INSECTICIDE RESISTANCE IN SPIRALLING WHITEFLY, Aleurodicus dispersus RUSSELL (HEMIPTERA: ALEYRODIDAE) AND ITS MANAGEMENT

by AURA SENSON (2017-11-063)

THESIS

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DEPARTMENT OF AGRICULTURAL ENTOMOLOGY COLLEGE OF AGRICULTURE VELLAYANI, THIRUVANANTHAPURAM-695 522 KERALA, INDIA

DECLARATION

I, hereby declare that this thesis entitled "INSECTICIDE RESISTANCE IN SPIRALLING WHITEFLY, *Aleurodicus dispersus* RUSSELL (HEMIPTERA: ALEYRODIDAE) AND ITS MANAGEMENT" is a bonafide record of research work done by me during the course of research and 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.

Aweren

AURA SENSON

(2017 - 11 - 063)

Vellayani,

CERTIFICATE

Certified that this thesis entitled "INSECTICIDE RESISTANCE IN SPIRALLING WHITEFLY, *Aleurodicus dispersus* RUSSELL (HEMIPTERA: ALEYRODIDAE) AND ITS MANAGEMENT" is a record of research work done independently by Ms. Aura Senson 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.

Vellayani,

Dr. Ambily Paul

(Major advisor, Advisory Committee) Assistant Professor AINP on Pesticide Residues Dept. of Agricultural Entomology, College of Agriculture, Vellayani

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CERTIFICATE

We, the undersigned members of the advisory committee of

Ms. Aura Senson, a candidate for the degree of Master of Science in Agriculture with major in Agricultural Entomology, agree that the thesis entitled "INSECTICIDE RESISTANCE IN SPIRALLING WHITEFLY, *Aleurodicus dispersus* RUSSELL (HEMIPTERA: ALEYRODIDAE) AND ITS MANAGEMENT" may be submitted by Ms. Aura Senson, in partial fulfilment of the requirement for the degree.

Dr. Ambily Paul (Chairman, Advisory Committee) Assistant professor AINP on Pesticide Residues Department of Agricultural Entomology College of Agriculture, Vellayani.

Dr. Anitha N (Member, Advisory Committee) Professor and Head Department of Agricultural Entomology College of Agriculture, Vellayani.

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Dr. Amritha V. S. Assistant Professor and Principal investigator AICRP on Honey Bees & Pollinators Department of Agricultural Entomology College of Agriculture, Vellayani

Dr. Nisha M. S. Assistant Professor Department of Nematology College of Agriculture, Vellayani

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LIST OF ABBREVIATIONS AND SYMBOLS USED

| % | Per cent |
|--------------------|---|
| @ | At the rate of |
| ADI | Acceptable daily intake |
| a.i. | Active ingredient |
| bw d ⁻¹ | Body weight per day |
| CIB & RC | Central Insecticide Board and Registration Committee |
| CD | Critical Difference |
| DAS | Days after spraying |
| EC | Emulsifiable concentrate |
| et al | And others |
| FAO | Food and Agriculture Organization |
| G | Gram |
| GC- MS | Gas Chromatograph- Mass Spectrometry |
| HAT | Hours after treatment |
| h | Hours |
| ha ⁻¹ | Per hectre |
| HPLC | High Performance Liquid Chromatography |
| IRAC | Insecticide Resistance Action Committee |
| kg | Kilogram |
| L | Litre |

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| LC- MS | Liquid Chromatography- Mass spectroscopy |
|----------|--|
| LOQ | Limit of quantification |
| Mg | Milligram |
| ml | Millilitre |
| MPI | Maximum Permissible Intake |
| OD | Oil Dispersion |
| ppm | Parts per million |
| QuEChERS | Quick, Easy, Cheap, Effective, Rugged and Safe |
| RBD | Randomized Block design |
| RSD | Relative Standard Deviation |
| SC | Suspension Concentrate |
| SP | Soluble powder |
| TMRC | Theoretical Maximum Residue Contribution |
| SD | Standard Deviation |
| sp | Species |
| viz., | Namely |
| WHO | World Health Organization |
| WDG | Water dispersible granules |
| WG | Wettable Granules |
| ZC | Zeon Capsules |

Introduction

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1. INTRODUCTION

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India is facing a grave danger in accidental introduction of many exotic pest species which has the higher potential to drown agrarian ecosystem. The spiralling whitefly, *Aleurodicus dispersus* Russell is one such introduction that has shattered the agricultural production system and has forced the farmers to use insecticides irrationally (Kodandaram *et al.*, 2016). It was considered as a neglected pest but in recent years it has attained the status of major pest due to its wider host range.

About 481 plants belonging to 295 genera and 90 families have been recorded as the host of spiralling whitefly around the world which comprises of several vegetables, fruits, ornamentals and avenue trees. In India, 253 plant species of 176 genera and 60 families are accounted as the host (Srinivasa, 2000). Farmers used to spray different insecticides including non-recommended ones with varying doses against this notorious pest. Excessive dependence on insecticides has resulted in resistance, ecological disturbances and higher cost to the growers. Comparing resistance levels in different location is a prerequisite while making decisions in insect pest management programme, as insecticide resistance is increasing swiftly due to the continuous use of chemicals. Several research works have been carried out across the world on the insecticide resistance against *Bemisia* tabaci Gennadius (Kranthi et al., 2002). However, the published works on insecticide resistance in spiralling whitefly, A. dispersus are so meagre even though they are causing severe damage in many crops especially vegetables. Studies conducted in College of Agriculture, Vellayani on insecticide resistance has revealed the development of resistance in different pests such as Maruca vitrata Fabricius (Sreelekshmi, 2014) in cowpea, Spodoptera litura (Fab) in amaranthus (Sreelekshmi, 2017) and Aphis craccivora Koch in cowpea (Hampaiah, 2018).

Pesticide residue in food commodities and their entry in to the food chain has become a major cause of concern all over the world. In order to assess the health hazards posed by insecticides, it is essential to study the facts about pesticide behaviour, their persistence/ dissipation in crops. Apart from dissipation studies, risk assessment studies should also be carried out to know the actual hazards caused by pesticides. Risk assessment can be described as the scientific understanding and measurement of chemical hazards and ultimately the risks associated with them.

A prevalent resistance management plan is the need of the hour for the successful management of spiralling whiteflies, as the extent of infestation by *Aleurodicus* in different crops is higher in Kerala. The present study on insecticide resistance in spiralling whitefly in Kerala is a maiden attempt. Thus the study analyses the extent of insecticide resistance in spiralling whitefly, *A. dispersus* in tomato and suggests measures to tackle insecticide resistance.

