethoxam @ 0.01 %, Imidacloprid @ 0.005 %,	Swarnalata *et al*., 2015.
Faba bean	Acetamiprid @ 0.369 mg L^{-1}, Thiamethoxam @ 0.079 mg L^{-1}	Fouad *et al.,* 2016
Cowpea	Thiamethoxam @ 0.30 mg L^{-1} and imidacloprid @ 0.20 mL L^{-1}	Thamilarasi, 2016
Som plant	Imidacloprid @ 1ml per 5L	Ghosh *et al.,* 2016
Urdbean	Thiomethoxam@ 25 g. a.i. ha^{-1}, Flubendiamide + Thiacloprid @ 60+60 g. a.i. ha^{-1}, Emamectin benzoate @ 11 g. a.i. ha^{-1}	Rajawat *et al.*, 2017
Faba bean	Acetamiprid @ 3.192 mg L^{-1}, Thiamethoxam @ 0.824 mg L^{-1}.	Kandil *et al.,* 2017

2.4. DISSIPATION OF PESTICIDE RESIDUES IN DIFFERENT CROPS

The literature related to the persistence and degradation of insecticides on various crops are presented in Table 7.

Table 7. Dissipation of insecticides in vegetables

Sl. No	Crop	Insecticide	Dosage (g a.i. ha^{-1})	Initial concentration (mg Kg^{-1})	Days taken to reach BDL	Half-life (days)	Reference
1	Okra	Thiamethoxam	140	0.48	5	1.3	Singh and Kulshrestha, 2005
		Acetamiprid	75	0.34	7	2.3	
2	Chilli	Dimethoate	300	0.33	15	4.7	Reddy *et al.*, 2007
		Lambda cyhalothrin	50	0.62	15	6.4	
3	Tomato	Thiacloprid	48	0.76	10	-	Singh and Dikshit, 2007
			96	1.38	10	-	
4	Tomato	Thiamethoxam	140	0.18	10	4.2	Karmakar and Kulshrestha, 2009
			280	0.30	10	3.5	
5	Okra	Lambda cyhalothrin	15	0.64	-	5.2	Deen *et al.*,2009
6	Cowpea	Lambda cyhalothrin	15	8.76	15	0.95	Soliman, 2011
		Acetamiprid	10	6.57	21	3	
		Thiamethoxam	25	8.96	21	2.95	
7	Cabbage	Thiacloprid	180	-	-	1.3-1.6	Wang and Zhang, 2011
8	Okra	Imidacloprid	18	0.30	3	0.49	Patel *et al.*,

				36	1.23	7	1.13	2012
9	Green chilli	Imidacloprid		120	2.53	30	5.82	Mathew *et al*., 2012
				240	3.15	35	5.77	
10	Okra	Thiamethoxam		25	0.25	7	1.47	Chauhan *et al*., 2013
11	Cowpea	Acetamiprid		15	0.28	7	1.90	Thamilarasi, 2016
		Imidacloprid		20	0.32	3	0.82	
		Thiamethoxam		25	0.89	10	2.55	
		Thiacloprid		24	0.37	5	2.80	
	Salad cucumber	Acetamiprid		15	0.42	10	2.55	
		Imidacloprid		20	0.18	3	0.66	
		Thiamethoxam		25	0.16	7	1.39	
		Thiacloprid		24	0.13	3	1.28	
12	Amaranthus	Quinalphos		250	1.75	15	3.35	Sreelakshmi, 2017
		Thiacloprid		120	3.17	5	0.98	
13	Capsicum	Thiamethoxam	Open field	50	1.62	7	-	Pathipati *et al*., 2018
			Poly house	50	2.77	15	-	
14	Cowpea	Chlorantraniliprole		150	0.27	-	-	Reddy *et al*., 2018
		Thiamethoxam		150	0.64	-	-	
15	Cabbage	Quinalp-hos	Plains	500	0.24	5	-	Padmanabhan and Paul, 2018
			Hills	500	2.66	7	-	

2.4.1 Risk Assessment of Insecticides

Parmar *et al.* (2016) reported that maximum permissible limit for flubendiamide and thiacloprid was calculated to be 1.2 and 0.6 mg person^{-1} day^{-1} respectively. TMRC for flubendiamide and thiacloprid was only 0.036 and 0.086% of their MPI respectively in red gram. The summative TMRC calculated for these pesticides on different commodities is only 2% of the total MPI. Hence, these pesticides do not pose any serious health risk for consumers.

Based on studies conducted by Bhattacharyya *et al.* (2017), the TMRC values on zero day were in the range of 0.268- 0.498 mg adult^{-1}day^{-1} and 1.538-3.27 mg adult^{-1} d^{-1} for emamectin benzoate and fipronil, respectively, which were much lower than the MPI of both insecticides at standard recommended dose. Hence, the application of emamectin benzoate and fipronil formulation in chilli does not pose any health risk to consumers even at two hours after spraying.

Materials and Methods

3. MATERIALS AND METHODS

The present study on "Insecticide resistance in cowpea aphid, *Aphis craccivora* (Koch) and its management" aims to assess the insecticide resistance in field population of cowpea aphid, *A. craccivora*, to evaluate the efficacy of new generation insecticides against resistant population of *A. craccivora* and to determine the dissipation of insecticide residues in cowpea pods. Three different field populations of *A. craccivora* were selected from three locations *viz.* Vilavoorkal, Vellayani and Vallamcode and studied the development of insecticide resistance and also evaluated the efficacy of new generation insecticides against resistant population of *A. craccivora* and studied the dissipation of insecticides at Vallamcode. The materials utilized and the methods employed are presented here under in detail.

3.1. PRELIMINARY SURVEY TO GATHER THE INFORMATION REGARDING THE PESTICIDE USE AND THE DEVELOPMENT OF INSECTICIDE RESISTANCE

A purposive survey was carried out to study the details of pests, effectiveness of pesticides, awareness on insecticide resistance and pesticide use pattern among 50 cowpea farmers in Kalliyoor area of Thiruvananthapuram district during 2016-17. Each farmer was interviewed separately using a questionnaire (Appendix I).

3.2 ASSESSMENT OF INSECTICIDE RESISTANCE IN FIELD POPULATIONS OF *A. craccivora* ON VEGETABLE COWPEA

3.2.1. Biology of *A. craccivora*

Biology of cowpea aphid, *A. craccivora* was studied under laboratory conditions in Dept. of Agrl. Entomology, College of Agriculture, Vellayani. Cowpea plants of variety Geethika were raised in ice cream cups kept inside the insect proof net cage (Plate 1). When plants were two to three leaf stage, one first instar nymph each was released per plant. Thus 10 such plants were kept as replication. The nymphal

a. Insect proof cage with rearing containers

b. Adult female

c. Colony of *A. craccivora*

Plate 1. Biology of *A. craccivora* in cowpea

period, reproductive period, fecundity and rate of reproduction (nymphs/day/aphid) were observed. The plants were monitored daily for observations on different biological parameters. The young ones were counted and removed daily with a camel hair brush. The plant was replaced whenever required. The nymphal period was considered from the date of birth of the first instar to the date of last instar. The number of days for which aphid continued to reproduce constituted the reproductive period. Total life cycle was calculated by adding the above two phases of life. Fecundity was taken as the total number of young ones produced by a given aphid in her lifetime.

3.2.2 Assessment of Insecticide Resistance

The apterous adult females of cowpea aphid *A. craccivora* were collected from the infested cowpea plants from three different locations. The first insect population was taken from a field with no previous history of pesticide application (Vilavoorkal). The second was from the field where insecticides were applied but no known report of control failures *viz*. College of Agriculture, Vellayani and third population was taken from field where indiscriminate use of insecticides were followed and control failures had been observed (Vallamcode). Three sets of populations collected from three places were multiplied separately in insect proof net cages as explained in 3.2.1. The adult apterous aphids of F2 generation were taken for the study. The resistance/susceptibility of the population were tested by using the three insecticides selected from the survey (3.1) which belongs to three different group of insecticides having different mode of action. The details of the insecticides selected for the resistance study is given in Table 8.

3.2.3. Mass Rearing of *A. craccivora*

Cowpea aphid, *A. craccivora* was collected from cowpea growing fields from three different locations and reared under the conditions as described by Jhansi (2003). Vegetable cowpea seed (variety- Geethika) was sown in ice cream cups, which were filled with 1:1 ratio of sand and vermi compost and kept in insect proof

Table 8. Details of insecticides used for resistance study

Sl. no	Details of insecticides				
	Chemical name	Trade name	Chemical group	Mode of action as per IRAC, 2018	Dosage (g a.i,ha^{-1})
1	Quinalphos 25% EC	Ekalux	Organophosphates	Acetylcholinesterase (AChE) inhibitors	250
2	Fenvalerate 20% EC	Fenval	Synthetic pyrethroids	Sodium channel modulators	25
3	Imidacloprid 17.8% SL	Confidence 555	Neonicotinoids	Nicotinic acetylcholine receptor (nAChR) competitive modulators	20

net cage (Plate 2). Sprouted plants were irrigated daily to maintain the succulence of seedlings. The aphid colonies were collected from the field and all the natural enemies were removed from the colony. With the help of camel hair brush, the apterous aphid females were transferred carefully to the leaves of potted cowpea plants. The aphids were observed daily for their progeny laid. Adult aphids were removed after their reproductive period and allow young one to feed on host plants. After 6-7 days of multiplication of aphid, the new born apterous female aphids were transferred to new cowpea plants, which were considered as F1 generation. The aphid population from three different locations were multiplied separately in different cowpea plants and kept in different net cages. F2 generation reared under laboratory conditions were used for further bioassay studies. The aphid colonies collected from three locations were maintained throughout the period of experiment.

3.2.4 Study on the Toxicity of Insecticides to *A. craccivora* collected from Different Locations

The bioassay was conducted as per the procedure explained by Reddy *et al.* (2014).

Design - CRD

Treatments - 22 (Three insecticides, each at seven levels + water spray as control)

Replications – 3

The treatments with dose were given below.

T_1- Quinalphos 0.02 %	T_8- Fenvalerate 0.0013 %	T_{15}- Imidacloprid 0.002 %
T_2 - Quinalphos 0.03 %	T_9- Fenvalerate 0.0025 %	T_{16}- Imidacloprid 0.003 %
T_3 -Quinalphos 0.04 %	T_{10}- Fenvalerate 0.005 %	T_{17}- Imidacloprid 0.004 %
T_4- Quinalphos 0.05 %	T_{11}- Fenvalerate 0.01 %	T_{18}- Imidacloprid 0.005 %
T_5- Quinalphos 0.06 %	T_{12}- Fenvalerate 0.02 %	T_{19}- Imidacloprid 0.006 %
T_6- Quinalphos 0.07 %	T_{13}- Fenvalerate 0.03 %	T_{20}- Imidacloprid 0.007 %
T_7- Quinalphos 0.08 %	T_{14}- Fenvalerate 0.04 %	T_{21}- Imidacloprid 0.008 %

T_{22}- Control (water spray)

a. Maintainance of field populations of *A. craccivora*

b.

c.

b & c Cowpea seedlings with *A. craccivora*

Plate 2. Mass rearing of *A. craccivora*

A series of concentrations of each commercial insecticide were prepared in aqueous solution and these insecticides were sprayed on two week old cowpea plants grown in ice cream cups. Allowed the plants to dry for 30 minutes. Twenty apterous aphids (F_2 generation) each from three locations were released into cowpea plants which was considered as one replication. Control plants were sprayed with water. Mortality was noted at 0.5, 0.75, 1, 2, 3, 6, 12 and 24 hours after treatment. Aphids failing to exhibit coordinated forward movement when probed with a soft camel hair brush were considered as dead. Percentage mortality was calculated by using Abbott formula (Abbot, 1925).

Corrected mortality =

$$\frac{\text{Observed mortality in treatment} - \text{observed mortality in control}}{100 - \text{Observed mortality in control}} \times 100$$

Toxicity values (LC_{50}) and (LC_{90}) were calculated by using probit analysis (Finney, 1971). The data were exposed to statistical analysis and the corresponding LC_{50} and LC_{90} values for every individual interval was attained by using the logarithmic model.

Mortality percentage = $a \times x^b$

$$LC_{50} = \exp \frac{(\log_{50} - a)}{b} \qquad LC_{90} = \exp \frac{(\log_{90} - a)}{b}$$

x = concentration of insecticide

a = intercept

b = regression coefficient

fiducial limits were estimated by using

$b \pm t[SE(b)]$

Resistance ratio = $\frac{LC_{50} \text{ or } LC_{90} \text{ of resistance population}}{LC_{50} \text{ or } LC_{90} \text{ of susceptible population}}$

The population of *A. craccivora* found to be resistant were taken for further study.

3.3 EVALUATION OF EFFICACY OF NEW GENERATION INSECTICIDES AGAINST THE RESISTANT POPULATION OF *A. craccivora* IN LABORATORY CONDITION

A. craccivora population found resistant to three insecticides collected from location- II and III were utilized for the evaluation of efficacy of new generation insecticides. Six insecticides were tested under laboratory conditions at recommended doses to find out the comparative efficacy against the resistant population of *A. craccivora* .The details of these insecticides are given in Table 9.

Design: CRD

Treatments: 7

Replications: 3

The laboratory evaluation was done based on the procedure explained as in experiment 3.3.

3.4 FIELD EVALUATION OF SELECTED NEW GENERATION INSECTICIDES AGAINST RESISTANT POPULATION OF *A. craccivora*

The effective three insecticides found out from expt. 3.3 were further tested in field for their efficacy in controlling the resistant population of *A.craccivora.*

Design : RBD

Treatments: 4

Replications: 5

Table 9. Details of new generation insecticides used for the management of resistant population of *A.craccivora.*

Treatment. No	Details of insecticides					
	Chemical name	Trade name	Chemical group	Mode of action as per IRAC, 2018	Dosage (g a.i,ha^{-1})	Field dose (mL / g L^{-1})
T_1	Acetamiprid 20 % SP	Rapid	Neonicotinoids	Nicotinic acetylcholine receptor (nAChR) competitive modulators	15	0.10
T_2	Thiamethoxam 25 % WG	Actara	Neonicotinoids	Nicotinic acetylcholine receptor (nAChR) competitive modulators	25	0.30
T_3	Thiacloprid 21.7 % SC	Splendour	Neonicotinoids	Nicotinic acetylcholine receptor (nAChR) competitive modulators	24	0.25
T_4	Dinotefuran 20% SG	Token	Neonicotinoids	Nicotinic acetylcholine receptor (nAChR) competitive modulators	25	0.30
T_5	Thiamethoxam 12.6 %+Lambda cyhalothrin 9.5 % ZC	Alika	Synthetic pyrethroids + Neonicotinoids	Sodium channel modulators and Nicotinic acetylcholine receptor (nAChR) competitive modulators	27.5	0.30
T_6	Dimethoate 30% EC	Rogorin	Organophosphates	Acetylcholinesterase (AChE) inhibitors	200	1.50
T_7	Control (water spray)					

22

This experiment was laid out in location - III from where the resistant populations of *A.craccivora* were collected. Details of the treatments are given in Table 10.

Table 10 – Details of the treatments for field evaluation.

Treatment No.	Chemical name	Trade name	Dosage (g a.i,ha^{-1})	Field dose (mL or g L^{-1})
T_1	Thiacloprid 21.7 % SC	Splendour	24	0.25
T_2	Thiamethoxam 12.6 % + Lambda cyhalothrin 9.5 % ZC	Alika	25	0.30
T_3	Thiamethoxam 25 % WG	Actara	27.5	0.30
T_4	Control			

3.4.1 Percent Reduction in *A. craccivora* Population

The number of aphids from each plants were assessed from 30 cm of the terminal twig with the unopened leaves and two opened leaves and the mean number was recorded (Thamilarasi, 2016). Percentage reduction in aphid population was calculated using the formula

Per cent reduction=

$$\frac{\text{Population in pre count} - \text{Population in treatment X 100}}{\text{Population in pre count}}$$

3.4.2 Estimation of *A. craccivora* Population by Scoring Method

Aphid population was assessed by scoring method as described by Banks (1954) and Rani (2001) as detailed below.

0	Zero (0)	No aphids.
1	Very light (V)	From one aphid to a small colony, confined to the very youngest leaves of the crown
2	Light (L)	Several aphid colonies are present on the stem and not confined to the uppermost leaves
3	Medium (M)	Aphids present in large numbers, not in recognizable colonies but diffuse and infesting a large proportion of leaves and stem.
4	Heavy (H)	Aphids present in large numbers, very dense, infesting all the leaves and stem, the latter usually being black with aphids

Ten plants were selected randomly in each plot and scored for aphid infestation visually following the standard scoring procedure mentioned in above.

3.5. ESTIMATION OF RESIDUES OF INSECTICIDES IN COWPEA PODS

Mature cowpea pods were taken from previous experimental plots sprayed with thiacloprid @ 24 g a.i.ha^{-1}, lambda cyhalothrin + thiamethoxam @ 27.5 g a.i.ha^{-1} and thiamethoxam @ 25 g a.i.ha^{-1} (expt.3.4) and pods were sampled 0, 1, 3, 5, 7, 10, 15 and 20 days after spraying. The determination of pesticide residues was done in the Pesticide Residue Research and Analytical laboratory, AINP on Pesticide Residues, College of Agriculture, Vellayani.