Thus the study entitled "Insecticide resistance in spiralling whitefly, *Aleurodicus dispersus* Russell (Hemiptera: Aleyrodidae) and its management was carried out with following objectives:

- To assess the insecticide resistance in field population of spiralling whitefly, A. dispersus
- To evaluate the efficacy of new generation insecticides against resistant population of A. dispersus.
- To determine the persistence and dissipation rate of new generation insecticides in tomato.

Review of Literature

2. REVIEW OF LITERATURE

The spiralling whitefly, *A. dispersus* was introduced to India from Central America during 1993 (Palaniswamy *et al.*, 1995). This alien species got acclimatized to the new habitat due to the absence of natural enemies. Wide host range and rapid spread has compelled farmers to rely on vague chemical management strategies ultimately aiding the whiteflies to develop the ability to tolerate insecticides which was lethal to them (Dhaliwal and Koul, 2017).

2.1. ORIGIN AND DISTRIBUTION OF A. dispersus

Spiralling whitefly, *A. dispersus* was considered to be originated in the wet tropics of the central and South American regions (Russell, 1965). They were reported from the following countries (Table 1)

| Insect | Countries where A. dispersus was reported | References |
|----------------------|--|------------------------|
| Spiralling whitefly, | Caribbean, Costa Rica, | Waterhouse and Norris, |
| A. dispersus | Panama, Ecaudor, Peru, | 1989 |
| | Brasil, Florida and | |
| | Canary Islands. | |
| | American Samoa, Palau, | Nechols, 1981; Lauofo |
| | Majura, Pohnpei, | and Iwamoto,1982; |
| | Mariana island, Saipan, | Kumashiro et al., 1983 |
| 1 | Western Samoa, Fiji, | |
| | Nauru, Papua New | |
| | Guinea, Kiribati, | |
| | Tokelau, Tonga | |
| | Philippines | Martin and Lucas, 1984 |

Table 1. List of countries from where A. dispersus was reported.

In India, it was first reported from Kerala in 1993. Outbreak was seen on cassava during dry season of 1993-94 (Palaniswamy *et al.*, 1995). From Kerala it spread to the adjacent places such as Kanyakumari district of Tamil Nadu. Mani and Krishnamoorthy (1996) reported the incidence in Coimbatore and Banglore on Guava. It was also reported from Chattishgarh plains (Awasthi and Tomar, 2012).

2.1.1. Host Range

A. dispersus is a polyphagous pest with wide range of host plants. The first report of spiralling whitefly from Florida was on coconut (Russell, 1965). Throughout the world host range of spiralling whitefly includes 481 host plants of 295 genera and 90 families which comprises of several vegetables, fruits, ornamentals and avenue trees. The major host includes several vegetables, fruits, ornamentals and avenue trees coming under the families' fabaceae, asteraceae, malvaceae, myrtaceae, euphorbiacae and moraceae (Srinivasa, 2000). Host plants of *A. dispersus* was documented by Rani (2004) from Instructional Farm, College of Agriculture, Vellayani and reported a total of 50 plant species of which 15 recorded high infestation. List of host plants reported from India is given in the Table 2.

| Host | Place | References |
|----------------------------|------------|---------------------------|
| Cashew and guava | Tamil Nadu | David and Regu, 1995 |
| Cassava, annona, | Kerala | Palaniswami et al., 1995; |
| banana, okra, cassia, | × | Ranjith et al., 1996 |
| citrus, chillies, coconut, | | |
| fig, guava, jasmine, | | |
| leucinia, mango, rose, | | |
| sapota, coconut, brinjal, | | |
| tomato, pepper, jack, | | |

Table 2. Host plants of A. dispersus reported from India.

| cocoa, pigeon pea, | | |
|--------------------------|------------------|--------------------------|
| papaya, guava and castor | | |
| Cassava, cashew, | Peninsular India | Mani and Krishnamoorthy, |
| mulberry and cotton | | 1999 |

2.1.2. Damage and Population Dynamics of A. dispersus.

Whiteflies cause damage to the plants mainly by sucking the sap. Both the nymphs and the adults of spiralling whitefly colonises the abaxial surface of the leaves leading to yellowing of the leaves. In case of severe colonisation, they are also seen on the upper surface. The major symptoms that are observed in plants are yellow speckling, crinkling and curling of the leaves. Nymphs produce white waxy flocculent material which creates nuisance as it spreads readily by wind. Nymphs also excrete sticky honeydew which harbours sooty mould fungus, *Capnodium* spp. and reduces photosynthetic capacity of the plant (Geetha, 2000).

Population of spiralling whitefly are severely affected by the weather parameters. Reduction in population can be observed at the time of heavy sporadic rains and cool temperature. Population density of spiralling whitefly was at its peak during March- June in Karnataka where they recorded positive correlation to maximum temperature and negative correlation to relative humidity (Mani and Krishnamoorthy, 2000). According to the study conducted by Vijayasree *et al.* (2011) in Kerala, spiralling whitefly was observed to be a dry season pest with its major occurrence in the field from February to June and the highest infestation was recorded during May. It was also inferred that high temperature favoured the population build-up while high rainfall suppressed it.

2.1.3. Extent of Crop Loss

According to Wen et al. (1995), 80 per cent fruit loss was reported from guava in Taiwan. Ranjith et al. (1996) noticed severe damage to many crops by

spiralling whitefly in Kerala. In Tamil Nadu 53.10 per cent yield reduction was noticed in tapioca (Geetha, 2000) and 28.09 per cent loss in mulberry leaf yield which subsequently resulted in cocoon yield loss up to 48.09 per cent and 71.31 per cent monetary loss (Qadri *et al.*, 2010). Yield loss of 2.39 to 14.76 per cent was reported in coccinia from Kerala (Vijayasree *et al.*, 2011).

2.2. BIOLOGY OF SPIRALLING WHITEFLY

Adults of spiralling whitefly are white, much larger than other whitefly species with body length greater than 2- 3mm with dark reddish-brown eyes. Eggs are laid at right angles to the midrib of the leaves forming a spiralling pattern. They are elliptical smooth and yellow in colour. Adult longevity was found to be 39 days under laboratory condition (Waterhouse and Norris, 1989). Females lay 14-26 eggs which hatch in 7 to 10 days (Wijesekara and Kudagamage, 1990). Wen *et al.* (1994) observed shortened life span of 17 to 18.5 days with rise in temperature from 15 to 30°C. The total nymphal and pupal period ranges from 12 to 14 days and 2 to 3 days respectively (Geetha, 2000).

According to Rani (2004), egg period observed were 5.80 ± 0.60 days on cassava, 7.60 ± 0.49 days on tomato and 6.00 ± 0.45 days on chilli. There are four nymphal instars, where first instar or crawlers have functional legs while the other three are sedentary. The crawler period was found to be more on tomato (5.80 ± 0.40 days) when compared to cassava and chilli.