3.5.1 Validation of Method for Pesticide Residue Analysis

3.5.1.1 *Chemicals and reagents*

Certified reference material of thiacloprid (99.9 % purity), lambda cyhalothrin (98.7 % purity) and thiamethoxam (99.3 % purity) was purchased from Sigma-Aldrich Pvt. Ltd. Acetonitrile, water, methanol (HPLC grade), sodium chloride, anhydrous sodium sulphate and magnesium sulphate were supplied from Merck, Germany. Primary Secondary Amine (PSA) was procured from Agilent technologies, USA. Sodium chloride, anhydrous sodium sulphate and magnesium sulphate were activated in a muffle furnace at a temperature of 350 °C for 4 hr. and kept in desiccators. Commercial formulation of thiacloprid (Splendour 21.7 % SC), lambda cyhalothrin + thiamethoxam (Alika 9.5 + 12.6 % ZC) and thiamethoxam (Actara 25 % WG) were purchased from local market.

3.5.1.2 *Preparation of standards*

Standard stock solution of thiacloprid and thiamethoxam were prepared in methanol and lambda cyhalothrin was prepared in n- hexane. Calibration curve was made by injecting the standards prepared from different concentrations (0.01, 0.05, 0.10, 0.50 and 1.00 μg mL^{-1}) of standard solutions from stock solution by serial dilution. All standard solutions were stored at -20 °C before and after use.

3.5.1.3 *Recovery experiments*

The analytical method for residue estimation was validated for linearity, limit of detection (LOD), limit of quantification (LOQ), recovery and precision. Recovery studies were conducted by spiking different concentrations (0.05, 0.25 and 0.50 mg kg^{-1}) of analytical standards of thiacloprid, lambda cyhalothrin and thiamethoxam in untreated cowpea pods. Five replicates were analyzed at each spiking level and accuracy of analytical methods was determined based on repeatability and relative standard deviation which is mandatory for residue validation.

3.5.2 Estimation of Persistence and Degradation of Residues of Insecticides

Studies on the dissipation of pesticide was conducted in Vallamcode area of Thiruvananthapuram district. Thiacloprid @ 24 g a.i.ha^{-1}, lambda cyhalothrin + thiamethoxam @ 27.5 g a.i.ha^{-1} and thiamethoxam @ 25 g a.i.ha^{-1} were sprayed on cowpea pods by using a knap sack sprayer. Samples were collected on 0,1,3,5,7,10, 15 and 20 days after spraying or until residues reaches below detectable level. 2 kg of cowpea pods were chopped, homogenized, sub-sampled and extracted following the QuEChERS (Quick, Easy, Cheap, Effective, Rugged, Safe) method. The estimation of residues of thiacloprid and thiamethoxam was done using LC-MS/MS and the estimation of lambda cyhalothrin was done using GC-ECD.

3.5.2.1 ***Extraction and clean up***

QuEChERS method was adopted for residue extraction and clean up in cowpea. A well homogenized cowpea sample of 25 g was taken into 250 mL centrifuge bottle. The analyte was extracted by the addition of 50 mL acetonitrile of HPLC grade. The centrifuge bottles were closed tightly and homogenized with a high speed tissue homogenizer (Heidolph Silent Crusher-M) at 140000 rpm for 3 min, to which 10 g of activated sodium chloride was added and vortexed for 2 min to achieve good separation of acetonitrile layer. The homogenized mixture was centrifuged at 2500 rpm for 5 min. The extract of 12 mL was carefully transferred to a 50 mL centrifuge tube containing 6 g pre activated sodium sulphate. Vortexed for 2 min and the extracts were cleaned up by dispersive solid phase extraction (DSPE). 8 mL of supernatant was transferred to 15 mL centrifuge tube containing 0.20 g PSA and 1.20 g magnesium sulphate and vortexed for 2 min. The vortexed mixture was centrifuged at 2500 rpm for 5 min. 5 mL of supernatant liquid was transferred to turbo tube and evaporated to dryness under a gentle steam of nitrogen using a turbovap set at 40 °C and 7.5 psi nitrogen flow. The residues were reconstituted in 2 mL of methanol and filtered through a 0.2 micron PVDF filter prior to estimation in LC-MS/MS. A 4 ml of the extract was evaporated in a turbovap and made up to 1 ml using hexane for GC-ECD analysis.

3.5.2.2 ***Instrumentation***

3.5.2.2.1 ***LC-MS/MS***

The chromatographic separation was achieved using Waters Acquity UPLC system equipped with a reversed phase Atlantis d C-18 (100 ×2.1 mm, 5 µm particle size) column. A gradient system involving the following two eluent components: (A) 10 % methanol in water + 0.1 % formic acid + 5 mM ammonium acetate; (B) 10 % water in methanol + 0.1 % formic acid + 5 mM ammonium acetate was used as mobile phase for the separation of residues. The gradient elution was done as follows: 0 min isocratic 20 % B, increased to 90 % in 4 min, then raised to 95 % with 5 min and increased to 100 % B in 9 min, decreased to the initial composition of 20 % B in 10 min and hold to 12 min for re-equilibration. The flow rate remains constant at 0.8 mL min^{-1} and injection volume was 10 µL. The column temperature was maintained at 40 °C. The effluent from the LC system was introduced into triple quadrupole API 3200 MS/MS system equipped with an electrospray ionization interface (ESI), operating in the positive ion mode. The source parameters were temperature 600 °C, ion gas (GSI) 50 psi, ion gas (GS2) 60 psi, ion spray voltage 5,500 V, curtain gas 13 psi. Settings for retention of time, declustering potential, entrance potential, collision cell entrance potential, collision energy and collision cell exit potential of corresponding insecticides were mentioned in the Table 11.

3.5.2.2.2 *GC-ECD*

Estimation of residues of lambda cyhalothrin was performed using Gas Chromatograph (Shimadzu 2010 AT) equipped with Electron Capture Detector (ECD). Operating conditions of GC are, Column, DB- 5 capillary (0.25µm film thickness X 0.25 mm X 30 m), carrier gas- Nitrogen, column flow- 0.79 mL/min., injector temperature -250 ° C and detector temperature used was 300 ° C. The retention time of lambda cyhalothrin under the above conditions was 54.259 min. The residues of were lambda cyhalothrin confirmed in GC-MS (Shimadzu GC- MS QP 2010 Plus) with retention time of 50.25 min. Helium was used as carrier gas in GC-MS operated with Electron Impact Ionization (70eV). In GC-MS, injector temperature, column, column flow were similar to that of GC.

Table 11. Retension time (RT) and Multiple Reaction Monitoring (MRM) transitions of the insecticides in LC-MS/MS

Name of the compound	RT (min.)	MRM transitions		Declustering potential	Entrance potential	Collision Cell Entrance Potential	Collision Energy	Collision Cell Exit Potential
		Quantitative ion pair	Qualitative ion pair					
Thiacloprid	1.7	253→126	253→ 99	43	6	17	30	1
				43	6	17	61	1
Thiamethoxam	0.83	292→211	292→181	31	7	19	19	2
				31	7	19	32	2

The MS/MS conditions were optimized using direct infusion in to ESI source in positive mode to provide highest signal/noise ratio for the quantification ion of each analyte. Two MS/MS transitions were made in case of chemical interferences observed in the quantitation ion chromatogram and for qualitative purpose. The ion source temperature was 550 ° C with ion spray voltage of 5500 V. Chromatographic elution zones were divided into appropriate number of time segments. In each segment corresponding MS/MS transitions were monitored using multiple reactions – monitoring (MRM) mode.

3.5.2.3 *Residue quantification*

Based on the peak area of the chromatogram obtained for various insecticides, the quantity of residue was determined as detailed below.

Pesticide residue ($\mu g\ g^{-1}$) = Concentration obtained from chromatogram by using calibration curve × Dilution factor

$$\text{Dilution factor} = \frac{\text{Volume of the solvent added} \times \text{Final volume of extract}}{\text{Weight of sample} \times \text{Volume of extract taken for concentration}}$$

The persistence of insecticides is generally expressed in terms of half-life (DT_{50}) i.e., time for disappearance of pesticide to 50 per cent of its initial concentration, which was one of the safety parameter used in pesticide degradation studies.

3.5.3. Estimation of Harvest Time Residues

Cowpea pods, treated with insecticides were collected at the time of harvest for the determination of terminal residue by LCMS / MS and GC-ECD techniques as per the Pesticide Residue Analysis Manual of ICAR (Sharma, 2013).

3.5.4 Risk Assessment of Different Insecticides in Cowpea

Dietary risk assessment of thiacloprid 24 g a.i.ha^{-1}, lambda cyhalothrin + thiamethoxam 27.5 g a.i.ha^{-1} and thiamethoxam 25 g a.i.ha^{-1} were estimated through Acceptable Daily Intake (ADI), Maximum Permissible Intake (MPI) and Theoretical Maximum Residue Concentration (TMRC). Daily consumption value of cowpea was considered as 90 g d^{-1} (Huan *et al.,* 2016). MPI was calculated based on Acceptable Daily Intake for each pesticide, which was fixed from WHO and average body weight of an adult human being which was considered as 55 Kg (Bhattacharyya *et al*., 2017). Based on the residue values TMRC has been calculated and assessment of health impact of insecticide residues on cowpea were made. If TMRC is less than MPI, the particular insecticide will not cause any health impact.

TMRC= Maximum residue level obtained at recommended dose on 0th day of application X total intake of food per day

MPI= Acceptable daily intake X average body weight (55) Kg of an adult

Results

4. RESULTS

4.1. PRELIMINARY SURVEY TO GATHER THE INFORMATION ON THE PESTICIDE USE AND THE DEVELOPMENT OF INSECTICIDE RESISTANCE

4.1.1. Details of Pests, Effectiveness of Pesticides and Awareness of Insecticide Resistance

Results of the survey on details of pests, effectiveness of pesticides and awareness of insecticide resistance are represented in the Table 12. Among the surveyed farmers, 60 per cent of farmers opined that pod borers were the major pests while 40 per cent responded aphids as the major pests of cowpea. No one responded bugs as major pests of cowpea. In case of insecticide use, flubendiamide (92 %), imidacloprid (80 %) and fenvalerate (80 %) were the most used insecticides followed by chlorpyriphos (68 %), quinalphos (60 %), Lambda- cyhalothrin (48 %) ethion+ cypermethrin (40 %), flonicamid (40 %), fipronil (32 %) and thiamethoxam (28 %). Sixty per cent of the farmers reported the control failures.

According to 60 per cent of farmers, pod borers were the most difficult pest to control whereas 40 per cent of the farmers reported that aphids were difficult to control. The present survey revealed that insectcides *viz.* lambda- cyhalothrin and fenvalerate having the highest control failures (40 % each), followed by imidacloprid (12 %) and quinalphos (8 %). However, only 20 per cent of the farmers are aware about insecticide resistance. No farmers collecting information on insecticide resistance from training classes and media, whereas 20 per cent farmers collected information from other farmers.

Table 12. Details of pests, effectiveness of pesticides and awareness of insecticide resistance among cowpea farmers in Kalliyoor area of Thiruvananthapuram district during 2016-17.

Particulars		Farmers responded (%)
Major pests attacking cowpea	a) Pod borers	60
	b) Bugs	0
	c) Aphids	40
Insecticides widely used against cowpea pests	Imidacloprid	80
	Fenvalerate	80
	Quinalphos	60
	Thiamethoxam	28
	Fipronil	32
	Flonicamid	40
	Ethion + Cypermethrin	40
	Flubendiamide	92
	Lambda - cyhalothrin	48
	Chlorpyriphos	68
Control failures reported by the continuous application	Yes	60
	No	40
Pests difficult to control	Pod borers	60
	Bugs	0
	Aphids	40
Which insecticide against control failures obtained	Fenvalerate	40
	Lambda- cyhalothrin	40
	Imidacloprid	12
	Quinalphos	8
Awareness regarding insecticide resistance	Aware	20
	Unaware	80
Source of information on insecticide resistance	Training classes	0
	Media	0
	Other farmers	20

4.1.2. Information on Pesticide Use Pattern

Information on pesticide use pattern is given in the Table 13. The survey revealed that 84 per cent of farmers collected technical information on pesticide from technical officers and 16 per cent of farmers collected technical information from company representatives. No farmers were collecting information from progressive farmers or through own decision and from media. Majority of farmers (80 %) purchased insecticides from the Vegetable and Fruit Promotion Council Keralam (VFPCK), while only 20 per cent of farmers were purchased insecticides from pesticides shops. Majority of farmers (88 %) are getting information on dose of pesticides from Vegetable and Fruit Promotion Council Keralam (VFPCK) and remaining 12 per cent of farmers getting information from pesticide shops.

Twenty eight per cent of farmers were spraying insecticides as prophylactic while 72 per cent sprayed insecticides only after the pest occurrence only. Among surveyed farmers only 28 per cent of farmers were doing manual mixing of insecticides, remaining 72 per cent of farmers were not following manual mixing of insecticides. Only 20 per cent of farmers are adopting Integrated Pest management strategies and not a single farmer in surveyed area is practicing any biological control measures.

4.2. ASSESSMENT OF INSECTICIDE RESISTANCE IN FIELD POPULATIONS OF *A. craccivora* ON VEGETABLE COWPEA

4.2.1. Biology of *A.craccivora*

The results of the study on biological parameters of aphid, *A. craccivora* are shown in Table 14 (Plate 3). The nymphal and reproductive periods of *A. craccivora* were 5.40 days and 6.20 days respectively. Total life cycle was 12.30 days. Fecundity and rate of reproduction (Nymphs/day/aphid) of aphid in cowpea were 52.10 and 8.24 nymphs respectively.

Table 13. Information on pesticide use pattern among cowpea farmers in Kalliyoor area of Thiruvananthapuram district during 2016-17.

Particulars		Farmers responded (%)
Source of technical information on pesticide	Technical officers	84
	Company representatives	16
	Other progressive farmers	0
	Own decisions	0
	Media	0
Source of insecticides	Pesticide shops	20
	VFPCK	80
	Directly from companies	0
Source of information on dose of pesticides	Technical officers	0
	Pesticide shops	12
	VFPCK	88
	Other progressive farmers	0
	Own decisions	0
	Media	0
Prophylactic use of insecticides	Yes	28
	No	72
Manual mixing of insecticides	Yes	28
	No	72
Application of IPM strategies	Yes	20
	No	80
Practicing any biological control measures	Yes	0
	No	100

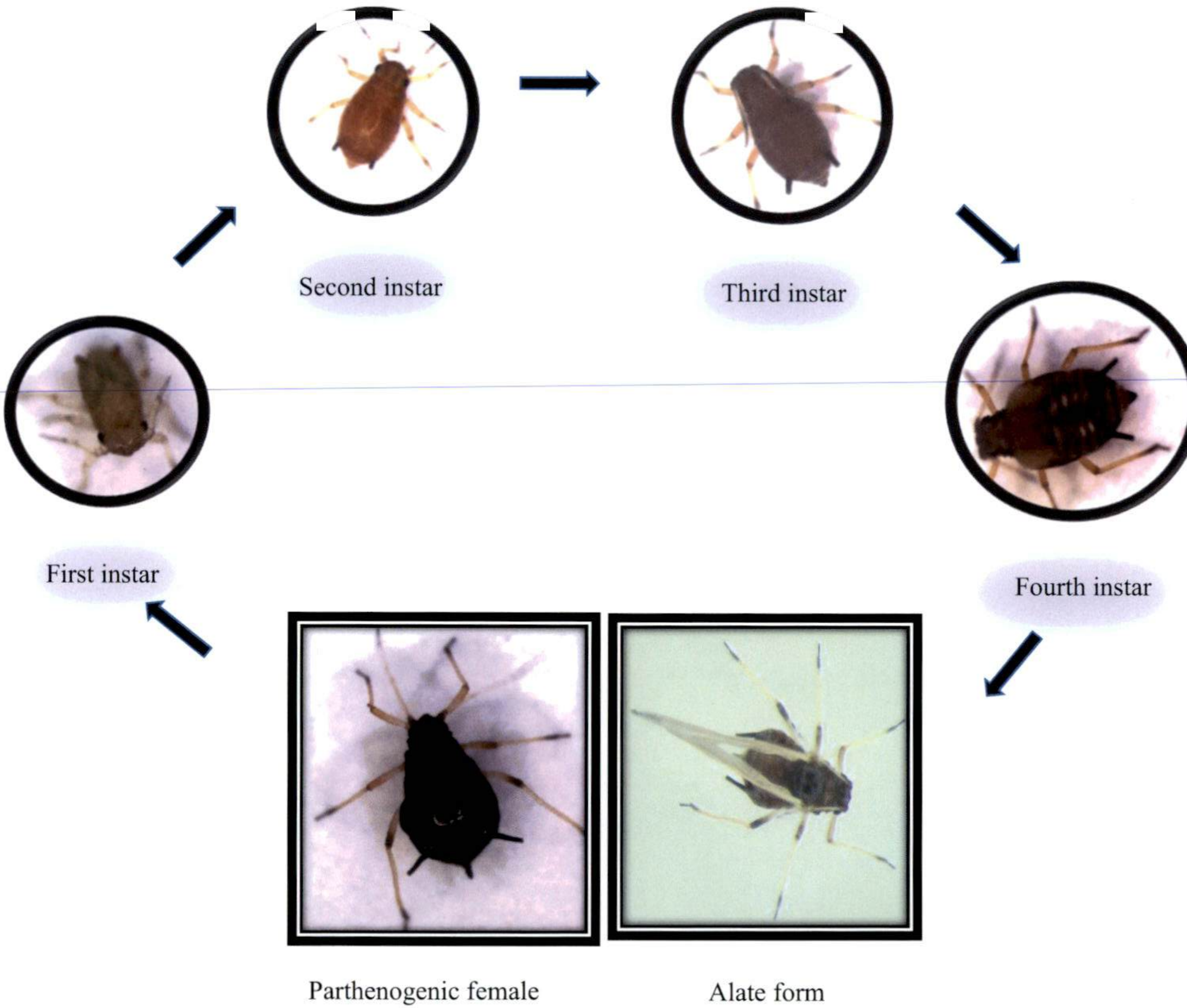


Plate 3. Life stages of *A. craccivora*

Table 14. Biological parameters of aphid, *A. craccivora* on cowpea

Sl. No	Biological parameters	*Duration (Days ± SE) /nymphs
1	Nymphal period	5.40 ± 0.22
2	Reproductive period	6.20 ± 0.13
3	Total life cycle	12.30 ± 0.15
4	Fecundity / aphid	52.10 ± 0.71
5	Rate of reproduction (Nymphs/day/aphid)	8.24 ± 0.32

SE- Standard Error; *Mean of 10 replications

4.2.2 Assessment of insecticide Resistance

4.2.2.1 *Quinalphos*

The results on the toxicity of quinalphos to the population of *A. craccivora,* collected from three different locations are presented in Table 15. LC_{50} values of quinalphos in *A. craccivora* population from location I were 852.21, 710.52, 440.08, 121.50, 11.10 and 1.74 ppm at 0.5, 0.75, 1, 2, 3 and 6 hours after treatment respectively. LC_{50} values of aphid population from location –II were 1071.94, 1010.31, 631.63, 268.66, 116.11 and 2.92 ppm at 0.5, 0.75, 1, 2, 3 and 6 hours after treatment respectively and in location –III LC_{50} values were 1287.05, 1105.11, 1036.11, 384.30, 214.16 and 2.97 ppm at 0.5, 0.75, 1, 2, 3 and 6 hours after treatment respectively. Based on observation, aphid population collected from Vilavoorkal was treated as susceptible strain. Resistance ratio was worked out based on LC_{50} values of aphids collected from Vilavoorkal. Resistance ratio based on LC_{50} of quinalphos to population of *A. craccivora* from location –II shown 1.21, 1.42, 1.44, 2.21, 10.46 and 1.67 fold resistance at 0.5, 0.75, 1, 2, 3 and 6 hours after treatment respectively against the *A. craccivora* population from location I.

Table 15. Toxicity of quinalphos to the populations of *A. craccivora* collected from three different locations

HAT	Location	LC_{50} (ppm)	Fiducial Limit (95% CI)		LC_{90} (ppm)	Fiducial Limit (95% CI)		χ^2	Slope ± SE	Resistance ratio	
			Lower	Upper		Lower	Upper			Based on LC_{50}	Based on LC_{90}
0.5	Vilavoorkal	852.21	497.26	36647.01	2210.06	1191.98	10857.93	0.142	1.20±0.102	1	1
	COA, Vellayani	1071.94	652.88	12727.6	2613.91	1460.98	35756.55	0.069	2.10±0.115	1.26	1.18
	Vallamcode	1287.05	799.33	18868.42	3032.86	1706.97	52347.25	0.213	2.06±0.132	1.51	1.37
0.75	Vilavoorkal	710.52	448.41	3344.02	1866.37	1090.99	10033.47	0.378	2.37±0.101	1	1
	COA, Vellayani	1010.31	619.12	11878.57	2574.29	1439.62	35343.30	0.190	2.10±0.113	1.42	1.38
	Vallamcode	1105.11	730.79	4940.20	2654.14	1590.01	14020.35	0.100	2.36±0.131	1.56	1.42
1	Vilavoorkal	440.08	297.71	1213.06	1496.92	928.61	5080.68	0.676	2.69±0.096	1	1
	COA, Vellayani	631.63	429.87	2042.82	1990.15	1215.73	8087.57	0.622	2.53±0.107	1.44	1.35
	Vallamcode	1036.11	676.49	6670.92	2732.75	1591.36	21612.97	0.054	2.21±0.127	2.35	1.83
2	Vilavoorkal	121.50	18.87	198.85	898.02	631.61	1762.32	1.986	3.66±0.093	1	1
	COA, Vellayani	268.66	166.00	413.99	1293.75	895.70	2754.38	0.977	3.40±0.103	2.21	1.44
	Vallamcode	384.30	294.55	516.92	1327.13	991.71	2253.64	0.361	4.13±0.120	3.16	1.48

3	Vilavoorkal	11.10	4.35	80.89	664.52	492.00	1122.89	3.404	4.22±0.093	1	1
	COA, Vellayani	116.11	28.33	205.78	1058.68	757.19	2036.43	0.623	3.66±0.103	10.46	1.59
	Vallamcode	214.16	17.42	322.89	1359.13	964.78	2789.25	0.090	3.42±0.117	19.29	2.05
6	Vilavoorkal	1.74	0.85	56.77	476.53	378.82	672.60	7.606	5.50±0.093	1	1
	COA, Vellayani	2.92	1.12	79.34	614.44	491.56	869.07	4.118	5.27±0.104	1.67	1.29
	Vallamcode	2.97	1.17	109.74	782.95	623.05	1150.06	5.659	4.72±0.119	1.71	1.64

Resistance ratio= LC of field strain (Resistant or Mode. resistant population)/LC of susceptible population; χ^2 is a measure of heterogeneity,
χ^2 table value at 5 df = 11.07
χ^2 is non-significant at: $p< 0.05$
CI- Confidence interval
HAT- Hours after treatment.

Similarly, resistance ratio based on LC_{50} of quinalphos to *A. craccivora* population from location –III calculated as 1.51, 1.56, 2.35, 3.16, 19.29 and 1.71 fold resistance at 0.5, 0.75, 1, 2, 3 and 6 hours after treatment respectively.

Similarly LC_{90} values of quinalphos against the aphid population from location I were 2210.06, 1866.37, 1496.92, 898.02, 664.52 and 476.53 ppm and in location –II LC_{90} values were 2613.91, 2574.29, 1990.15, 1293.75, 1058.68 and 614.44 ppm after 0.5, 0.75, 1, 2, 3 and 6 hours of treatment respectively. In location –III LC_{90} values were 3032.86, 2654.14, 2732.75, 1327.13, 1359.13 and 782.95 ppm after 0.5, 0.75, 1, 2, 3 and 6 hours of treatment respectively. Resistance ratio of *A. craccivora* from location –II calculated as 1.18, 1.38, 1.35, 1.44, 1.59 and 1.29 fold resistance against the population from location I and resistance ratio of population from location –III as 1.37, 1.42, 1.83, 1.48, 2.05 and 1.64 fold resistance after 0.5, 0.75, 1, 2, 3 and 6 hours of treatment respectively.

4.2.2.2 *Fenvalerate*

Toxicity of fenvalerate to the population of *A. craccivora,* collected from three different locations were presented in Table 16. Population of *A. craccivora* collected from location I and location II, LC_{50} values of fenvalerate were 300.72, 250.87, 196.36, 102.31, 60.76, 22.33, 10.15, 2.32 ppm and 959.30, 898.25, 888.59, 561.04, 272.20, 66.17, 60.40 and 12.60 ppm at 0.5, 0.75, 1, 2, 3, 6, 12 and 24 hours after treatment respectively. LC_{50} values of fenvalerate against aphid collected from location –III were 896.06, 863.60, 819.70, 682.65, 546.82, 434.62, 393.16 and 335.27 ppm at 0.5, 0.75, 1, 2, 3, 6, 12 and 24 hours after treatment respectively. In the *A. craccivora* population taken from location II, resistance ratios were 3.19, 3.58, 4.53, 5.48, 4.48, 2.97, 5.95 and 5.43. Resistance ratios of aphid population from location –III calculated as 2.98, 3.44, 4.17, 6.67, 9.00, 19.46, 38.73 and 144.51 fold resistance against the aphid population from location after 0.5, 0.75, 1, 2, 3, 6, 12 and 24 hours of treatment respectively.

Table 16. Toxicity of fenvalerate to the populations of *A. craccivora* collected from three different locations

HAT	Location	LC_{50} (ppm)	Fiducial Limit (95% CI)		LC_{90} (ppm)	Fiducial Limit (95% CI)		χ^2	Slope ± SE	Resistance ratio	
			Lower	Upper		Lower	Upper			Based on LC_{50}	Based on LC_{90}
0.5	Vilavoorkal	300.72	188.90	1117.38	826.51	490.80	3413.18	1.120	2.54±0.088	1	1
	COA, Vellayani	959.30	617.65	4303.97	2322.23	1376.40	11947.67	0.047	2.39±0.115	3.19	2.81
	Vallamcode	896.06	737.12	1362.57	1975.42	1458.57	3674.91	0.613	3.65±0.180	2.98	2.39
0.75	Vilavoorkal	250.87	162.20	761.40	751.05	458.39	2573.41	2.530	2.70±0.086	1	1
	COA, Vellayani	898.25	587.78	3522.34	2234.49	1342.59	10119.65	0.070	2.46+0.114	3.58	2.96
	Vallamcode	863.60	713.58	1293.84	1948.29	1441.19	3607.88	0.423	3.66±0.178	3.44	2.59
1	Vilavoorkal	196.36	132.41	455.10	645.52	413.31	1713.05	3.115	3.04±0.085	1	1
	COA, Vellayani	888.59	558.14	7043.41	2434.09	1383.91	23063.15	0.191	2.17±0.111	4.53	3.77
	Vallamcode	819.70	675.51	1246.46	1971.33	1439.38	3842.39	0.284	3.50±0.175	4.17	3.05
2	Vilavoorkal	102.31	67.05	172.30	470.12	324.67	935.32	7.194	3.71±0.082	1	1
	COA, Vellayani	561.04	383.47	1760.93	1937.62	1183.44	7972.03	0.118	2.52±0.106	5.48	4.12
	Vallamcode	682.65	584.12	877.22	1645.31	1276.40	2643.16	0.344	4.21±0.173	6.67	3.50

3	Vilavoorkal	60.76	28.12	96.33	378.18	272.79	661.33	5.90	4.22±0.082	1	1
	COA, Vellayani	272.20	139.85	495.0	1525.47	981.88	4589.59	0.26	2.79±0.103	4.48	4.03
	Vallamcode	546.82	469.45 5	639.627	1346.59	1102.42 8	1887.839	0.349	5.07±0.171	9.00	3.56
6	Vilavoorkal	22.33	11.74	48.60	296.94	221.45	476.46	5.258	4.68±0.081	1	1
	COA, Vellayani	66.17	15.57	132.56	683.27	545.80	968.80	1.259	5.33±0.103	2.97	2.30
	Vallamcode	434.62	364.39	493.51	1042.66	904.51	1291.74	0.523	6.49±0.172	19.46	3.51
12	Vilavoorkal	10.15	6.45	32.83	236.05	182.89	345.84	7.416	5.40±0.082	1	1
	COA, Vellayani	60.40	13.87	122.41	622.16	505.60	848.97	0.449	5.74±0.104	5.95	2.64
	Vallamcode	393.16	313.31	453.69	1006.98	873.92	1248.17	0.472	6.39±0.171	38.73	4.27
24	Vilavoorkal	2.32	0.38	26.83	239.13	183.30	359.04	7.157	5.16±0.082	1	1
	COA, Vellayani	12.60	7.11	81.08	562.80	458.12	765.16	2.237	5.69±0.105	5.43	2.35
	Vallamcode	335.27	244.61	397.99	922.19	807.82	1123.62	0.365	6.56±0.172	144.51	3.86

Resistance ratio= LC of field strain (Resistant or Mode. resistant population)/LC of susceptible population; χ^2 is a measure of heterogeneity
χ^2 table value at 5 df = 11.07
χ^2 is non-significant at: $p< 0.05$
CI- Confidence interval
HAT- Hours after treatment.

LC_{90} values of fenvalerate against aphid collected from location I and II were 826.51, 751.05, 645.52, 470.12, 378.18, 296.94, 236.05, 239.13 ppm and 2322.23, 2234.49, 2434.09, 1937.62, 1525.47, 683.27, 622.16, 562.80 ppm at 0.5, 0.75, 1, 2, 3, 6, 12 and 24 hours after treatment respectively. In location –III LC_{90} values of fenvalerate against aphid were 1975.42, 1948.29, 1971.33, 1645.31, 1346.59, 1042.66, 1006.98 and 922.19 ppm respectively. Resistance ratios of aphid population collected from location II were recorded as 2.81, 2.96, 3.77, 4.12, 4.03, 2.30, 2.64 and 2.35 at respective time intervals. Whereas, ratios were recorded as 2.39, 2.59, 3.05, 3.50, 3.56, 3.51, 4.27 and 3.86 for location –III after 0.5, 0.75, 1, 2, 3, 6, 12 and 24 hours of treatment respectively.

4.2.2.3 *Imidacloprid*

Toxicity of imidacloprid to the population of *A. craccivora,* collected from three different locations were depicted in Table 17. LC_{50} values of imidacloprid against aphid collected from location I and II were 114.23, 88.75, 56.21, 18.54, 2.56, 0.54 ppm and 127.54, 118.25, 75.42, 35.55, 18.63, 1.52 ppm at 0.5, 0.75, 1, 2, 3 and 6 hours after treatment respectively. Similarly, imidacloprid recorded LC_{50} against aphid population from location–III were 128.71, 110.51, 103.61, 38.43, 21.42 and 4.28 ppm respectively. Resistance ratios of location II were 1.10, 1.33, 1.34, 1.92, 7.28 and 2.81. Whereas resistance ratios for population from location–III were 1.13, 1.25, 1.84, 2.07, 8.35 and 7.92 after 0.5, 0.75, 1, 2, 3 and 6 hours of treatment respectively.

Likewise imidacloprid showed LC_{90} against aphid population from location I recorded LC_{90} values were 272.82, 226.62, 181.23, 109.59, 82.19 and 59.93 ppm and in location II LC_{90} values were 305.85, 292.55, 224.64, 148.29, 123.44 and 73.80 ppm after 0.5, 0.75, 1, 2, 3 and 6 hours of treatment respectively. In location–III LC_{90} values were 303.29, 265.41, 273.28, 132.71, 135.91 and 77.74 ppm respectively. Resistance ratios of aphid population collected from location II were

Table 17. Toxicity of imidacloprid to the populations of *A. craccivora* collected from three different locations

HAT	Location	LC_{50} (ppm)	Fiducial Limit (95% CI)		LC_{90} (ppm)	Fiducial Limit (95% CI)		χ^2	Slope ± SE	Resistance ratio	
			Lower	Upper		Lower	Upper			Based on LC_{50}	Based on LC_{90}
0.5	Vilavoorkal	114.23	74.58	592.01	272.82	161.40	1669.03	0.069	2.30±0.131	1	1
	COA, Vellayani	127.54	78.88	2604.56	305.85	170.90	7402.91	0.113	2.04±0.131	1.10	1.12
	Vallamcode	128.71	79.93	1886.80	303.29	170.70	5234.61	0.213	2.07±0.132	1.13	1.11
0.75	Vilavoorkal	88.75	57.67	382.54	226.62	134.74	1137.63	0.222	2.40±0.113	1	1
	COA, Vellayani	118.25	74.75	1254.89	292.55	166.39	3719.80	0.137	2.11±0.130	1.33	1.29
	Vallamcode	110.51	73.08	494.02	265.41	159.00	1402.03	0.100	2.36±0.131	1.25	1.17
1	Vilavoorkal	56.21	39.509	140.48	181.23	114.91	570.87	0.312	2.76±0.107	1	1
	COA, Vellayani	75.42	53.43	217.67	224.64	140.54	838.31	0.346	3.48±0.123	1.34	1.24
	Vallamcode	103.61	67.65	667.09	273.28	159.14	2161.29	0.054	2.21±0.127	1.84	1.51
2	Vilavoorkal	18.54	6.82	27.57	109.59	79.09	203.09	0.915	3.81±0.103	1	1
	COA, Vellayani	35.55	24.27	51.18	148.29	105.10	300.87	0.441	3.48±0.118	1.92	1.35
	Vallamcode	38.43	29.46	51.69	132.71	99.17	225.36	0.361	4.13±0.120	2.07	1.21

3	Vilavoorkal	2.56	0.98	11.51	82.19	61.91	135.74	3.547	4.23±0.103	1	1
	COA, Vellayani	18.63	6.31	28.60	123.44	90.22	228.77	0.227	3.71±0.117	7.28	1.50
	Vallamcode	21.42	1.74	32.29	135.91	96.48	278.92	0.090	3.42±0.117	8.37	1.65
6	Vilavoorkal	0.54	0.086	7.96	59.93	48.23	83.58	7.204	5.41±0.104	1	1
	COA, Vellayani	1.52	0.81	11.32	73.80	59.68	103.99	5.004	5.05±0.119	2.81	1.24
	Vallamcode	4.28	1.83	13.62	77.74	62.62	110.53	2.249	5.02±0.119	7.92	1.30

Resistance ratio= LC of field strain (Resistant or Mode. resistant population)/LC of susceptible population; χ^2 is a measure of heterogeneity,
χ^2 table value at 5 df = 11.07
χ^2 is non-significant at: $p < 0.05$
CI- Confidence interval
HAT- Hours after treatment.

1.12, 1.29, 1.24, 1.35, 1.50 and 1.24. Whereas the resistance ratios for aphid collected from location–III were 1.11, 1.17, 1.51, 1.21, 1.65 and 1.30 after 0.5, 0.75, 1, 2, 3 and 6 hours of treatment respectively..