2.3. MANAGEMENT OF WHITEFLY

Chemical management of spiralling whitefly is necessary at the initial stages so as to manage the heavy infestation. In cassava 92.66 to 98.61 per cent mortality was obtained by the application of triazophos against spiralling whitefly (Geetha, 2000). Triazophos 40 EC at 0.06 %, dimethoate 30 EC at 0.05 % and profenofos was found to manage spiralling whitefly effectively in mulberry (Kumari, 2011). Spinosad 45 SC @ 0.2 ml L⁻¹ and cynantraniliprole 10 OD @ 0.3 ml L⁻¹ showed 96.19 per cent adult mortality at 48 hours after treatment (HAT) which was similar to indoxacarb 15.8 EC @ 0.3 ml L^{-1} which resulted in 92.37 per cent adult mortality during rabi season (Pushpalatha and Balikai, 2015).

On cassava acephate and triazophos were found to be efficient in reducing 90 per cent spiralling whitefly population (Boopathi *et al.*, 2017). According to Mani (2017), dichlorovos 0.08 % was obtained as the best chemical against the various stages of whitefly along with clothianidin, imidacloprid and thiamethoxam. Kumar and Singh (2018) reported that imidacloprid was most effective in minimising whitefly population (78.28 per cent) followed by thiamethoxam and acetamiprid. Management of other whitefly species are given in Table 3.

| Species | Crop | Chemicals Recommended | Reference |
|-----------|---------|--|-----------------------------------|
| B. tabaci | Brinjal | Profenofos 10 EC @800 ml ha ⁻¹ | Singh <i>et al.</i> , 2003 |
| B. tabaci | Cotton | Bifenthrin 10 EC @ 1000 ml ha ⁻¹ Indoxacarb 14.5 SC @ 500 ml ha ⁻¹ | Balakrishnan <i>et al.</i> , 2009 |
| B. tabaci | Brinjal | Fipronil 50 SC @50 g a.i ha ⁻¹ Indoxacarb 14.5 SC @ 770 g a.i ha ⁻¹ Bifenthrin 10 EC @ 25 g a.i ha ⁻¹ | Sinha and Viswanath, 2011 |
| B. tabaci | Brinjal | Acephate 75 SP @ 0.75 g L ⁻¹ Imidacloprid 17.8 SL @ 0.75 ml L ⁻¹ | Konar <i>et al.</i> , 2011 |
| B. tabaci | Brinjal | Spiromesifen 0.024% Diafenthiuron 0.05% Triazophos 0.08% | Shaikh <i>et al.</i> , 2014 |
| B. tabaci | Brinjal | Imidacloprid 70 WG @ 0.2 g L ⁻¹ Fipronil 50 SC @ 2 ml L ⁻¹ Buprofezin 40 SC @ 2 ml L ⁻¹ | Das and Islam, 2014 |
| B. tabaci | Brinjal | Imidacloprid 17.8 SL Acephate 75 SP | Yadav and Kumawat, 2014 |
| B. tabaci | Brinjal | Imidacloprid 17.8 SL @ 0.004% Dimethoate 30 EC @ 0.03% | Bharati et al., 2015 |

Table 3. Management of different whitefly species.

| B. tabaci | Tomato | Imidacloprid @ 20 g a.i ha ⁻¹ | Jha and Kumar, |
|-----------|---------|--|--------------------|
| | | Profenophos 40% + Cypermethrin 4% | 2017 |
| | | @ 44 g a.i ha ⁻¹ | |
| B. tabaci | Brinjal | Thiamethoxam 25 WG @ 100 g ha ⁻¹ | Kumar et al., 2017 |
| | | Imidacloprid 17.8 SL @ 100 ml ha ⁻¹ | |
| B. tabaci | Okra | Imidacloprid 17.8 SL @ 35.6 g a.i ha ⁻¹ | Berwa et al., 2017 |
| | | Diafenthiuron 50 WP $@$ 300 g a.i ha ⁻¹ | |
| B. tabaci | Chilli | Acetamiprid 20 SP @ 0.004% | Mokal et al., 2018 |
| | | Thiamethoxam 25 WG @ 0.01% | |
| | | Spinosad 45 SC @ 0.014% | |

2.4. INSECTICIDE RESISTANCE IN WHITEFLIES

Extensive use of insecticides has resulted in the phenomena known as insecticide resistance which has led to the failure of many conventional insecticides (Denholm *et al.*, 1998) Insecticide resistance as defined by IRAC refers to a heritable character in an insect that provides unsuccessful control by an insecticide when applied at the recommended dose (IRAC, 2018).

Insecticide resistance is observed in whiteflies of which reports on resistance in *B. tabaci* are more. World-wide studies have been conducted in *B. tabaci* about its resistance to organophosphate, carbamates, pyrethroids, neonicotinoids and other new generation molecules (Ahmed *et al.*, 2002). It is considered to be fifth top resistant insect species, which has got resistance to 54 insecticides (Sparks and Nauen, 2015).

2.4.1. Resistance to Cyclodienes, Organophosphates and Carbamates.

Resistance of whiteflies to cyclodienes, organophosphates and carbamates are presented in Table 4.

| Pest | Host plant | Location | Insecticide | Intensity of resistance | Reference |
|--------------|---------------|----------|------------------------------|-------------------------------|--------------------------|
| B. tabaci | Cotton | Israel | Monocrotophos, | 6-9 fold | Byrne <i>et</i> |
| | | | profenofos and chlorpyriphos | | al.,1994 |
| B. tabaci | Cotton | Israel | Methomyl | 5- fold | Horowitz et al., 1994 |
| B. tabaci | Cotton | Pakistan | Monocrotophos, | 105- fold | Cahill et |
| Biotype -A | | | Profenofos, | 56- fold, | al., 1995 |
| 2 (M) | | | Chlorpyriphos | 67- fold | |
| Bemisia | Melon | USA | Endosulfan and | 1 to 1.5- | Prabhakar |
| argentifolia | | | Chlorpyriphos | fold | et al., 1997 |
| B. tabaci | Cotton | India | Oxydemeton | 59-66- | Singh et |
| | | | methyl | fold | al., 1999 |
| B. tabaci | Squash | Pakistan | Dimethoate | 324-fold | Ahmed et |
| | Cotton | | | 782-fold | al., 2002 |
| | Brinjal | | | 283-fold | |
| B. tabaci | Cotton | Pakistan | Monocrotophos | 68- fold | Ahmed et |
| | | | | | al., 2002 |
| B. tabaci | Brinjal | Pakistan | Acephate | 550-fold | Ahmed et |
| | | | | | al., 2002 |
| B. tabaci | Cotton | India | Methomyl | 15-80 Fold | Kranthi et |
| | | | Monocrotophos | 6-13 fold | al., 2001 |
| B. tabaci | Tomato | China | Chlorpyriphos | 8.94-fold | Wang et |
| | | | | | al., 2017 |

Table 4. Resistance of whiteflies against cyclodienes, organophosphates and carbamates

2.4.2. Resistance to Synthetic Pyrethroids

B. tabaci collected from Poinsettia in UK had 160-fold resistance to cypermethrin, 110-fold to bifenthrin and 380-fold against etofenprox (Cahill *et al.*, 1995). Resistance of 550-fold was recorded against deltamethrin in whiteflies collected from cotton in Pakistan followed by 35 and 19- fold resistance to bifenthrin and fenpropathrin in brinjal respectively and 19-fold resistance to lambda-cyhalothrin (Ahmed *et al.*, 2002). According to Kranthi *et al.* (2002), in North India *B. tabaci* from cotton showed 5-45-fold resistance to cypermethrin.