4.3 EVALUATION OF EFFICACY OF NEW GENERATION INSECTICIDES AGAINST THE RESISTANT POPULATION OF *A. craccivora* IN LABORATORY CONDITION

4.3.1 Mortality of *A. craccivora* Collected from Location II

The data on the per cent mortality of *A.craccivora* collected from location II treated new generation insecticides are presented in Table 18. The highest mortality (45 %) was observed in insects treated with lambda cyhalothrin + thiamethoxam 27.5 g a.i.ha^{-1} and was found to be significantly higher than the rest of the treatments after 0.5 hour of treatment, followed by thiamethoxam 25 g a.i.ha^{-1} (38.33 %). Acetamiprid 15 g a.i.ha^{-1} and dimethoate 200 g a.i.ha^{-1} (check) recorded 36.67 per cent mortality and was on par with the thiamethoxam 25 g a.i.ha^{-1}. Dinotefuran 25 g a.i.ha^{-1} recorded 31.67 per cent followed by thiacloprid 24 g a.i.ha^{-1} (16.67 %) and these were significantly different from other treatments. All treatments were found to be better when compared to control which recorded no mortality after 0.5 hour of treatment.

Lambda cyhalothrin + thiamethoxam 27.5 g a.i.ha^{-1} treated aphids showed 63.33 per cent mortality after 0.75 hour of treatment. Acetamiprid 15 g a.i.ha^{-1} recorded 53.33 per cent mortality and which was on par with thiamethoxam 25 g a.i.ha^{-1} (51.66 %) and similarly dinotefuran 25 g a.i.ha^{-1} (41.67 %) was on par with dimethoate (check) 200 g a.i.ha^{-1} (40 %). Thiacloprid 24 g a.i.ha^{-1} recorded 31.67 per cent mortality and was significantly different from other treatments and lesser than rest of treatments except water spray (control) which recorded no mortality after 0.75 hour of treatment.

Table 18. Mortality of *A. craccivora* collected from location II (moderate resistant population) treated with new generation insecticides

Treatment	Dosage (g a.i. ha^{-1})	Mortality (%)* HAT				
		0.5	0.75	1	2	3
Acetamiprid 20 % SP	15	36.67 (37.26)	53.33 (46.91)	76.67 (61.15)	100 (89.72)	100 (89.72)
Thiamethoxam 25 % WG	25	38.33 (38.25)	51.67 (45.96)	61.67 (51.75)	88.33 (70.12)	100 (89.72)
Thiacloprid 21.7 % SC	24	16.67 (24.04)	31.67 (34.23)	31.67 (34.23)	63.33 (52.74)	78.33 (62.29)
Dinotefuran 20% SG	25	31.67 (32.23)	41.67 (40.20)	46.67 (43.09)	61.67 (51.75)	76.67 (61.15)
Lambda cyhalothrin 9.5 % + Thiamethoxam 12.6 % ZC	27.5	45 (42.13)	63.33 (52.74)	80 (63.54)	100 (89.72)	100 (89.72)
Dimethoate 30% EC (check)	200	36.67 (37.26)	40 (39.21)	40 (39.21)	100 (89.72)	100 (89.72)
Water spray (control)		0 (0.284)	0 (0.284)	0 (0.284)	0 (0.284)	0 (0.284)
CD at 5%		3.303	3.169	3.871	2.308	1.855

Figures in parenthesis are angular transformed values; HAT – Hours after treatment

*Mean of 3 replications

After one hour of treatment lambda cyhalothrin + thiamethoxam 27.5 g a.i.ha^{-1} recorded 80 per cent mortality and was on par with acetamiprid 15 g a.i.ha^{-1} (76.67 %). Thiamethoxam 25 g a.i.ha^{-1} recorded 61.67 per cent mortality followed by dinotefuran 25 g a.i.ha^{-1} (46.67 %) and dimethoate (check) 200 g a.i.ha^{-1} (40 %). Thiacloprid 24 g a.i.ha^{-1} recorded the lowest mortality (31.67 %) which was significantly different from other treatments and superior to control.

Lambda cyhalothrin + thiamethoxam 27.5 g a.i.ha^{-1}, acetamiprid 15 g a.i.ha^{-1} and dimethoate (check) 200 g a.i.ha^{-1} treated insects recorded cent per cent mortality after 2 hours of treatment followed by thiamethoxam 25 g a.i.ha^{-1} (88.33 %). Thiacloprid 24 g a.i.ha^{-1} showed 63.33 per cent mortality and which was on par with dinotefuran 25 g a.i.ha^{-1} (61.67 %) and water spray recorded no mortality.

After 3 hours of treatment, lambda cyhalothrin + thiamethoxam 27.5 g a.i.ha^{-1}, acetamiprid 15 g a.i.ha^{-1}, thiamethoxam 25 g a.i.ha^{-1} and dimethoate (check) 200 g a.i.ha^{-1} treated aphids recorded cent per cent mortality. Thiacloprid 24 g a.i.ha^{-1} recorded 78.33 per cent mortality and was on par with dinotefuran 25 g a.i.ha^{-1} (76.67 %) and all treatments were superior to water spray which had no mortality.

4.3.2 Mortality of Resistant Population of *A.craccivora* Collected from Location III

Results associated with per cent mortality of *A.craccivora* population collected from location III against new generation insecticides are presented in Table 19. After 0.5 hour of treatment significant highest mortality (28.33 %) was recorded in lambda cyhalothrin + thiamethoxam 27.5 g a.i.ha^{-1} , acetamiprid 15 g a.i.ha^{-1} and thiacloprid 24 g a.i.ha^{-1} treated aphids followed by thiamethoxam 25 g a.i.ha^{-1} (25 %) which was on par with dimethoate (check) 200 g a.i.ha^{-1} (23.33 %).

Table 19. Mortality of *A. craccivora* from location III (Resistant population) treated with new generation insecticides

Treatment	Mortality (%)* HAT				
	0.5	0.75	1	2	3
Acetamiprid 20 % SP @ 15 g a.i. ha^{-1}	28.33 (32.15)	38.33 (38.25)	53.33 (46.91)	71.67 (57.85)	88.67 (68.66)
Thiamethoxam 25 % WG @ 25 g a.i. ha^{-1}	25 (29.92)	31.67 (34.23)	41.67 (40.20)	70 (56.83)	90 (71.96)
Thiacloprid 21.7 % SC @ 24 g a.i. ha^{-1}	28.33 (32.15)	43.33 (41.16)	55 (47.87)	71.67 (57.85)	91.67 (73.40)
Dinotefuran 20% SG @ 25 g a.i. ha^{-1}	15 (22.59)	26.67 (31.07)	36.67 (37.26)	76.67 (61.15)	88.33 (70.12)
Lambda cyhalothrin 9.5 % + Thiamethoxam 12.6 % ZC @ 27.5 g a.i. ha^{-1}	28.33 (32.15)	43.33 (41.16)	63.33 (52.74)	91.67 (73.40)	100 (89.72)
Dimethoate 30% EC @ 200 g a.i. ha^{-1} (check)	23.33 (28.85)	28.33 (32.15)	38.33 (38.25)	73.33 (58.93)	88.33 (70.12)
Water spray (control)	0 (0.28)	0 (0.28)	0 (0.28)	0 (0.28)	0 (0.28)
CD at 5%	4.288	2.846	3.149	3.878	4.845

Figures in parenthesis are angular transformed values; HAT – Hours after treatment

*Mean of 3 replications

The lowest mortality was recorded in dinotefuran 25 g a.i.ha^{-1} (15 %), which was significantly different from other treatments and superior to control.

Lambda cyhalothrin + thiamethoxam 27.5 g a.i.ha^{-1} and thiacloprid 21.7 % SC 24 g a.i.ha^{-1} treated aphids showed significantly higher mortality (43.33 per cent) after 0.75 hour of treatment, followed by acetamiprid 15 g a.i.ha^{-1} (38.33 %). Thiamethoxam 25 g a.i.ha^{-1} recorded 31.67 per cent mortality and was on par with dimethoate (check) 200 g a.i.ha^{-1} (28.33 %), which was on par with dinotefuran 25 g a.i.ha^{-1} (26.67 %).

After 1 hour of treatment the highest mortality was observed in lambda cyhalothrin + thiamethoxam 27.5 g a.i.ha^{-1} treated insects with 63.33 per cent and was found to be significantly better than the rest of the treatments followed by thiacloprid 24 g a.i.ha^{-1} (55 %) which was on par with acetamiprid 15 g a.i.ha^{-1} (53.33 %). Thiamethoxam 25 g a.i.ha^{-1} showed 41.67 per cent mortality which was on par with dimethoate (check) 200 g a.i.ha^{-1} (38.33 %) and dinotefuran 25 g a.i.ha^{-1} (36.67 %) whereas control had no mortality.

The highest mortality was observed in lambda cyhalothrin + thiamethoxam 27.5 g a.i. ha^{-1} (91.67 %) treated aphids after 2 hours of treatment and was significantly different from rest of the treatments. Dinotefuran 25 g a.i.ha^{-1} recorded 76.67 per cent mortality, which was on par with dimethoate (check) @ 200 g a.i.ha^{-1} (73.33 %), thiacloprid 24 g a.i.ha^{-1} (71.67 %) and acetamiprid 15 g a.i.ha^{-1} (71.67) these were on par with thiamethoxam 25 g a.i.ha^{-1} (70.00 %). All treatments were superior to water spray which had no mortality.

Lambda cyhalothrin + thiamethoxam 27.5 g a.i.ha^{-1} recorded cent per cent mortality after 3 hours of treatment and was significantly different from other treatments. Thiacloprid 24 g a.i.ha^{-1} showed 91.67 per cent mortality, which was on par with thiamethoxam 25 g a.i.ha^{-1} (90 %), acetamiprid 15 g a.i.ha^{-1} (88.67), dimethoate (check) 200 g a.i.ha^{-1} (88.33 %) and dinotefuran 25 g a.i.ha^{-1} (88.33 %). All treatments were superior to control which recorded no mortality.

4.4. FIELD EVALUATION OF SELECTED NEW GENERATION INSECTICIDES AGAINST RESISTANT POPULATION OF *A. craccivora*

4.4.1 Per cent Reduction in *A.craccivora* Population

Field evaluation of selected insecticides against resistant population of *A. craccivora* was carried out in a farmer's field at Vallamcode where highly resistant population of *A. craccivora* was found (Plate 4). The results are presented under Table 20.

The highest per cent reduction of aphid population was noticed in lambda cyhalothrin + thiamethoxam 27.5 g a.i.ha^{-1} (19.72 %) after 0.25 day of spraying and was significantly superior to all other treatments. Thiacloprid 24 g a.i.ha^{-1} treated plot, 15.74 per cent reduction in aphid population was observed and it was on par with thiamethoxam 25 g a.i.ha^{-1} which recorded 14.14 per cent reduction and all other treatments were superior to control.

After 0.5 day of spraying lambda cyhalothrin + thiamethoxam 27.5 g a.i.ha^{-1} recorded 85.13 per cent reduction in aphid population which was significantly different from other treatments. Thiamethoxam 25 g a.i.ha^{-1} showed 76.14 per cent reduction which was on par with thiacloprid 24 g a.i.ha^{-1} (74.92 %).

Lambda cyhalothrin + thiamethoxam 27.5 g a.i.ha^{-1} recorded maximum per cent reduction of aphid population one day after spraying followed by thiamethoxam 25 g a.i.ha^{-1} (93.97 %) and thiacloprid 24 g a.i.ha^{-1} (91.50 %).

Cent per cent reduction in aphid population was recorded three days after spraying and retained up to 10 days after spraying and all treatments were found to be non-significant.

a. Flower infestation

b. Leaf infestation

c. Stem infestation

d. pod infestation

Plate 4. Infestation of *A. craccivora* in cowpea plant

Table 20. Mortality of resistant population of *A.craccivora* treated with new generation insecticides under field conditions

Treatments	Percentage reduction in aphid population after spraying (DAS)#						
	0.25	0.5	1	3	5	7	10
Thiacloprid 21.7 % SC @ 24 g a.i. ha^{-1}	15.74 (23.34)	74.92 (59.96)	91.50 (73.09)	100 (89.72)	100 (89.72)	100 (89.72)	100 (89.72)
Lambda cyhalothrin 9.5 % + Thiamethoxam 12.6 % ZC @ 27.5 g a.i. ha^{-1}	19.72 (26.36)	85.13 (67.43)	97.63 (81.28)	100 (89.72)	100 (89.72)	100 (89.72)	100 (89.72)
Thiamethoxam 25 % WG @ 25 g a.i. ha^{-1}	14.14 (22.03)	76.14 (60.84)	93.97 (75.87)	100 (89.72)	100 (89.72)	100 (89.72)	100 (89.72)
Control *	0 (0.28)	7.50 (15.80)	16.17 (23.40)	29.51 (32.75)	49.15 (44.58)	61.74 (52.29)	68.91 (56.98)
CD(0.05)	2.011	3.23	4.29	3.23	6.77	9.45	9.19

Figures in parenthesis are angular transformed values; DAS – Days after spraying

*Percentage increase in aphid population; #Mean of 15 plants.

4.4.2 Estimation of *A.craccivora* by Scoring Method

The results of the study on effect of new generation insecticides against resistant population of *A. craccivora* under field conditions in terms of scores are presented in Table 21.

After 6 hours of treatment, the lowest score was recorded in thiacloprid 24 g a.i.ha^{-1} (1.26) followed by thiamethoxam 25 g a.i.ha^{-1} (1.50), control (1.64) and lambda cyhalothrin + thiamethoxam 27.5 g a.i.ha^{-1} (2.47). Twelve hours after treatment, thiacloprid 24 g a.i.ha^{-1} (0.80) recorded less score followed by thiamethoxam 25 g a.i.ha^{-1} (0.91) and lambda cyhalothrin + thiamethoxam 27.5 g a.i.ha^{-1} (1.17). Whereas the highest score observed in control was 1.64.

Table 21. Effect of new generation insecticides against resistant population of *A. craccivora* by scoring method under field conditions

Treatments	Dosage (g a.i. ha^{-1})	*Mean score of aphid population at different time intervals (HAT)				
		Pre count	6	12	24	48
Thiacloprid 21.7 % SC	24	1.40	1.26	0.80	0.60	0
Lambda cyhalothrin 9.5 % + Thiamethoxam 12.6 % ZC	27.5	2.65	2.47	1.17	0.65	0
Thiamethoxam 25 % WG	25	1.58	1.50	0.91	0.83	0
Control		1.64	1.64	1.64	1.85	1.94

HAT- Hours after treatment; *Mean of 15 plants

After 24 hours of treatment, less score was observed in thiacloprid 24 g a.i.ha^{-1} (0.60) followed by lambda cyhalothrin + thiamethoxam 27.5 g a.i.ha^{-1} (0.65) and thiamethoxam 25 g a.i.ha^{-1} (0.83). Whereas in control increase in the score was observed *viz.* 1.85. Zero score was noticed in all treatments which were treated with new generation insecticides after 48 hours of treatment whereas in control increase in the aphid score (1.94) was observed.

4.5 ESTIMATION OF RESIDUES OF INSECTICIDES IN COWPEA PODS

4.5.1 Method Validation for Pesticide Residue Analysis

Results of the validation for the estimation of different insecticides in cowpea showed satisfactory recovery for the compounds which are fortified. Validation of method was accomplished with good linearity (0.01-1 µg mL^{-1}) and recovery. Mean recovery of all the insecticides under study was within the acceptance range of 70-120 per cent at three levels of fortification (0.05, 0.25 and 0.5 µg mL^{-1}). Repeatability of the recovery results as shown by the relative standard deviations (RSD) was below 20 per cent, established that the method was sufficiently reliable for pesticide residue analysis and the results are presented in Table 22, 23 and 24.

In thiacloprid, the mean per cent recoveries were 74.40, 85.33 and 76.33 per cent at three respective fortification levels with relative standard deviation of 2.64, 4.63 and 2.00 per cent respectively. In case of thiamethoxam, mean per cent recoveries were 106.33, 112.28 and 105.13 per cent at three fortification levels with relative standard deviation values were 9.28, 0.74 and 1.15 per cent respectively. The mean per cent recovery of lambda -cyhalothrin were 106, 104 and 106 per cent at three fortification levels and relative standard deviation values were 2.83, 1.92 and 0.94 per cent.

Table 22. Per cent recovery of thiacloprid fortified at different levels using QuEChERS method

LOQ (mg kg^{-1})	Recovery (%)			Mean recovery (%) ± SD (mg kg^{-1})	RSD (%)
	R_1	R_2	R_3		
0.05	76	75	72.2	74.40±1.97	2.64
0.25	85.2	87.6	83.2	85.33±5.13	4.63
0.5	76	78	75	76.33±1.53	2.00

Table 23. Per cent recovery of thiamethoxam fortified at different levels using QuEChERS method

LOQ (mg kg^{-1})	Recovery (%)			Mean recovery (%) ± SD (mg kg^{-1})	RSD (%)
	R_1	R_2	R_3		
0.05	116.8	105	97.2	106.33±9.87	9.28
0.25	113.2	112	111.6	112.28±0.83	0.74
0.5	105	106.4	104	105.13±1.21	1.15

Table 24. Per cent recovery of Lambda cyhalothrin fortified at different levels using QuEChERS method

LOQ (mg kg^{-1})	Recovery (%)			Mean recovery (%) ± SD (mg kg^{-1})	RSD (%)
	R_1	R_2	R_3		
0.05	103	109	106	106 ± 3.00	2.83
0.25	104	106	102	104 ± 2.00	1.92
0.5	106	105	107	106 ± 1.00	0.94

Limit of Quantification (LOQ) - 0.05 mg kg^{-1}, R- Replication, SD – Standard Deviation, RSD – Relative Standard Deviation

4.5.2 Estimation of Persistence and Degradation of Residues of Insecticides and their Half-lives

The results on mean residue, dissipation percentage and half-lives of selected new generation insecticides are presented in Table 25.

4.5.2.1 *Thiacloprid 21.7 % SC (as single insecticide)*

The mean initial deposit of 0.39 mg kg^{-1} was recorded on cowpea pods after two hours of spraying and it got dissipated to 0.37 mg kg^{-1} on first day with a dissipation percentage 5.13. On third day after spraying, the residue dissipated to 0.25 mg kg^{-1} with a dissipation percentage of 35.90. Residue dissipated to 0.23 mg kg^{-1} with dissipation percentage of 41.02 on fifth day after spraying. On seventh day after spraying residues of thiacloprid reached to below quantifiable levels with a half-life of 4.37 days.

4.5.2.2 *Lambda cyhalothrin 9.5 % + Thiamethoxam 12.6 % ZC (insecticide mixture)*

4.5.2.2.1 *Lambda cyhalothrin 9.5 %*

In the insecticide mixture initial deposit of lambda cyhalothrin at two hours after spraying was 0.19 mg kg^{-1} which dissipated to 0.06 mg kg^{-1} with dissipation percentage of 68.42 on first day after spraying. On the third day, residues got dissipated to below quantifiable levels with a half-life of 0.31 days.

4.5.2.2.2 *Thiamethoxam 12.6 %*

In case of thiamethoxam the mean initial deposit of 0.42 mg kg^{-1} was recorded on cowpea pods after two hours of spraying which was reduced to 0.08 mg kg^{-1} with dissipation percentage 80.95 on next day. On the third day after spraying, the residues reached below quantification levels and half-life of thiamethoxam was 0.37 days.

Table 25. Residue of insecticides in cowpea pods

Days after Spraying (DAS)	Thiacloprid 21.7 % SC		Lambda cyhalothrin 9.5 % + Thiamethoxam 12.6 % ZC				Thiamethoxam 25 % WG	
			Lambda cyhalothrin 9.5 %		Thiamethoxam 12.6 %			
	Mean residue ± SD (mg kg^{-1})	Dissipation (%)	Mean residue ± SD (mg kg^{-1})	Dissipation (%)	Mean residue ± SD (mg kg^{-1})	Dissipation (%)	Mean residue ± SD (mg kg^{-1})	Dissipation (%)
Before application	BQL	-	BQL	-	BQL	-	BQL	-
0 (2 h after spraying)	0.39±0.02	-	0.19±0.04	-	0.42±0.04	-	2.21±0.02	-
1	0.37±0.03	5.13	0.06±0.03	68.42	0.08±0.03	80.95	1.09±0.03	50.68
3	0.25±0.01	35.90	BQL	-	BQL	-	0.48±0.02	78.28
5	0.23±0.02	41.02	BQL	-	BQL	-	0.25±0.03	88.69
7	BQL	-	BQL	-	BQL	-	BQL	-
Half-life (Days)	4.37		0.31		0.37		1.21	

BQL – Below Quantification Level, Limit of Quantification (LOQ) - 0.05 mg kg^{-1}, SD – Standard Deviation

4.5.2.3 *Thiamethoxam 25 % WG (as single insecticide)*

An initial deposit of 2.21 mg kg^{-1} was recorded after two hours of spraying. One day after spraying, the residues were reduced to 1.09 mg kg^{-1} with dissipation percentage of 50.68. On third day after spraying the residues were dissipated to 0.48 mg kg^{-1} with dissipation percentage of 78.28. Nearly 88.69 per cent of residues were degraded on fifth day after spraying and residue recorded is 0.25 mg kg^{-1}. After seventh day of spraying, residues reached to below quantifiable levels and the half-life of thiamethoxam worked out was 1.21 days.

4.5.3 Estimation of Harvest Time Residues of Insecticides

Harvest time residues of thiacloprid @ 24 g a.i.ha^{-1}, lambda cyhalothrin + thiamethoxam @ 27.5 g a.i.ha^{-1} and thiamethoxam @ 25 g a.i.ha^{-1} were found to be below quantification levels in cowpea pods at the time of harvest (Table 26).

4.5.4 Risk Assessment of Different Insecticides in Cowpea

Risk assessment of different insecticides in cowpea were calculated and expressed in Table 27, 28 and 29.

4.5.4.1 *Thiacloprid 21.7 % SC*

ADI of thiacloprid was 0.01 mg kg^{-1} bw d^{-1}. The mean residue of thiacloprid in cowpea fruits from 0^{th} to 5^{th} day after spraying were 0.39, 0.37, 0.25 and 0.23 mg kg^{-1} respectively. Maximum permissible intake (MPI) was 550 μg g^{-1} person $^{-1}$ d^{-1}, by taking 90 g as daily consumption of cowpea fruits by man, TMRC values were calculated. TMRC values from 0^{th} to 5^{th} day after spraying were 35.1, 33.3, 22.5 and 20.7 μg g-^{1} person $^{-1}$ d^{-1} respectively, which were lower than the MPI of thiacloprid as 550 μg g-^{1} person $^{-1}$ d^{-1}.

Table 26. Harvest time residue of insecticides in cowpea pods

Days after Spraying (DAS)	Thiacloprid 21.7 % SC	Thiamethoxam 12.6 % + Lambda cyhalothrin 9.5 % ZC		Thiamethoxam 25 % WG
		Lambda cyhalothrin 9.5 %	Thiamethoxam 12.6 %	
	Mean residue ± SD (mg kg^{-1})	Mean residue ± SD (mg kg^{-1})	Mean residue ± SD (mg kg^{-1})	Mean residue ± SD (mg kg^{-1})
7	BQL	BQL	BQL	BQL
10	BQL	BQL	BQL	BQL

BQL – Below Quantification Level, Limit of Quantification (LOQ) - 0.05 mg kg^{-1}, SD – Standard Deviation

4.5.4.2 ***Lambda cyhalothrin 9.5 % + Thiamethoxam 12.6 % ZC (insecticide mixture)***

The prescribed ADI of lambda cyhalothrin and thiamethoxam were 0.02 and 0.08 mg kg^{-1} bw d^{-1} respectively. The mean residue from 0^{th} to 3^{th} day after spraying lambda cyhalothrin were 0.19, 0.06 and 0.05 mg kg^{-1}. In the case of thiamethoxam the residues present in 0^{th} to 1^{st} day after spraying were 0.42 and 0.08 mg kg^{-1} respectively. MPI of lambda cyhalothrin and thiamethoxam were 1100 and 4400 µg g^{-1} person $^{-1}$ d^{-1} respectively. In lambda cyhalothrin TMRC values were 17.1, 5.4 and 4.5 µg g-^{1} person $^{-1}$ d^{-1} and in thiamethoxam TMRC values were 37.8 and 7.2 µg g^{-1} person $^{-1}$ d^{-1} respectively. TMRC< MPI and hence, lambda cyhalothrin + thiamethoxam as insecticide mixture doesn't likely to pose any health problems to consumers.

4.5.4.3 ***Thiamethoxam 25 % WG***

ADI value of thiamethoxam to man was 0.08 mg kg^{-1} bw d^{-1}. Mean residues from 0^{th} to 5^{th} day after spraying were 2.21, 1.09, 0.48 and 0.25 mg kg^{-1} respectively. MPI and daily consumption values were 4400 µg g-^{1} person $^{-1}$ $d^{-1}$and 90 g per day respectively. From 0^{th} to 5^{th} day after spraying TMRC values were 198.9, 98.1, 43.2 and 22.5 µg g-^{1} person $^{-1}$ d^{-1} respectively which were lower than MPI value (4400 µg g-^{1} person $^{-1}$ d^{-1}). Hence thiamethoxam does not seem to pose any health impact on consumers even after 2 hours of spraying.

Table 27. Risk assessment of thiacloprid 21.7 % SC in cowpea

ADI (mg kg^{-1} bw d^{-1})	Average body weight (kg^{-1})	DAS	Daily consumption (g d^{-1})	MPI (µg g^{-1} person $^{-1}$ d^{-1})*	Mean residue (µg g^{-1})	TMRC (µg g^{-1} person $^{-1}$ d^{-1})
0.01	55	0	90	550	0.39	35.1
0.01	55	1	90	550	0.37	33.3
0.01	55	3	90	550	0.25	22.5
0.01	55	5	90	550	0.23	20.7

DAS – Days after spraying; ADI- Acceptable daily intake; MPI- Maximum permissible intake; TMRC- Theoretical maximum residue concentration * MPI= ADI x Average body weight x 1000

Table 28. Risk assessment of thiamethoxam 25 % WG in cowpea

ADI (mg kg^{-1} bw d^{-1})	Average body weight (kg^{-1})	DAS	Daily consumption (g d^{-1})	MPI (µg g^{-1} person $^{-1}$ d^{-1})*	Mean residue (µg g^{-1})	TMRC (µg g^{-1} person $^{-1}$ d^{-1})
0.08	55	0	90	4400	2.21	198.9
0.08	55	1	90	4400	1.09	98.1
0.08	55	3	90	4400	0.48	43.2
0.08	55	5	90	4400	0.25	22.5

DAS – Days after spraying; ADI- Acceptable daily intake; MPI- Maximum permissible intake; TMRC- Theoretical maximum residue concentration * MPI= ADI x Average body weight x 1000

Table 29. Risk assessment of lambda cyhalothrin 9.5 % + Thiamethoxam 12.6 % ZC (insecticide mixture) in cowpea

ADI (mg kg^{-1} bw d^{-1})		Average body weight (kg^{-1})	DAS	Daily consumption (g d^{-1})	MPI (µg g^{-1} person $^{-1}$ d^{-1})*		Mean residue (µg g^{-1})		TMRC (µg g^{-1} person $^{-1}$ d^{-1})	
Lambda cyhalothrin 9.5 %	Thiamethoxam 12.6 %				Lambda cyhalothrin 9.5 %	Thiamethoxam 12.6 %	Lambda cyhalothrin 9.5 %	Thiamethoxam 12.6 %	Lambda cyhalothrin 9.5 %	Thiamethoxam 12.6 %
0.02	0.08	55	0	90	1100	4400	0.19	0.42	17.1	37.8
0.02	0.08	55	1	90	1100	4400	0.06	0.08	5.4	7.2
0.02	-	55	3	90	1100	-	0.05	-	4.5	-

DAS – Days after spraying; ADI- Acceptable daily intake; MPI- Maximum permissible intake; TMRC- Theoretical maximum residue concentration * MPI= ADI x Average body weight x 1000

Discussion

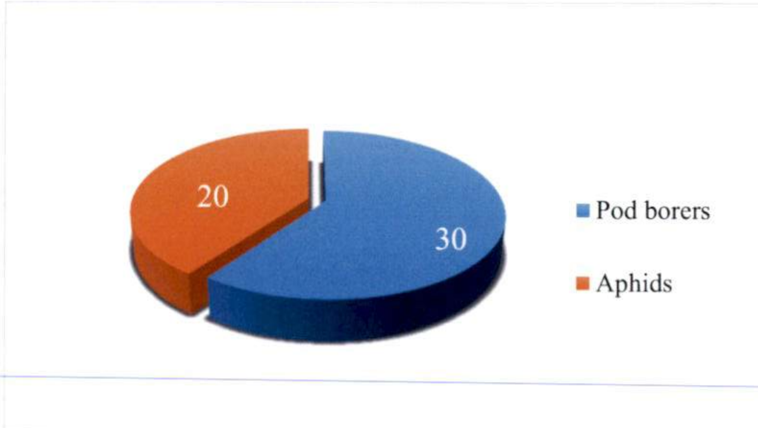
5. DISCUSSION

The cowpea aphid (*A. craccivora*) is one of the most important sucking insect pest attacking legumes particularly cowpea and is a major threat to cowpea growers throughout the country. Management of the aphid basically relies on chemical control using insecticides such as organophosphates, carbamates, pyrethroids and neonicotinoids. Simultaneously several control failures have been reported from many fields and the status of insecticidal resistance in *A.craccivora* seems quite serious. Development of insecticide resistance has been recognized as the top environmental problem for nearly two decades throughout the world. The information gathered from the present study on the insecticide resistance in the field populations of *A. craccivora* and the efficacy of new generation insecticides against the resistant population of *A. craccivora* are discussed here under.

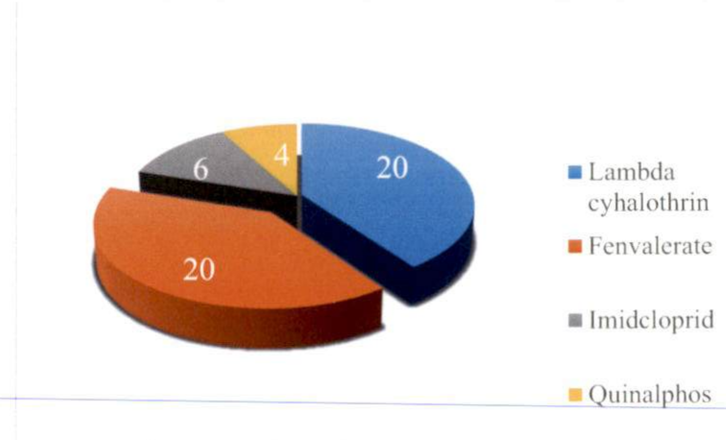
5.1. PRELIMINARY SURVEY TO GATHER THE INFORMATION ON THE PESTICIDE USE AND THE DEVELOPMENT OF INSECTICIDE RESISTANCE

Resistance to any xenobiotic molecule is a basic biological phenomenon found in every living organism, from micro-organisms to man. Excessive and indiscriminate use of insecticides has led to problems of insecticide resistance, pest resurgence, accumulation of harmful residues and toxicity to non-target organisms.

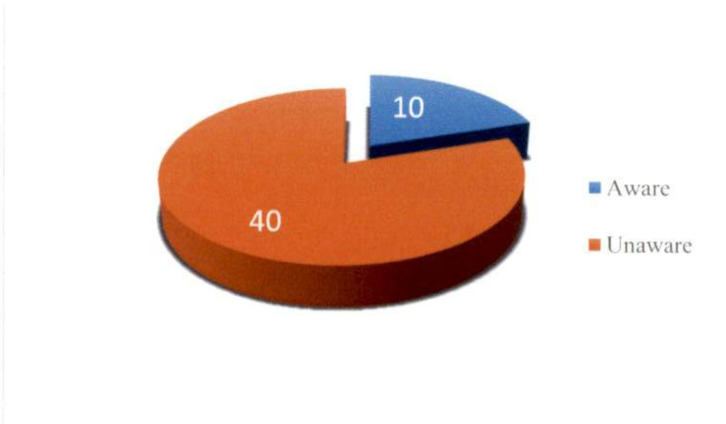
In order to gather the information on details of pest, effectiveness plant protection chemicals, awareness on insecticide resistance, and information on pesticide use pattern, a preliminary survey was conducted among 50 farmers of Kalliyoor using a questionnaire. Among surveyed farmers, 30 farmers opined that pod borers were the major pests of cowpea, whereas 20 farmers responded aphids as major pests and the management was also very difficult (Fig.1). The control failures revealed the development of insecticide resistance in pests of cowpea. In Kerala, study on the control failures of pod borer, *M. vitrata* in cowpea have been conducted by Sreelakshmi (2014) which was the pioneer work in Kerala on insecticide



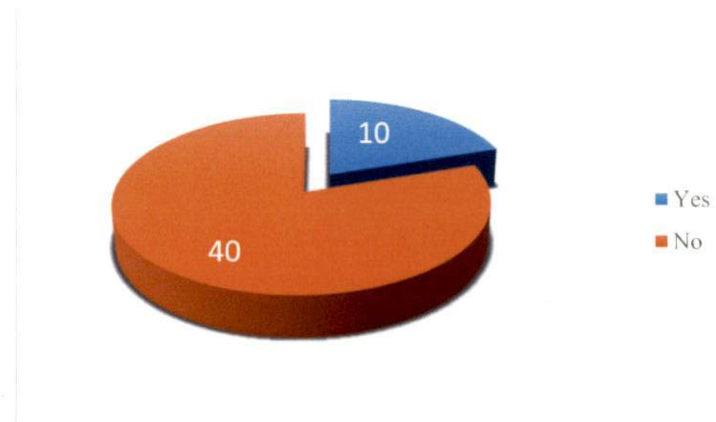
Pests difficult to control



Control failures reported against different insecticides



Awareness regarding insecticide resistance



Application of IPM strategies

- Total number of farmers surveyed - 50

Figure 1. Response of farmers on details of pests, effectiveness of plant protection chemicals and awareness of insecticide resistance

resistance in crop pest. The present study concentrated on control failures in *A. craccivora* and its management using new generation insecticides. From the survey it is clear that fenvalerate and lambda cyhalothrin are the insecticides which showed control failures against aphids. Besides that two insecticides imidacloprid and quinalphos also showed control failures. More or less similar trend in insecticide use pattern was reported by Sreelakshmi (2014). Survey revealed the wide use of insecticides in cowpea which was also evident from the presence of pesticide residues in cowpea sampled from Kalliyoor (PAMSTEV, 2017).

The overall results of the survey revealed the occurrence of control failures in pests of cowpea against insecticides like fenvalerate, lambda cyhalothrin, imidacloprid and quinalphos. Since fenvalerate and lambda cyhalothrin belongs to same group *viz.* synthetic pyrethroids having similar mode of action, fenvalerate has been selected for the present study. Further studies were taken up to find out the insecticide resistance in *A. craccovora* against fenvalerate, imidacloprid and quinalphos having three different groups with different mode of action.

5.2. ASSESSMENT OF INSECTICIDE RESISTANCE IN FIELD POPULATIONS OF *A. craccivora* ON VEGETABLE COWPEA

5.2.1. Biology of *A.craccivora*

The studies on assessment of insecticide resistance in *A. craccivora* needs a continuous supply of F_2 generation of aphids. To meet this requirement and to acclimatize with rearing method, studies on biology of *A. craccivora* was conducted in the laboratory.