In China, whitefly *B. tabaci* collected from cotton, tomato and capsicum has showed a resistance factor of 7-86 fold against bifenthrin and 20-246 fold against cypermethrin (Luo *et al.*, 2010). Wang *et al.* (2017) reported 9.54-fold resistance to bifenthrin by *B. tabaci* collected from Hunan region.

2.4.3. Resistance to New Generation Insecticides

Neonicotinoids has replaced the older chemicals for the management of whiteflies of which Imidacloprid is the popular one. They represent a class of novel insecticides. After the widespread use of these chemicals, resistance build up took place at a faster rate due to its systemic action and persistent nature (Mullins, 1993).

B. tabaci collected from three different hosts' melon, lettuce and cole crops from Imperial valley, California were studied for its resistance development to imidacloprid. Here the resistance development was at a slow pace of 4-folds at F4 generation, 34-folds at F16, 78-folds by F24 and the maximum was obtained as 82fold at F27 (Prabhakar *et al.*, 1997). According to Horowitz *et al.* (1999), *B. tabaci* from cotton and greenhouse ornamental crops showed 5 to 10-fold resistance to acetamiprid after three years of infestation. *B. tabaci* in greenhouse vegetables from Spain reported 116-fold resistance to imidacloprid, 100-fold against thiamethoxam and 74-fold to acetamiprid (Nauen *et al.*, 2002). In China *B. tabaci* from different provinces were tested for its resistance factor to thiamethoxam and imidacloprid by Wang *et al.* (2010). Strains from city of Yangcheng reported 1900 and 1200- fold resistance to imidacloprid and thiamethoxam respectively, Jiangsu Province had 38-86 and 29-120- fold resistance, 1100 and 520- fold resistance from Zhejiang province and 450 and 300- fold resistance from Yunnan province for imidacloprid and thiamethoxam. *B. tabaci* from host capsicum had 33- fold resistance to acetamiprid, 83.8- fold to imidacloprid and greater than 166- fold resistance to thiamethoxam (Luo *et al.*, 2010). 15-fold resistance was reported against acetamiprid in Israel in *B. tabaci* collected from cotton plant (Horowitz and Ishaaya, 2014). *B. tabaci* obtained from three hosts tomato, pepper and cucumber in China showed 59.93-fold resistance to imidacloprid, 48.56- fold to thiamethoxam, 58.22-fold to acetamiprid and 124.96-fold to nitenpyram.

Resistance to buprofezin was first detected in Netherland from greenhouse crops. Buprofezin is thiadiazine chitin-synthesis inhibitor widely used for controlling *B. tabaci* in cotton fields. From China *B. tabaci* biotype Q showed 3.75-fold resistance to abamectin and 11- fold resistance to pyriproxyfen, a phenyl ether juvenile hormone mimic (Denholm *et al.*, 1998). Resistance to buprofezin was reported in glass house whitefly, *Trialeurodes vaporarium* Westwood by Gorman *et al.* (2002). According to Wang *et al.* (2010), low resistance factor to Spinosad (0.5 to 6.4) was obtained and none showed resistance to abamectin whereas 2 to 25-fold resistance was obtained against fipronil in China.

B. tabaci collected from ornamentals in greenhouse in Israel showed 12fold resistance to buprofezin (Horowitz *et al.*, 1994). In case of pyriproxifen 550fold resistance was shown by *B. tabaci* collected from rose in Israel (Denholm *et al.*, 1998). *B. tabaci* from cotton in Israel also showed 500-fold resistance against pyriproxifen (Horowitz and Ishaaya, 2014).

2.5. DISSIPATION OF PESTICIDE RESIDUE IN DIFFERENT CROPS

Table 5 presents various reports on persistence and degradation of insecticides on various crops.

2.6. RISK ASSESSMENT OF INSECTICIDES

Risk assessment studies are purely theoretical calculations to confirm the safety of the consumer when provided with agricultural products treated with chemical pesticides. Study conducted by Kumar *et al.* (2018) reported that chilli (both dried and fresh) sprayed with thiacloprid was safe for consumption at the day of application as Theoretical maximum residue contribution (TMRC) was lower than maximum permissible intake value (MPI). Risk assessment studies conducted by Padmanabhan (2018) on cabbage and cauliflower from plain and hills treated with chlorantraniliprole, flubendiamide, indoxacarb, quinalphos, cypermethrin, acetamiprid and thiamethoxam were safe but insecticides *viz.*, fipronil and diamethoate were observed to be unsafe as TMRC values, 72.8, 38.4 and 13.6 μ g person⁻¹ day⁻¹.

| T | | | | | | |
|----------|--------------------|---------------------------|---------------|----------|------------|--------------------------|
| | | ſ | Initial | Days | -J.1 J1-11 | |
| Cron | Incertivides | Dosage | Concentration | taken to | Half- life | References |
| CIOD | | (g a.i ha ⁻¹) | (ma ha-1) | reach | (Days) | |
| | | | (Sy Sm) | BDL | | |
| Chilli | Dimethoate | 300 | 0.33 | 15 | 4.7 | Reddy et al 2007 |
| | Lambda cyhalothrin | 50 | 0.62 | 15 | 6.4 | |
| Tomato | Thiaclourid | 48 | 0.76 | 10 | I | Singh and Dikshit. 2007 |
| | and or and | 96 | 1.38 | 10 | 1 | |
| Tomato | Thiamethovam | 140 | 0.18 | 10 | 4.2 | Karmakar and |
| | | 280 | 0.30 | 10 | 3.5 | Kulshrestha, 2009 |
| | Spiromesifen | 100 | 0.609 | 10 | 1.71 | |
| Chilli | Imidacloprid | 17.8 | 1.27 | 10 | 2.08 | Varghese, 2011 |
| | Acetamiprid | 20 | 2.44 | 20 | 2.27 | |
| Tomato | Acetamiprid | 2.5 g L ⁻¹ | 0.98 | 15 | 1.04 | |
| | Dinotefuran | 1.25 g L ⁻¹ | 0.83 | 15 | 1.72 | El din <i>et al</i> 2012 |
| Cucumber | Acetamiprid | 2.5 g L ⁻¹ | 1.26 | 15 | 1.18 | |
| | Dinotefuran | 1.25 g L ⁻¹ | 0.53 | 15 | 3.18 | |
| | | | | | | |