The mode of reproduction in *A.craccivora* was exclusively through parthenogenesis throughout the year. In the present study, the nymphal and reproductive periods of *A. craccivora* were found to be 5.40 and 6.20 days respectively. However, in Puducherry, Angayarkanni and Nadarajan (2008), reported that nymphal and reproductive periods of *A. craccivora* were 7.60 and 6.57 days respectively. The difference in duration of life stages may be due to variation in climatic conditions.

Yadav *et al.* (1991) described that nymphal period of green peach aphid, *M. persicae* for red and pale green morphs ranged from 6 to 7 and 7 to 8 days respectively while the reproductive period was 13.80 and 8.67 days respectively. In the present study, total life cycle, fecundity and rate of reproduction (nymphs/day/aphid) of *A. craccivora* in cowpea were 12.30 days, 52.10 and 8.24 nymphs respectively. Several authors reported total life cycle, fecundity and rate of reproduction of *A. craccivora* from different localities as 8.9-20.3 days, 65.2 and 4.85 nymphs (Joshi *et al.*,1998) and 13.39 days, 53.02 and 8.07 nymphs in Puducherry (Angayarkanni and Nadarajan, 2008).

5.2.2. Assessment of Insecticide Resistance

Natural selection by an insecticide allows some insects with resistance genes to survive and pass the resistance trait on to their offspring. The percentage of resistant insects in population continues to multiply whereas susceptible insects which are eliminated by the insecticide. Eventually, resistant insects out numbers susceptible ones and the pesticide is no longer effective (Gour and Sridevi, 2017).

Cowpea aphid, *A. craccivora* is one of the major constraints in increasing the production and productivity of cowpea in Kerala. The pest is managed by the application of insecticides such as organophosphates, carbamates, pyrethroids and neonicotinoids. However, several control failures have been reported from many fields and the status of insecticidal resistance in *A.craccivora* seems quite serious globally. Among aphids 24 species have developed resistance, most of the cases were observed in *M. persicae* (469 cases) followed by *A. gossypii* (268 cases). However in *A. craccivora,* two cases were reported to develop resistance against demeton-S-methyl and dimethoate in Tajikistan (APRD, 2018). The information available on insecticide resistance against cowpea aphid under Indian condition is so meager. No study has been carried out in Kerala on insecticide resistance against *A. craccivora*. Hence this study was conducted to assess the insecticide resistance in *A. craccivora* in cowpea.

The present study conducted to assess the development of insecticide resistance in three field populations (F_2 generation) of *A. craccivora* in cowpea. The first aphid population was from Vilavoorkal (no insecticide application), second from College of Agriculture, Vellayani (no control failures reported after insecticide application) and third from Vallamcode (heavy application of insecticides and control failures reported).

Based on the data, Vilavoorkal population was selected as reference strain (as susceptible population). Population collected from Vallamcode showed the highest resistance to synthetic pyrethroid insecticide *viz.* fenvalerate (Sodium channel modulator) followed by imidacloprid (neonicotinoid -Nicotinic acetylcholine receptor (nAChR) competitive modulator) and quinalphos (organophosphate-Acetylcholinesterase (AChE) inhibitor) (Fig. 2).

The result showed that resistance ratio of aphid population against fenvalerate ranged from 2.97-19.46 fold (based on LC_{50} values) and 2.30-3.51 fold (based on LC_{90} values) at 6 hours after treatment when compared to field check. The highest LC_{50} of 434.62 ppm was recorded in population collected from Vallamcode followed by College of Agriculture, Vellayani (66.17 ppm) and Vilavoorkal (22.33 ppm). Vallamcode population recorded the highest LC_{90} of 1042.66 ppm followed by College of Agriculture, Vellayani (683.27 ppm) and Vilavoorkal (296.94 ppm). Percentage increase in toxicity of *A. craccivora* population as compared to reference strain in different insecticides was shown in Figure. 3.

The development of resistance may be due to the intensive and recurrent use of fenvalerate for the management of aphid, which is evident from the preliminary survey conducted along with the present studies. In 2014 itself, Sreelakshmi reported

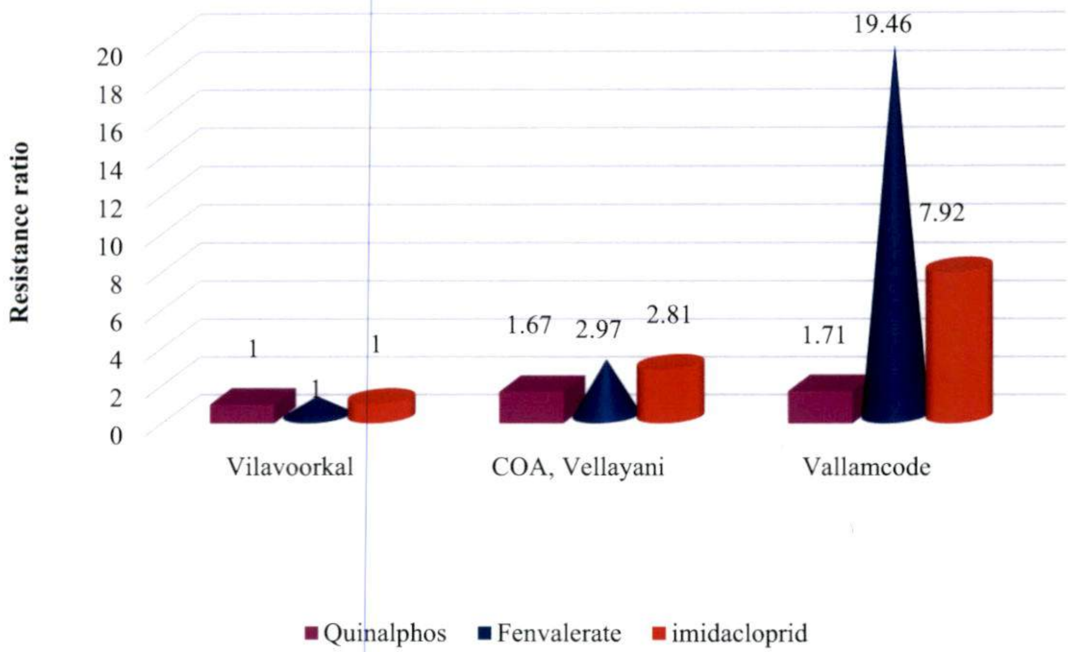


Figure 2. Resistance ratio of *A. craccivora* population against different insecticides

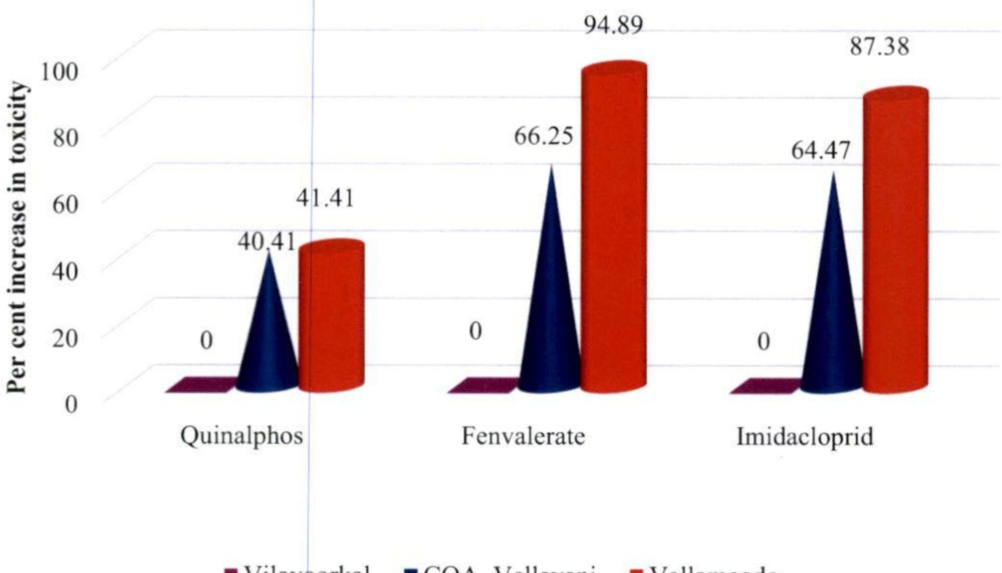


Figure 3. Percentage increase in toxicity of *A. craccivora* population as compared to reference strain in different insecticides

the wide use of synthetic pyrethroid especially fenvalerate and lambda cyhalothrin among cowpea farmers of Kalliyoor. In general, *A. craccivora* reproduces continuously by parthenogenesis in warmer climates, the resistant genotypes once selected by insecticide pressure keep on reproducing without the resistance being diluted by crossing with susceptible insects and in the absence of natural enemies such as coccinellids and syrphids, resistant aphid populations can increase at a rapid rate (Ahmad *et al.,* 2003).

Generally, development of resistance was easy in case of synthetic pyrethroids than the organophosphates and carbamates, because synthetic pyrethroid molecules constitute as a single isomer, which may induce the production of detoxifying enzyme and in that way resistance will develop rapidly. Whereas in the case of organophosphates and carbamates, it does not exist as a stereo isomer so the insect has to develop several mechanisms, which need many enzyme systems to cause detoxification of the insecticide (Sreelakshmi, 2014). Pyrethroid resistance in several species of arthropods is reported during the past decade, in spite of the fact that pyrethroids have been in use for a limited period of time in comparison to the other synthetic insecticides. Most knowledge of pyrethroid resistance has been derived from studies with house fly. Gour and Sridevi, 2017 suggested the reason behind the reduced susceptibility as decreased sensitivity of the nerve membrane and decreased availability of pyrethroid at the primary target site, which may be mediated by several mechanisms. They also reported that the reduced penetration is a possible mechanism of resistance against several insecticides in a number of species of insects. The mechanism in house fly was found to be a single factor (*pen*), located on the third Chromosome which was found in a permethrin - selected strain of the house fly.

In Pakistan, high levels of pyrethroid resistance, seen in the field population of *A. gossypii*, it might be a consequence of multiple resistance involving more than one mechanism (Ahmad *et al.,* 2003). According to Wang *et al.* (2007) in China, F_2

generation of *A. gossypii* had developed 1223.2 fold resistance against fenvalerate when compared with susceptible strain and resistance was increased up to 29,035.6 fold at F_{17} generation on cotton. The present results were in coherence with a study made by Mokbel (2013) in Egypt and he revealed that field population of *A. craccivora* showed recognizable resistance to lambda- cyhalothrin (21.4 fold) and high tolerance level to fenvalerate (8.46 fold). Sreelakshmi and Paul (2016) revealed that a resistance ratio of 2.28 to 7.94 folds for chlorpyriphos and lambda cyhalothrin respectively in resistant population of *M. vitrata* in cowpea in Kerala.

In the present study, resistance ratios varied from 2.81-7.92 fold for imidacloprid with respect to LC_{50} values and 1.24-1.30 fold with respect to LC_{90} values at 6 hours after treatment. LC_{50} of 4.28 ppm was recorded in Vallamcode population, which was the highest among three populations followed by College of Agriculture, Vellayani (1.52 ppm) and Vilavoorkal (0.54 ppm).

Multiple mechanisms worked together throughout the resistance development in insects involving mutations on the target genes, body wall penetrations, behavioral resistance, and metabolism of insecticides through detoxification enzymes (Devonshire *et al.,* 1998). However, the major group of insecticides used for aphid control belong to neonicotinoids. Resistance levels were significantly increased, when field populations were pressured with indiscriminate use of insecticide. However many pests developed resistance to pesticides of neonicotinoid class through enhanced activity of P450 and this over expression or amplification in P450 genes could result in the increase of the activity. Microarray analysis to all known detoxification genes of *M. persicae* shown constitutive over expression of CYP6CY3 in the 5191A clone. Using genomic DNA as template, qPCR showed that the over-expression was due to gene amplification, and this mechanism also providing it protection from the plant secondary metabolite nicotine in tobacco (Li *et al.,* 2016).

According to Liu *et al.* (2003), studies on the imidacloprid resistance in field population of *N. lugens* revealed that the resistance increased by 11.35 times in 25 generations and the resistance ratio reached 72.83 compared to laboratory susceptible strain. Wen *et al.,* (2009) revealed that field populations of brown plant hopper (*N. lugens*) had developed a moderate to high level of resistance to imidacloprid (2.5 folds). Rao *et al.* (2014) studied the development of resistance in different population of Asian Citrus Psyllid, *Diaphorina citri* Kuwayama in different areas of Maharasthra. They reported that Nagpur population showed increase in resistance level to imidacloprid (Resistance ratio=1.42 in 2011 and 1.47 in 2012) over the resistance level of 2010, similarly Amravati population showed increase in resistance level to imidacloprid (RR=3.00). Contradictory to the present study, Mokbel (2013) reported that the field strain of *A. craccivora* showed higher susceptibility to imidacloprid (0.77 fold). In Republic of Korea imidacloprid resistant (IMI-R) strain of *A. gossypii* showed a little cross-resistance (15 times) to fenvalerate (Koo *et al.,* 2014).

Resistance ratios of quinalphos ranged from 1.67-1.71 fold with respect to their LC_{50} and 1.29-1.64 fold with respect to their LC_{90} values when compared with field check at 6 hours after treatment. Population collected from Vallamcode showed the highest LC_{50} of 2.97 ppm against quinalphos followed by population collected from College of Agriculture, Vellayani (2.92 ppm) and Vilavoorkal (1.74 ppm).

Considering the median lethal concentration values, field populations of *A. craccivora* were suceptible to organophosphate insecticide *viz.* quinalphos than imidacloprid and fenvalerate. Compared to the commonly used insecticides in cowpea, quinalphos was the less used insecticide by farmers. (Sreelakshmi, 2014). It is in accordance with the result of preliminary survey conducted in the present study. It may be the reason for susceptibility of *A. craccivora* to quinalphos.

According to Belal *et al.* (2009) development of resistance in *A. craccivora* to malathion took 34 generations to become measurable (10.72 fold). In Egypt, Mokbel (2013) studied insecticide resistance in cowpea aphid and he reported that tolerance was observed with malathion (resistance ratios of 4.74). Contradictory to present findings conducted by Sreelakshmi (2017) revealed that population of *S. litura* collected from Kovilnada showed a resistance ratio of 10.41 fold against quinalphos in Kerala,

5.3 EVALUATION OF EFFICACY OF NEW GENERATION INSECTICIDES AGAINST THE RESISTANT POPULATION OF *A. craccivora* IN LABORATORY CONDITION

Results of the previous experiment revealed the development of insecticide resistance in the field populations of *A. craccivora* collected from Vallamcode against fenvalerate and imidacloprid followed by population collected from College of Agriculture, Vellayani. For the effective management of resistance in the population of *A. craccivora* from Vallamcode, a laboratory experiment was conducted to evaluate the efficacy of new generation insecticides against resistant population of *A. craccivora viz.* acetamiprid 15 g a.i.ha^{-1} (Nicotinic acetylcholine receptor (nAChR) competitive modulators), thiamethoxam 25 g a.i.ha^{-1} (Nicotinic acetylcholine receptor (nAChR) competitive modulators), thiacloprid 24 g a.i.ha^{-1} (Nicotinic acetylcholine receptor (nAC-hR) competitive modulators), dinotefuran 25 g a.i.ha^{-1} (Nicotinic acetylcholine receptor (nAChR) competitive modulators), thiamethoxam + lambda cyhalothrin 27.5 g a.i.ha^{-1} (Sodium channel modulators + Nicotinic acetylcholine receptor (nAChR) competitive modulators) and dimethoate (as check) 200 g a.i.ha^{-1} (Acetyl cholinesterase (AChE) inhibitors). The result showed that higher mortality was observed in *A.craccivora* treated with thiamethoxam + lambda cyhalothrin 27.5 g a.i.ha^{-1} (100 %) followed by thiacloprid 24 g a.i.ha^{-1}(91.67 %) and thiamethoxam 25 g a.i.ha^{-1} (90.00 %) after 3 hours of treatment. Therefore, these insecticides were selected for field evaluation against resistant population of *A. craccivora.* IRAC (2018) pointed out that successful Insecticide Resistance Management is to prevent or delay the evolution of resistance to insecticides, or to help to regain susceptibility in insect pest populations in which resistance has already arisen. Alternations, sequences or rotations of compounds from different mode of action groups provide a sustainable and effective approach to IRM.

The effective new generation insecticide obtained from laboratory experiment in the present study was a combination product, thiamethoxam + lambda cyhalothrin 27.5 g a.i.ha^{-1} followed by thiacloprid 24 g a.i.ha^{-1} and thiamethoxam 25 g a.i.ha^{-1}. All the effective treatments include compounds from neonicotinoid group. The neonicotinoid group insecticides having several key features that led to their rapid adoption *viz.* lower binding efficiencies to vertebrate compared to invertebrate receptors, indicating selective toxicity to arthropods, systemic nature, high persistence, versatility in application, high water solubility and assumed lower impacts on fish and other vertebrates. Hence, the neonicotinoid group insecticides can be used to manage resistant populations of insect pests and also to delay the development of insecticide resistance due to their peculiar mode of actions (Ahmad, 2009).

Efficacy of neonicotinoids (thiamethoxam and thiacloprid) in managing resistant population of *A. craccivora* was mainly due to their systemic nature and they were easily taken up by leaves and translocated to all parts of the plant and makes them effectively toxic to herbivorous insects such as aphids and toxicity persists for a variable period of time depends on the plant, its growth stage, and the amount of pesticide applied (Simon-Delso, 2015).