Table 5. Dissipation of insecticides in vegetables

| | Acetamiprid | | 15 | 0.42 | 10 | 2.55 | |
|----------------|---------------|--------------|------|------|----|------|------------------|
| Commo | Imidacloprid | | 20 | 0.32 | 3 | 0.82 | |
| Cowpea | Thiamethoxam | | 25 | 0.89 | 10 | 2.55 | |
| | Thiacloprid | | 24 | 0.37 | 5 | 2.80 | Thamilarasi 2016 |
| | Acetamiprid | | 15 | 0.42 | 10 | 2.55 | |
| Salad | Imidaclorprid | | 20 | 0.18 | 3 | 0.66 | |
| cucumber | Thiamethoxam | | 25 | 0.16 | 7 | 1.39 | |
| | Thiacloprid | | 24 | 0.13 | 3 | 1.28 | |
| A monomphie | Quinalphos | | 250 | 1.75 | 15 | 3.35 | Sreelekshmi 2017 |
| AIIIai ainiius | Thiacloprid | | 120 | 3.17 | 5 | 0.98 | |
| | Onder Control | Plains | 500 | 0.24 | 5 | 1 | Padmanahhan 2018 |
| Cabbage | Quinalphos | Hills | 500 | 2.66 | 7 | 1 | |
| Cowpea | Thiacloprid | | 24 | 0.39 | 7 | 4.37 | |
| | Lambda | Lambda | 27.5 | 0.19 | 3 | 0.31 | |
| | cyhalothrin + | cyhalothrin | | | | | Mammin 2018 |
| | thiamethoxam | thiamethoxam | | 0.42 | 3 | 0.37 | 11auptau, 2010 |
| | | | | | | | |
| | Thiamethoxam | | 25 | 2.21 | 7 | 1.21 | |

Materials and Methods

3. MATERIALS AND METHODS

The study on "Insecticide resistance in spiralling whitefly, *A. dispersus* (Hemiptera: Aleyrodidae) and its management" was conducted to assess the insecticide resistance in the field population of spiralling whitefly, *A. dispersus* and to manage it with new generation insecticides. Population of spiralling whitefly was collected mainly from three different locations, College of Agriculture, Vellayani, farmer's field at Kalliyoor and Sreekaryam. The laboratory and field experiments on insecticide resistance were conducted at Department of Agricultural Entomology, College of Agriculture, Vellayani and farmer's field at Kalliyoor. Studies on dissipation of residues were carried out at Pesticide Residue Research and Analytical laboratory, College of Agriculture, Vellayani.

3.1 DOCUMENTATION OF WHITEFLIES IN DIFFERENT HOSTS

Whiteflies belonging to different genera in various crops were observed and documented during January- August 2018.

3.2ASSESSMENT OF INSECTICIDE RESISTANCE IN FIELD POPULATIONS OF SPIRALLING WHITEFLY *A. dispersus* IN TOMATO

Adults of spiralling whitefly, *A. dispersus* were collected from three different locations. The first population was taken from a field with no pesticide application (Sreekaryam). The second population was from a field where insecticides were applied but no known reports of control failure (Kalliyoor) and the third population was taken from a field where application of insecticides and control failure were observed (College of Agriculture, Vellayani). Three insecticides from three different groups with different mode of action was selected to test the resistance/ susceptibility of the populations (Table 6). The three different fields and insecticides selected were based on the previous survey conducted during 2017-18 (Sreelekshmi, 2017; Padmanabhan, 2018; Hampaiah, 2018)

Table 6. Details of insecticides used for resistance study.

| | Dosage (g a.i ha ⁻¹) | 250 | 25 | 20 |
|-------------------------|-------------------------------------|---|---------------------------|--|
| | Mode of Action as per IRAC,2018 | Acetylcholine esterase (AChE) inhibitors | Sodium channel modulators | Nicotínic acetylcholine receptor (nAChR) competitive modulators |
| Details of Insecticides | Chemical group | Organophosphates | Synthetic pyrethroids | Neonicotinoids |
| | Trade name | Ekalux | Fenval | Confidence 555 |
| | Chemical Name | Quinalphos 25% EC | Fenvalerate 20% EC | Imidacloprid 17.8% SL |

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3.2.1 Study on the Toxicity of Selected Insecticides to *A. dispersus* Collected from Different Locations

Design: CRD

Treatments-22 (Three insecticides, each at seven different concentrations + control) Replications- 3

Table 7 shows the details of treatments with doses.

Tomato seedlings were raised without applying any insecticides. Leaf dip method was followed for conducting bioassay (Sreelekshmi, 2017). A series of concentrations of each commercial insecticide were prepared in aqueous solution and the tomato leaves were dipped for 25 seconds in each treatment and shade dried. After proper drying, the leaves were placed in plastic jars and twenty adult whiteflies from each location were released. Leaves dipped in water were considered as the control. Mortality was noted after 0.8, 1, 3, 6, 12 and 24 HAT and was confirmed by probing the adult whiteflies with soft camel hair brush. Whiteflies failing to show coordinated forward movement were considered dead. Abbott's formula (Abbott, 1925) was used to calculate the percentage mortality.

| | | Observed mortality i | n treatment – Observ | ed mortality in |
|-----------|---|----------------------|----------------------|-----------------|
| Corrected | = | | contr | ol ×100 |
| mortality | | 100 | - Observed mortality | in control |

The observed mortality was used to calculate relative toxicity to these chemicals in terms of LC_{50} and LC_{90} . Toxicity values LC_{50} and LC_{90} were calculated using probit analysis (Finney, 1971). The population showing the lowest LC_{50} was considered as susceptible population (reference strain).

Mortality Percentage = $a \times x^b$

LC $_{50} = \exp(\log_{50} - a) \div b$ LC $_{90} = \exp(\log_{90} - a) \div b$

x= Concentration of insecticide

a= intercept

b= regression coefficient

| Treatment | Insecticides | Dose (%) |
|-----------------|-----------------|----------|
| T ₁ | Quinalphos | 0.02 |
| T ₂ | Quinalphos | 0.03 |
| T ₃ | Quinalphos | 0.04 |
| T4 | Quinalphos | 0.05 |
| T5 | Quinalphos | 0.06 |
| T ₆ | Quinalphos | 0.07 |
| T ₇ | Quinalphos | 0.08 |
| T ₈ | Fenvalerate | 0.0013 |
| T9 | Fenvalerate | 0.0025 |
| T ₁₀ | Fenvalerate | 0.005 |
| T ₁₁ | Fenvalerate | 0.01 |
| T ₁₂ | Fenvalerate | 0.02 |
| T ₁₃ | Fenvalerate | 0.03 |
| T ₁₄ | Fenvalerate | 0.04 |
| T ₁₅ | Imidacloprid | 0.002 |
| T ₁₆ | Imidacloprid | 0.003 |
| T ₁₇ | Imidacloprid | 0.004 |
| T ₁₈ | Imidacloprid | 0.005 |
| T ₁₉ | Imidacloprid | 0.006 |
| T ₂₀ | Imidacloprid | 0.007 |
| T ₂₁ | Imidacloprid | 0.008 |
| T ₂₂ | Control (Water) | |

Table 7. Details of different treatments for resistance study.

Fiducial limits were calculated using

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

Resistance ratio = LC_{50} or LC_{90} of resistance population

LC50 or LC90 of susceptible population

Further study was carried out using the resistant population.

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

Population of *A.dispersus* which was found resistant to three insecticides were used for the evaluation of new generation insecticides. Seven insecticides were tested against the resistant population at recommended dose to find its efficacy. The details of these insecticides are given in Table 8.

Design: CRD

Treatments: 8

Replications: 3

The laboratory evaluation was done as in experiment 3.2 and mortality was noted after 0.25, 0.5, 0.75, 1, 1.25 and 1.5 HAT. Percentage mortality was calculated using Abbott's formula (Abbott, 1925)

3.4 FIELD EVALUATION OF SELECTED NEW GENERATION INSECTICIDES AGAINST POPULATION OF WHITEFLIES.

3.4.1 A. dispersus

Three effective insecticides from experiment 3.3 were further tested in field to test their efficacy in managing the resistant population of *A.dispersus*.

Design: RBD Treatments: 4 Replications: 5

Table 8. Details of new generation insecticides used for the management of resistant population of A. dispersus in laboratory.

| Treatment | | | Details of Insecticides | | | |
|-----------|--|------------|---|-----------------------------------|---------------------------|---------------------|
| No | Chemical Name | Trade Name | Mode of action as per IRAC,2018 | Chemical group | Dosage | Field Dose |
| | | | | | (g a.i ha ⁻¹) | (ml or g L^{-1}) |
| T1. | Buprofezin 25% SC | Apple | Inhibitors of chitin biosynthesis, type 1 | Insect growth regulator | 75 | 0.6 |
| Т2. | Clothianidin 50%WDG | Dantostu | Nicotinic acetylcholine receptor (nAChR) competitive modulators | Neonicotinoids | 20 | 0.08 |
| Т3. | Cynantraniliprole 10.26%OD | Benevia | Ryanodine receptor modulators | Diamides | 06 | 1.8 |
| Τ4. | Dinotefuran20%SG | Osheen | Nicotinic acetylcholine receptor (nAChR) competitive modulators | Neonicotinoids | 25 | 0.3 |
| T5. | Flonicamid 50%WG | Ulala | Chordotonal organ modulators - undefined target site | Flonicamid | 75 | 0.3 |
| T6. | Thiamethoxam 25%WG | Actara | Nicotinic acetylcholine receptor (nAChR) competitive modulators | Neonicotinoids | 50 | 0.2 |
| Т7. | Thiamethoxam 12.6% + Lambda cyhalothrin9.5%ZC | Alika | Nicotinic acetylcholine receptor (nAChR) competitive modulators and Sodium channel modulators | Neonicotinoids and pyrethroids | 33+15.75 | 0.3 |
| T8. | Control (water) | | | | | |

The experiment was done at College of Agriculture, Vellayani from where the resistant population was collected. Details of the treatments are given in Table 9.

| Treatment | Chemical Name | Trade | Dosage | Field Dose |
|----------------|---------------------|----------|---------------------------|---------------------|
| No | | Name | (g a.i ha ⁻¹) | (ml or g L^{-1}) |
| T ₁ | Thiamethoxam 12.6% | Alika | 33+15.75 | 0.30 |
| | + lambda | | | |
| | cyhalothrin 9.5% ZC | | | |
| T ₂ | Clothianidin 50% | Dantostu | 20 | 0.08 |
| | WDG | | | |
| T ₃ | Flonicamid 50% WG | Ulala | 75 | 0.30 |
| T ₄ | Control - water | | | |

Table 9: Details of the treatment for field evaluation

3.4.2 Aleurothrixus trachoides

The efficacy of new generation insecticides mentioned in experiment 3.4.1 was tested against the population of *A. trachoides* in tomato which was seen along with *A. dispersus* in the field.

The number of adult whiteflies were estimated by counting individuals on the abaxial and adaxial surface of three leaves from top, middle and bottom regions of the plant. (Boopathi *et al.*, 2017).

3.5 ESTIMATION OF INSECTICIDE RESIDUE IN TOMATO FRUITS.

Experimental plot (Plate 1) was laid out at farmer's field, Kalliyoor.

Design: RBD

Treatments: 4

Replications: 5

Three effective insecticides observed in field experiment for the management of spiralling whitefly, *A. dispersus* were sprayed on the tomato plants at the time of



Plate 1. View of experimental plot planted with tomato

fruit initiation. Second spray was given 10 days after first spray and samples were taken at a time interval of 0, 1, 3, 5, 7, 10, 15 and 30 days after spraying. Plants in the control were treated with water. The determination of pesticide residue was carried out at 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 (CRM) of lambda cyhalothrin, thiamethoxam, clothianidin and flonicamid were purchased from Sigma- Aldrich Pvt. Ltd. Acetonitrile, water, methanol (HPLC grade), sodium chloride and anhydrous sodium sulphate were supplied from Merck, Germany. Primary secondary amine 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^o C for 4 h and kept in desiccators. Commercial formulations of these chemicals were purchased from local market.

3.5.1.2 Preparation of Standards

Standard stock solutions of clothianidin, flonicamid 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 (1, 0.5, 0.25, 0.1, 0.05, 0.025, 0.01 μ g ml⁻¹) of standard solutions from stock solution by serial dilution. All standard solutions were stored at -20^oC 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, 0.1 and 0.25 mg kg⁻¹) of analytical standards of lambda cyhalothrin and thiamethoxam, clothianidin, and flonicamid in untreated tomato fruits. Three replicates were

analysed 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 Insecticide

Tomato fruit samples were collected as explained in 3.5. Samples were chopped, crushed, sub-sampled and extracted following the QuEChERS (Quick, easy, cheap, effective, rugged, safe) method (Sharma, 2013). The residue estimation of thiamethoxam, clothianidin and flonicamid were done in LC-MS/MS (Liquid chromatography- Mass spectrometer) and lambda cyhalothrin in GC- ECD (Gas chromatography- electron capture detector).