The present findings were in agreement with results of research work carried out by different scientists *viz.* Reddy *et al.* (2014) and Abd-Ella (2014). They reported that thiamethoxam and acetamiprid were found to be effective in managing the population of *A. craccivora*. Whereas the latest neonicotinoid, dinotefuran was found to be less effective in managing the aphid population, this in line with the result of the present study. Thamilarasi (2016) conducted a study and reported that thiamethoxam

0.30 g L^{-1} and imidacloprid 0.20 mL L^{-1} were found to be effective in managing sucking pests in cowpea and salad cucumber under polyhouse condition.

According to the study conducted by Misra (2002), thiamethoxam @ 25 g a.i.ha^{-1} was significantly superior in controlling the cotton aphid. Gore *et al.* (2010) reported that thiamethoxam as the most effective insecticide against safflower aphid followed by imidacloprid, dimethoate, acephate and diafenthuron. Shinde *et al.* (2011), reported that neonicotinoid group of insecticide (thiomethoxam) recorded mortality in the range of 84-98 per cent in okra aphids indicated that they are more effective than organophosphate insecticides. These are in agreement with the present findings. More or less similar results were observed in the study made by Rajawat (2017). He reported that thiomethoxam 25% WG was found most effective against *A.craccivora* with a record of 88.24 per cent reduction in population followed by thiacloprid 21.7% SC in Madhya Pradesh.

5.4 FIELD EVALUATION OF SELECTED NEW GENERATION INSECTICIDES AGAINST RESISTANT POPULATION OF *A. craccivora*

Field experiment was laid out to evaluate the efficacy of the selected three new generation insecticides *viz.* thiamethoxam + lambda cyhalothrin 27.5 g a.i.ha^{-1}, thiacloprid 24 g a.i.ha^{-1} and thiamethoxam 25 g a.i.ha^{-1} selected from laboratory studies. The results showed that all treatments were effective in controlling *A. craccivora* when compared to control. After spraying of thiamethoxam + lambda cyhalothrin 27.5 g a.i.ha^{-1}, thiacloprid 24 g a.i.ha^{-1} and thiamethoxam 25 g a.i.ha^{-1}, aphid population reduced to zero.

According to the study of Chalam *et al.* (2003) in Andhra Pradesh, new generation insecticides such as acetamiprid was highly effective followed by diafenthiuron, thiamethoxam and were recommended for managing the resistant population of *A. gossypii*. Sreelakshmi (2014) reported that spraying of emamectin benzoate @ 10 g ai ha^{-1} or indoxacarb + acetamiprid @100 g ai ha^{-1} or spinosad @ 75 g ai ha^{-1} could effectively manage the resistant population of *M. vitrata* in cowpea in Kerala. Sreelakshmi (2017) revealed that emamectin benzoate, indoxacarb, spinetroam, chlorantraniliprole and flubendiamide were found to be effective to manage the resistant population of *S. litura.*

Result well informed the effectiveness of combination product (thiamethoxam + lambda cyhalothrin) which comes under category of pre-packed mixtures having one synthetic pyrethroid and one neonicotinoid compound. Delay in resistance development is mainly due to synergistic joint action and broad spectrum of activity. This is in line with the study of Das (2014) and he revealed the potency of mixture, fenvalerate and fenitrothion for 14 generations of *M. persicae*. After 14 generations of selection the level of resistance to fenvalerate was 52.6- fold, fenitrothion 11.1-fold but, mixture developed only a 3.5-fold resistance. In another study by Samanta *et al.* (2017) reported that insecticide mixture of thiamethoxam and lambda-cyhathrin (ready-mix formulation) was most effective even at its lower dose level against pest complex of tea. Borude *et al.* (2018) revealed that minimum per cent of open boll damage (22.67%) due to bollworm complex at picking was observed in the plots treated with thiamethoxam 12.6% + lambda cyhalothrin 9.5% ZC .

5.5. ESTIMATION OF RESIDUES OF INSECTICIDES IN COWPEA PODS

The indiscriminate and repetitive use of insecticides especially in frequently exposed population of insects lead to accumulate the toxic pesticide residue in the agriculture produce and pose serious threat to health of the consumers (Reddy *et al.*, 2007). In the present findings, the residues of two single insecticides in the promising insecticide mixture *viz.* thiamethoxam and lambda cyhalothrin @ 27.5 g a.i.ha^{-1} dissipated within five days. However, thiacloprid @ 24 g a.i.ha^{-1} and thiamethoxam @ 25 g a.i.ha^{-1} when sprayed separately, dissipated within seven days. The half-lives for insecticides in the mixtures like thiamethoxam + lambda cyhalothrin calculated as

1.73 and 0.37 days while in case of single insecticide thiacloprid and thiamethoxam half-lives were 4.37 and 1.21 days respectively. This study revealed that when insecticides were sprayed as mixture, the half-life is less than that when sprayed as single insecticide. In pre packed mixtures, the concentration of each single insecticide is less as compared to concentration of insecticides when sprayed alone.

Dissipation study conducted by Barik *et al.* (2010) in paddy revealed that the initial residues of Alika (thiamethoxam + lamda cyhalothrin) were 0.26 and 0.50 mg kg^{-1} after two hours of spraying and thiamethoxam was dissipated within 20 days while lamdacyhalothrin was dissipated within five days. In West Bengal, Bhattacharyya *et al*. (2017) conducted a dissipation study on emamectin benzoate + fipronil in chilli and results revealed that the residue of emamectin benzoate dissipated completely after 1st day while, fipronil dissipated within 5 days. No much dissipation studies on thiamethoxam + lamda cyhalothrin has been conducted whereas the research work on dissipation of thiamethoxam and lamda cyhalothrin as single insecticides are available.

Present results are in coherence with the studies conducted by Singh and Kulshrestha (2005) in New Delhi, where the residues of thiamethoxam persisted more than 5 days but no residues were detected at 7 days in okra. In another study by Chauhan *et al.* (2013) in Haryana, reported that the initial deposits of 0.245 mg kg^{-1} reached below detectable level of 0.005 mg kg^{-1} at 15 days after application with a half-life period of 1.47 days in okra. This difference in degradation pattern may be due to the difference in meteorological factors prevailed in different agro climatic zones or during the cropping period.

Saimandir *et al*. (2009) revealed that half-life of thiacloprid was 11.10 days in brinjal. The dissipation studies of thiacloprid conducted by Sharma and Parihar (2013) reported that the residues reached below detection level on 5th day after application in tomato. In Kerala, Thamilarasi (2016) reported that the residues of thiacloprid and thiamethoxam dissipated within 5 and 10 days respectively in cowpea under polyhouse condition which is in line with the results of present study.

In the present study no residues of thiamethoxam + lambda cyhalothrin, thiamethoxam and thiacloprid were detected in mature pods at harvest time. It showed the safety of these insecticides in the harvested produce when offered for consumption.

Increasing awareness on the potential impact of insecticides, studies on risk assessment of insecticides in different crops are gaining much importance nowadays. Hence in the present investigation, risk assessment studies has been conducted along with dissipation studies. Study was conducted by comparing the theoretical maximum residue concentration (TMRC) with maximum permissible intake (MPI). The values of TMRC were lower than the MPI in all promising insecticides under the study. So these insecticides *viz.* thiamethoxam + lambda cyhalothrin, thiamethoxam and thiacloprid do not pose any serious health risk for consumers, crop protection and environmental contamination point of view. In conclusion, spraying of single insecticides *viz.* thiacloprid, thiamethoxam and the ready mixture of thiamethoxam + lambda cyhalothrin were found to be safe in vegetable cowpea and were found to be safe to the consumers.

The present findings are in agreement with the studies of Parmar *et al.* (2016) and Bhattacharyya *et al.* (2017) about safety of ready mixtures *viz.* flubendiamide + thiacloprid in red gram and emamectin benzoate + fipronil in chilli and these insecticide mixtures does not pose health risk to consumers.

In India, vegetable production is facing massive challenge to meet the future demands of growing population. Sucking pests are one of the major threats and cause significant economic damage to vegetable cultivation. Among sucking pests, cowpea aphid, *A. craccivora* not only cause damage by direct feeding by sucking sap and also act as vector for several plant pathogenic viruses. Conventional insecticides are widely used to control the sucking pests but most of them have failed due to lower efficacy, development of high folds of resistance and resurgence of the pests (Kodandaram *et al*., 2016).

The present study forms a maiden attempt in assessing the extent of insecticide resistance development in the field populations of *A. craccivora* in cowpea in Kerala. This investigation revealed the development of insecticide resistance in the field populations of *A. craccivora* against fenvalerate and imidacloprid. Results showed that aphid population collected from Location- II (College of Agriculture, Vellayani) was found to be moderately resistant and aphid population collected from Location- III, (Vallamcode) showed high resistance to fenvalerate and imidacloprid. In order to break the resistance in cowpea aphid, *A. craccivora* thiamethoxam + lambda cyhalothrin 27.5 g a.i.ha^{-1}, thiamethoxam 25 g a.i.ha^{-1} and thiacloprid 24 g a.i.ha^{-1} were found to be effective. Residue estimation of these effective insecticides showed that insecticides were dissipated within five days with half-lives of 0.37, 1.73, 1.21 and 4.37 days respectively. However, considering the less mammalian toxicity, good aphicidal activity, consumer safety and high dissipation rate, blue labelled insecticide thiamethoxam 25 g a.i.ha^{-1} followed by thiamethoxam + lambda cyhalothrin 27.5 g a.i.ha^{-1} and thiacloprid 24 g a.i.ha^{-1} could be used against *A. craccivora* in cowpea. New pesticides with more specific mode of action and reduced spectrums of activity may be more suited for large scale agricultural systems. Further studies have to be taken up to develop and popularize Insecticide Resistance Management strategies against *A. craccivora* by giving emphasis on efficient use of insecticides and to conserve the ecosystem for sustainable pest management.

Summary

6. SUMMARY

Cowpea is an important nutritious grain legume crop in tropical and sub-tropical countries. Cowpea aphid, *A. craccivora* is one of the devastating pests causing significant economic damage to all arial parts of cowpea plant. Application of more than one insecticide with short interval leads to deposition of huge amount of pesticide load in cowpea as well as in environment and develop resistance in insects. The present study was undertaken to conduct a preliminary survey among cowpea farmers to collect information regarding details of pests, pesticide use pattern and development of insecticide resistance, to assess the development of resistance in field population of *A. craccivora,* to evaluate the efficacy of new generation insecticides against resistant population of *A. craccivora* and to study the persistence and degradation of residues of insecticides on cowpea. The results are summarized here under.

- A preliminary survey conducted out among major cowpea farmers in Kalliyoor area of Thiruvananthapuram district revealed that 60 per cent of responded farmers opined, pod borers were the most difficult pest to control whereas 40 per cent of the farmers reported that aphids were difficult to control. The present survey revealed that lambda cyhalothrin and fenvalerate insecticides having the highest control failures (40 % each), followed by imidacloprid (12 %) and quinalphos (8 %).
- Studies on the biology of *A. craccivora* revealed that nymphal, reproductive periods and total life cycle were 5.40, 6.20 and 12.30 days respectively. Fecundity and rate of reproduction (Nymphs/day/aphid) in cowpea were 52.10 and 8.24 nymphs respectively.
- Bioassay was carried out in CRD to assess the insecticide resistance in field population of *A. craccivora* against quinalphos, fenvalerate and imidacloprid from three different locations *viz.* Location-I field with no previous history of pesticide application (Vilavoorkal), location-II field having insecticides

application but no known report of control failures (College of Agriculture, Vellayani) and location-III, field having heavy where application of insecticide and control failures had been observed (Vallamcode) against quinalphos, fenvalerate and imidacloprid. Population collected from Location-I was treated as susceptible to insecticides with resistance ratio-1. Population collected from Location- II was found to be moderately resistant with resistant ratios of 1.67, 2.97 and 2.81 and population collected from Location- III to be resistant with resistant ratios of 1.71, 19.46 and 7.94 for quinalphos, fenvalerate and imidacloprid respectively.

- Laboratory experiments conducted to evaluate the efficacy of new generation insecticides *viz.* acetamiprid 20 % SP @ 15 g a.i.ha^{-1}, thiamethoxam 25 % WG @ 25 g a.i.ha^{-1}, thiacloprid 21.7 % SC @ 24 g a.i.ha^{-1}, dinotefuran 20% SG @ 25 g a.i.ha^{-1}, thiamethoxam 12.6 % + lambda cyhalothrin 9.5 % ZC @ 27.5 g a.i.ha^{-1} and dimethoate 30% EC (as check) @ 200 g a.i.ha^{-1} against the resistant population of *A. craccivora.* The result revealed that higher mortality was observed in *A. craccivora* treated with thiamethoxam 12.6 % + lambda cyhalothrin 9.5 % ZC @ 27.5 g a.i.ha^{-1} (100 %), thiacloprid 21.7 % SC @ 24 g a.i.ha^{-1}(91.67 %) and thiamethoxam 25 % WG @ 25 g a.i.ha^{-1}(90.00 %) at 3 hours after treatment.
- Field experiment was carried out in RBD with four treatments *viz.* thiamethoxam 12.6 % + lambda cyhalothrin 9.5 % ZC @ 27.5 g a.i.ha^{-1}, thiacloprid 21.7 % SC @ 24 g a.i.ha^{-1} and thiamethoxam 25 % WG @ 25 g a.i.ha^{-1} including control against the resistant population of *A. craccivora.* Result showed that per cent mortality of *A. craccivora* recorded were 97.63, 93.97 and 91.50 per cent respectively at one day after spraying.
- Dissipation studies on residues of promising insecticides were conducted by analyzing the cowpea pods collected at 0, 1, 3, 5, 7 and 10 days after application of insecticides *viz.* thiamethoxam 12.6 % + lambda cyhalothrin 9.5

% ZC @ 27.5 g a.i.ha^{-1}, thiacloprid 21.7 % SC @ 24 g a.i.ha^{-1} and thiamethoxam 25 % WG @ 25 g a.i.ha^{-1} and it revealed that these insecticides were dissipated within five days with half-lives 0.37, 1.73, 1.21 and 4.37 days respectively.

- Risk assessment studies were conducted by utilizing dissipation data and compared theoretical maximum residue concentration (TMRC) with maximum permissible intake (MPI). The study revealed that three tested insecticides were found to be safe for consumption even after the same day of application.

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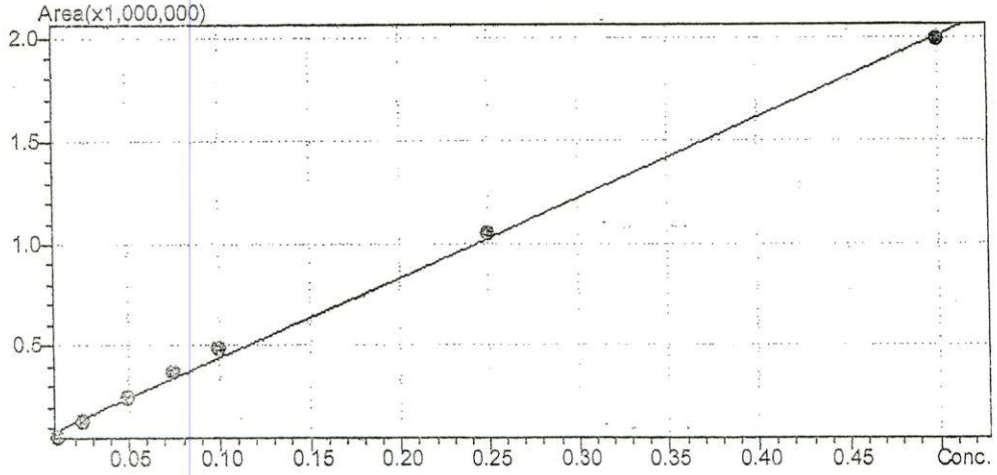
Appendices

Appendix-I

Proforma for survey on pesticide use pattern against cowpea aphid, *A. craccivora*

Sl. no.	Particulars	Response of farmers
1.	Location	
2.	Name and address of farmer	
3.	Age	
4.	Source of technical information regarding crop protection	
a	Technical officers	
b	Company representatives	
c	Other progressive farmers	
d	Own decisions	
e	Media	
5.	Name of plant protection chemicals used	
6.	Source of plant protection chemicals	
7.	Source of information on dose of pesticides	
a.	Technical officers	
b.	Pesticide shops	
c.	VFPCK	
d.	Other progressive farmers	
e.	Own decisions	
f.	Media	
8.	Is there any practice of manual mixing of pesticides and spraying?	Yes/No
9.	Is there any prophylactic application of PP chemicals	Yes/No
10.	Whether following integrated pest management strategies	Yes/No
11.	Practicing any biological control measures	Yes/No
12.	Any control failures noticed after the application of any pesticides	
13.	Name of pest which is very difficult to control	
14.	Do you aware of insecticide resistance	