3.5.2.1 Extraction and Clean up

Ground tomato fruit samples (25 g) were taken in a 250 ml centrifuge bottle. The analyte was extracted by adding 50 ml acetonitrile of HPLC grade. After proper shaking 10 g activated sodium chloride was added. This centrifuge bottle was closed tightly and centrifuged at 8000 rpm for 8 min. 16 ml supernatant was pipetted out and added to 50 ml centrifuge tube with 6 g activated sodium sulphate. It was then vortexed and 12 ml was pipetted out to 15 ml centrifuge tube containing 0.2 g PSA and 1.2 g magnesium sulphate. After mixing it with the help of vortex the mixture was centrifuged at 2500 rpm for 5 minutes. 4 ml was transferred to a turbo tube for GC- ECD analysis and 3 ml was pipetted for LC-MS/MS analysis. These tubes were evaporated using turbovap which uses a gentle steam of nitrogen at 40 °C and 7.5 psi nitrogen flow. The residue was reconstituted in 1.5 ml of methanol and filtered through 0.2 micron PVDF filter prior to estimation in LC-MS/MS and to 1 ml of n- hexane for GC-ECD analysis.

3.5.2.2 Instrumentation

LC-MS/MS

The chromatographic separation was done using Waters Acquity UPLC system equipped with a reverse phase Atlantis d c-18 (100×2.1 mm, 5μ m particle

size) column. The mobile phase for the separation of residues as a gradient system involved two components: (A) 10% methanol in water + 0.1% formic acid+ 5mM ammonium acetate; (B) 10% water in methanol +0.1% formic acid +5mM ammonium acetate. The gradient elution was done as: 0 min isocratic 20% B, increased to 90% in 4min, then raised to 95% with 5 min and 100% B in 9min which was decreased to the initial composition of 20% B in 10min and hold till 12 min for re- equilibrium. The flow rate remains constant at 0.8ml min⁻¹ and injection volume was 10µL. The column temperature was maintained at 40°C and effluent from the LC system was introduced into triple quadrapole API 3200MS/MS system equipped with an electrospray ionisation interface (ESI), operating in the positive ion mode. The source parameters were temperature 600 °C, ion gas (GSI) 50 psi, ion gas (GS2) 60psi, ion spray voltage 5500 V, curtain gas 13psi.

GC-ECD

Estimation of residues for lambda cyhalothrin was done by Gas chromatograph equipped with Electron capture detector (ECD). It includes column, DB-5 capillary (0.25µm film thickness X 0.25mm X 30m), carrier gas- Nitrogen, column flow- 0.79ml min⁻¹, injector temperature -250 °C and detector temperature 300 °C. Helium was used as carrier gas in GC-MS operated with electron impact ionisation (70eV). In GC-MS, injector temperature, column, column flow was similar to that of GC.

The MS/MS condition were optimised using direct infusion in to ESI source in positive mode to provide the 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 quantification ion chromatogram and for qualitative purpose. The ion source temperature was 550°C with ion spray voltage of 5500V. 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 given below.

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

Volume of the solvent added (ml) \times Final volume of extract (ml)

Dilution factor =

Weight of the sample $(g) \times$ Volume of extract taken for

Concentration (ml)

Half- life (DT_{50}) is the time taken for the disappearance of pesticide to 50 per cent of its initial concentration of each insecticide was found out using dissipation data.

3.5.3 Estimation of Harvest Time Residues

Fruits were harvested from each treatment for pesticide residue analysis as explained in 3.5. Harvest time residues in tomato fruit was determined using LCMS/MS and GC-ECD techniques as per the pesticide residue analysis manual of ICAR (Sharma, 2013).

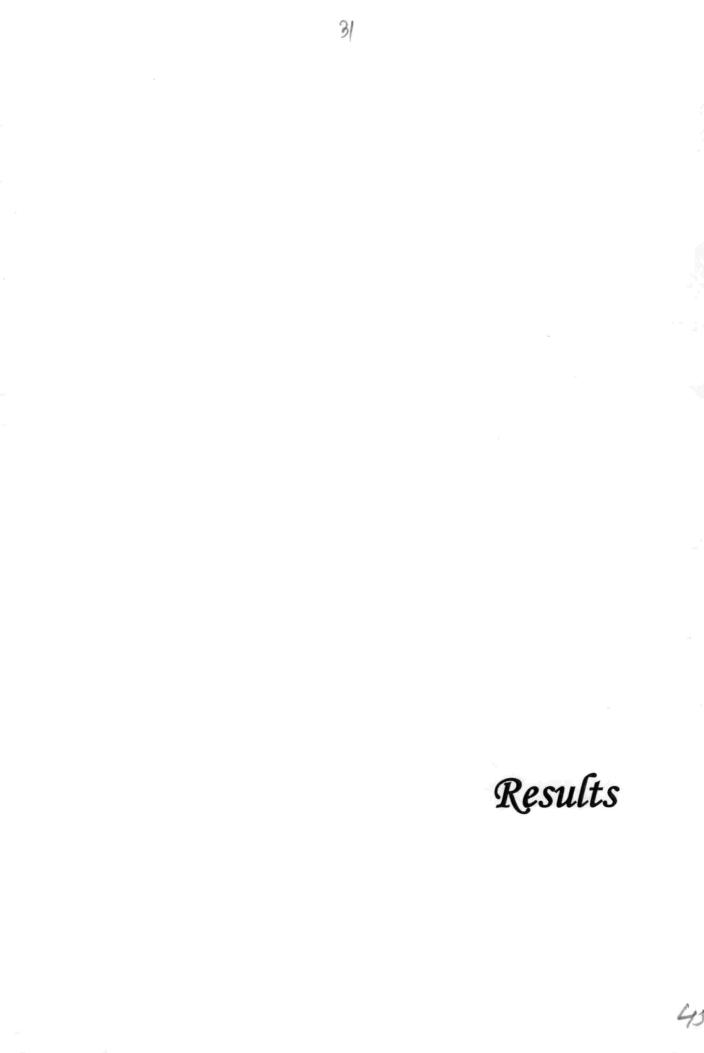
3.5.4 Risk Assessment of Different Insecticides in Tomato Fruit

Dietary risk of selected insecticides were estimated using Acceptable daily intake (ADI), Maximum permissible intake (MPI) and theoretical Maximum residue contribution (TMRC). Daily consumption of tomato was considered as 50 g per day (NSSO, 2014). Acceptable daily intake for each insecticides for calculating MPI has already been fixed by WHO and average body weight of an adult human being was considered as 60 kg (Katna *et al.*, 2017). Based on the residue values, TMRC were calculated and health impact of insecticide was analysed. If TMRC is less than MPI, the insecticide will not cause any harm to health.