Appendix-II

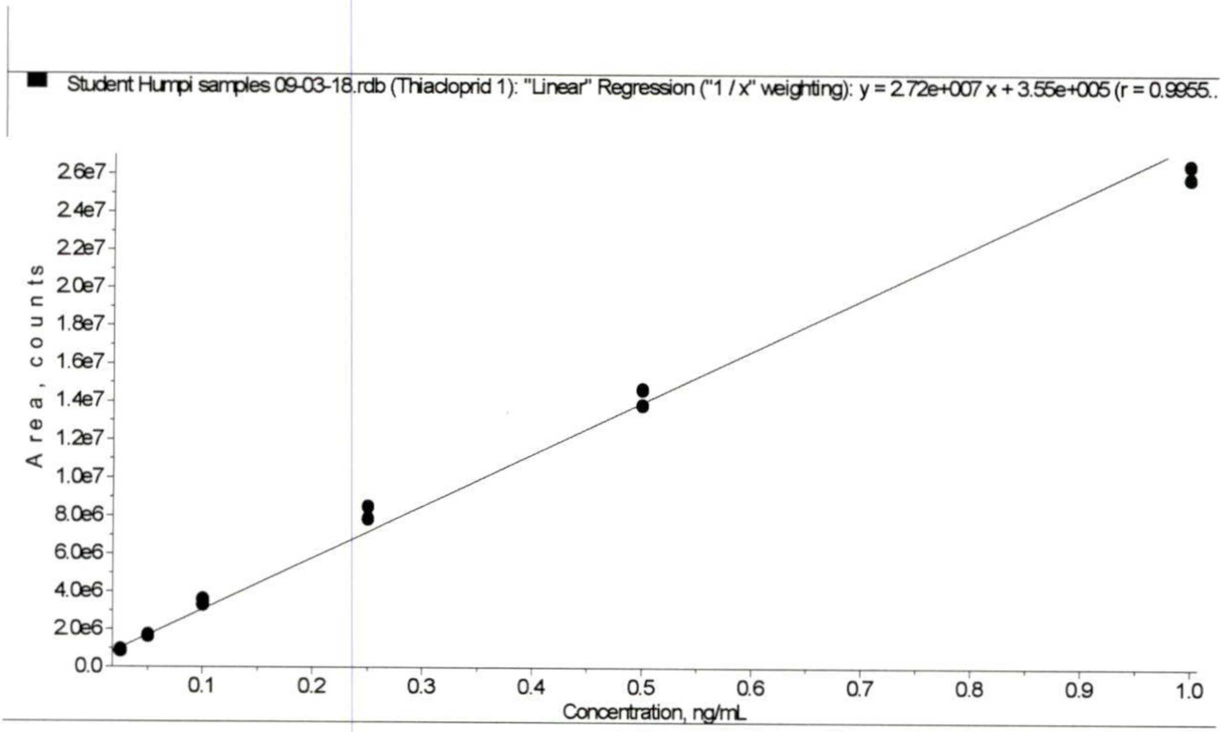


Y = aX + b, a = 3908186, b = 51866.38, R^2 = 0.9982965, R = 0.9991479

Mean RF: 4706060, RF SD : 431378.8, RF %RSD : 9.166453

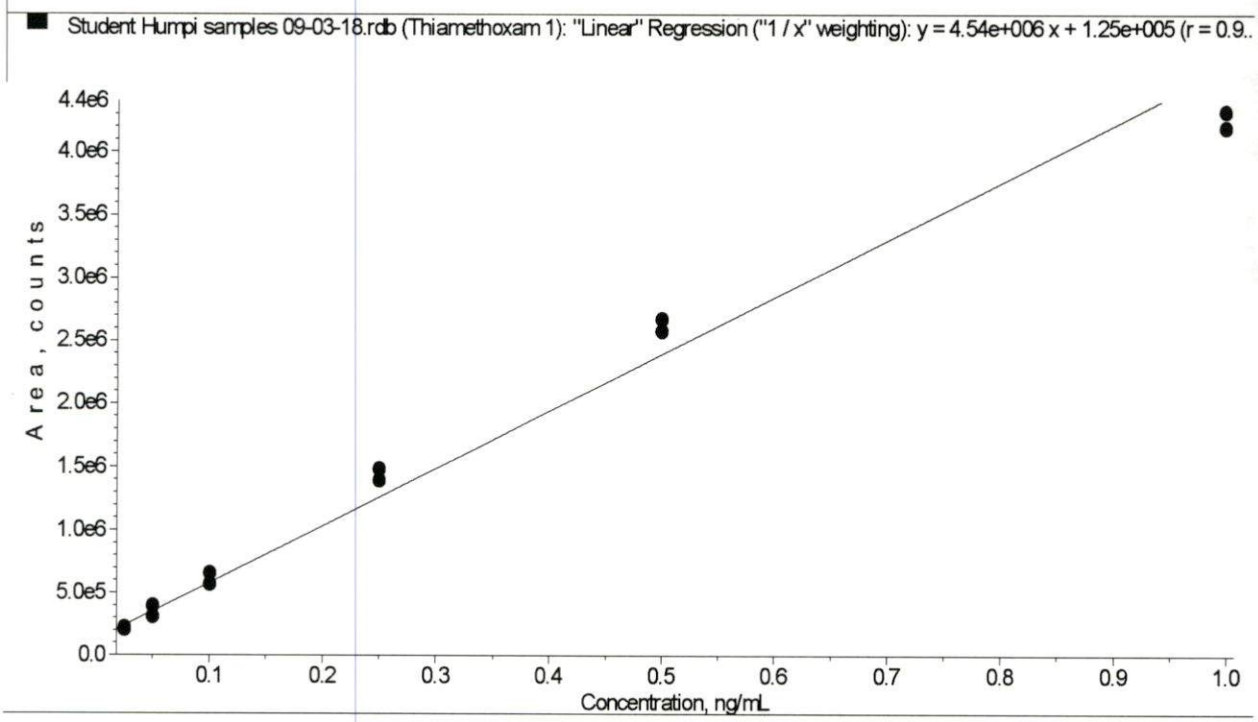
Calibration curve of Lamda cyhalothrin

Appendix-III



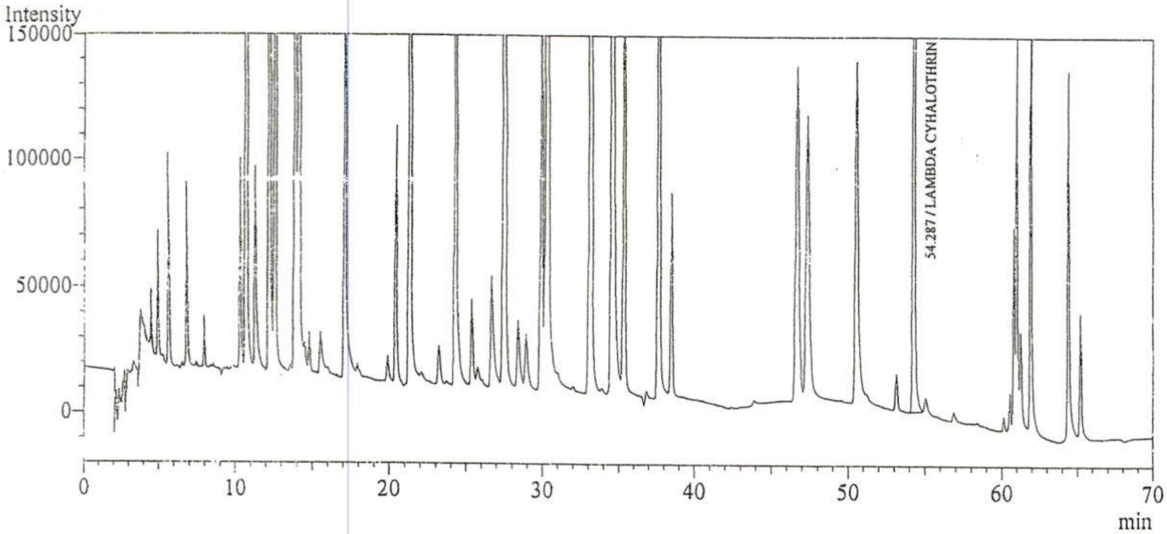
Calibration curve of thiacloprid

Appendix-IV



Calibration curve of thiamethoxam

Appendix-V

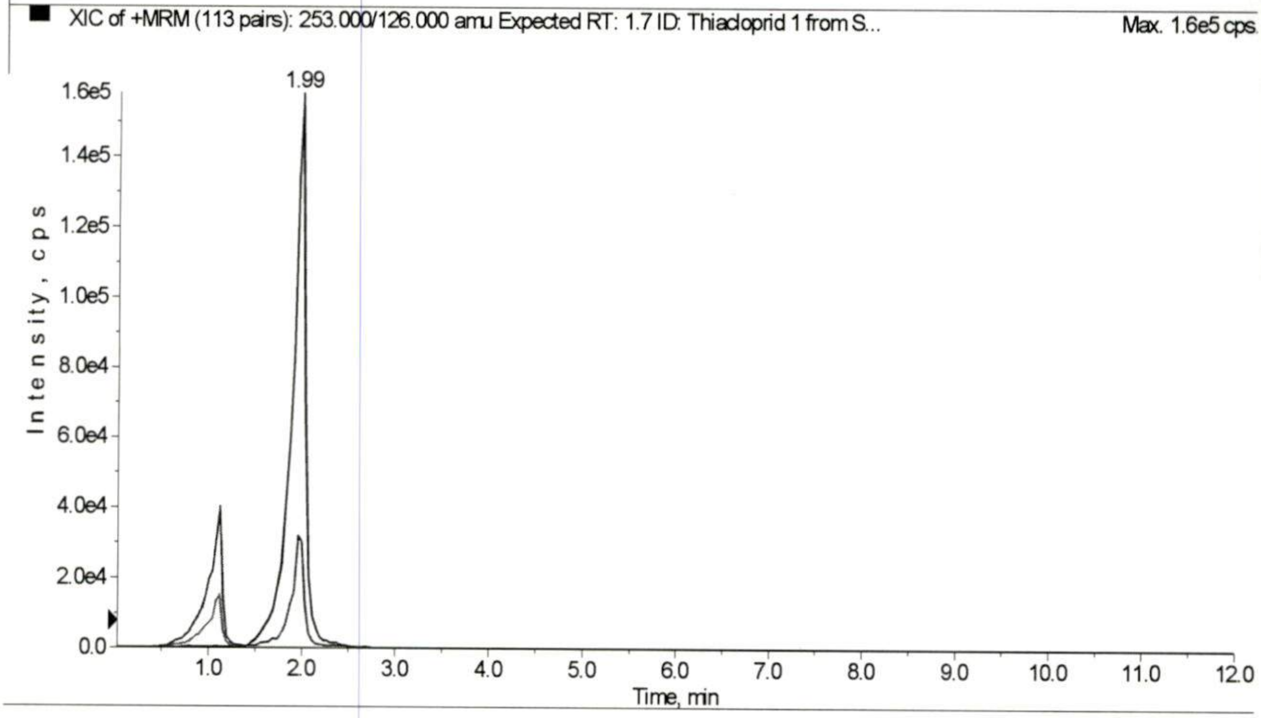


Peak Table - Channel 1

Peak#	Name	Ret.Time	Area	Conc.	Units
1	LAMBDA CYHALOTHRIN	54.287	2770576	0.667	ppm
Total			2770576		

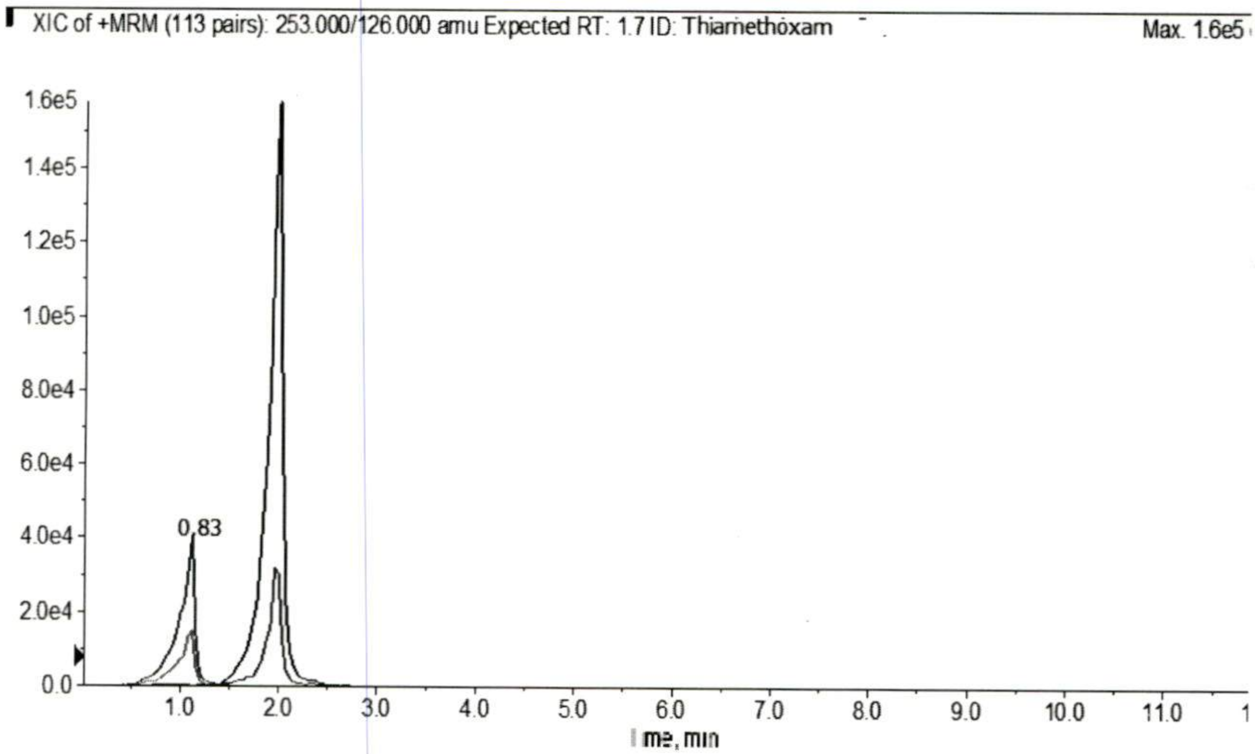
Calibration curve of lambda cyhalothrin

Appendix-VI



LC-MS/MS chromatogram of thiacloprid

Appendix-VII



LC-MS/MS chromatogram of thiamethoxam

Abstract

INSECTICIDE RESISTANCE IN COWPEA APHID, *Aphis craccivora* (KOCH) AND ITS MANAGEMENT

by

JANGAM HAMPAIAH

(2016-11-035)

ABSTRACT

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ABSTRACT

A study on "Insecticide resistance in cowpea aphid, *Aphis craccivora* (Koch) and its management" was conducted in College of Agriculture, Vellayani and farmer's field at Vallamcode, Thiruvananthapuram district during 2016-2018. The main objectives were to assess the insecticide resistance in field population of cowpea aphid, *A. craccivora* and to evaluate the efficacy of new generation insecticides against resistant population of *A. craccivora*.

A preliminary survey was carried out among major cowpea farmers in Kalliyoor area of Thiruvananthapuram district during 2017-18 revealed the continous use of single insecticide *viz*., fenvalerate, lambda cyhalothrin, imidacloprid and quinalphos and the occurrence of control failures in the field populations of *A.craccivora.*

Bioassay was carried out in CRD to assess the insecticide resistance in field population of *A. craccivora* collected from three different locations based on the intensity of insecticide application. A series of concentrations of three insecticides *viz*. fenvalerate, imidacloprid and quinalphos were prepared in aqueous solution and bio assay was done using F_2 generations of aphid populations collected from three locations. Results revealed that population collected from Location-I (Vilavoorkal) was found to be susceptible to insecticides with resistance ratio-1. Population collected from Location- II (Instructional farm, College of Agriculture, Vellayani) was found to be moderately resistant with resistant ratios of 1.67, 2.97 and 2.81 and aphid population collected from Location- III, (Vallamcode) showed more resistance with resistant ratios of 1.71, 19.46 and 7.94 for quinalphos, fenvalerate and imidacloprid respectively.

Laboratory experiments were conducted to evaluate the efficacy of new generation insecticides *viz.* acetamiprid 15 g a.i.ha^{-1}, thiamethoxam 25 g a.i.ha^{-1}, thiacloprid 24 g a.i.ha^{-1}, dinotefuran 25 g a.i.ha^{-1}, thiamethoxam + lambda cyhalothrin

27.5 g a.i.ha^{-1} and dimethoate (as check) 200 g a.i.ha^{-1} against the resistant population of *A. craccivora*. The result revealed that, significantly higher mortality was observed in *A. craccivora* treated with thiamethoxam + lambda cyhalothrin 27.5 g a.i.ha^{-1}(100 %) followed by thiacloprid 24 g a.i.ha^{-1} (91.67 %) and thiamethoxam 25 g a.i.ha^{-1}(90.00 %) which were on par with each other after 3 hours of treatment.

Field experiment was laid out in RBD at Vallamcode from where resistant population was collected with four treatments selected from laboratory experiment of aphids. The percent reduction recorded were 97.63, 93.97 and 91.50 respectively in thiamethoxam + lambda cyhalothrin 27.5 g a.i.ha^{-1}, thiamethoxam 25 g a.i.ha^{-1} and thiacloprid 24 g a.i.ha^{-1} treated plots at one day after spraying. Dissipation of residues of these effective insecticides was studied by analysing the pods collected at 0, 1, 3, 5, 7 and 10 days after application of insecticides at recommended dose and the result showed that insecticides were dissipated within five days with half-lives of 0.37, 1.73, 1.21 and 4.37 days respectively.

The present study revealed the development of insecticide resistance in the field populations of *A. craccivora* against fenvalerate and imidacloprid. By considering the less mammalian toxicity, good aphicidal activity, consumer safety and high dissipation rate, thiamethoxam 25 g a.i.ha^{-1} followed by thiamethoxam + lambda cyhalothrin 27.5 g a.i.ha^{-1} and thiacloprid 24 g a.i.ha^{-1} could be recommended against *A. craccivora* in cowpea. Further studies have to be taken up to develop and popularize Insecticide Resistance Management strategies against *A. craccivora* by giving emphasis on efficient use of insecticides and to conserve the ecosystem for sustainable pest management.