TMRC= Maximum residue level obtained at recommended dose × total intake of

food per day

MPI= Acceptable daily intake × average body weight of an adult



4. RESULTS

4.1. DOCUMENTATION OF WHITEFLIES IN DIFFERENT HOSTS

Whiteflies belonging to different genera documented from various crops are presented in table 10 and plate 2-7. The whitefly species observed were rugose spiralling whitefly, *Aleurodicus rugioperculatus* Martin, spiralling whitefly, *A. dispersus* and solanum whitefly, *Aleurothrixus trachoides* Back. Major host plants of spiralling whitefly recorded were tomato, chilli, cassava, papaya, mulberry, gauva and cera rubber.

The host species of rugose spiralling whitefly included banana, coconut, country badam, teak and jackfruit. The solanum whitefly was observed on tomato and chilli.

4.2. ASSESSMENT OF INSECTICIDE RESISTANCE IN FIELD POPULATION OF SPIRALLING WHITEFLY *A. dispersus* IN TOMATO.

The results showing the lethal concentration to kill 50% (LC₅₀) and 90% (LC₉₀) of population of *A. dispersus* and resistance ratio with reference to the susceptible population are given in the Tables 11-13.

4.2.1. Quinalphos

Whitefly population from Sreekaryam recorded the lowest LC_{50} value of 35.39 ppm with upper fiducial limit 41.13 ppm and lower fiducial limit 27.53 ppm. Population gathered from Vellayani had the highest LC_{50} value of 92.11 ppm with upper and lower fiducial limits 126.99 ppm and 77.49 ppm respectively. While whitefly population from Kalliyoor recorded a LC_{50} value of 40.65 ppm with upper and lower fiducial limits being 45.43 ppm and 34.85 ppm respectively (Table 11).

Similarly, LC₉₀ was also calculated for all the three populations. The lowest LC₉₀ value was shown by population from Sreekaryam which was 103.31 ppm with upper fiducial limit of 129.52 ppm and lower of 93.42 ppm. The highest LC₉₀ value was observed for whitefly population from Vellayani (208.74 ppm) with upper fiducial limit, 336.32 ppm and lower fiducial limit, 159.62 ppm followed by LC₉₀

Table 10: Whitefly species and their hosts.

| | Common Name | Scientific Name | Host] | Host Plants |
|---|---------------------|--------------------------|---------------|--------------------------|
| | | · | Crop | Scientific Name |
| 1 | Spiralling whitefly | Aleurodicus dispersus | Tomato | Solanum lycopersicum |
| | la. | | Chilli | Capsicum frutescens |
| | | | Cassava | Manihot esculenta |
| | | | Papaya | Carica papaya |
| | | | Mulberry | Morus rubra |
| | | | Guava | Psidium guajava |
| | | | Cera rubber | Hevea sp. |
| | Rugose spiralling | Aleurodicus | Banana | Musa sp. |
| | ATTOINTM | i ugioper cuiuius | Coconut | Cocus nucifera |
| | , | | Country badam | Terminalia catappa |
| | | | Teak | Tectona grandis |
| - | | | Jack | Artocarpus heterophyllus |
| | Solanum whitefly | Aleurothrixus trachoides | Tomato | Solanum lycopersicum |
| | | | Chilli | Capsicum frutescens |

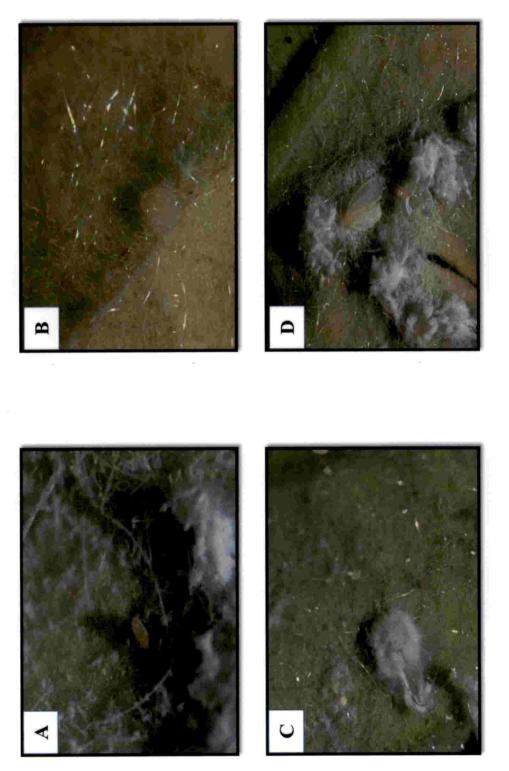
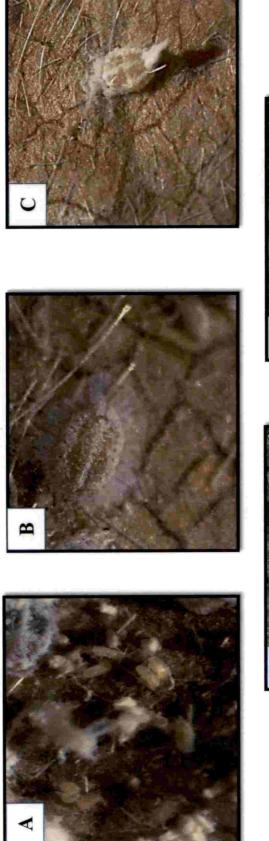


Plate 2. Spiralling whitefly, A. dispersus; (A) Egg; (B) and (C) Nymphal stages; (D) Adult

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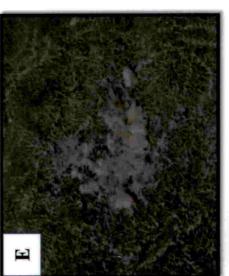
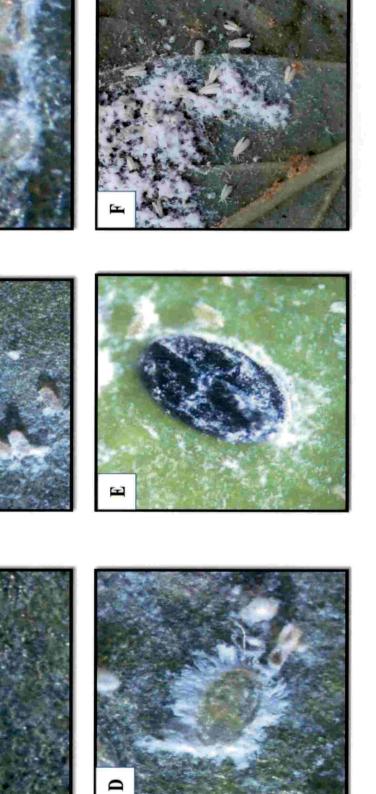




Plate 3. Rugose spiralling whitefly, A. rugioperculatus; (A) Egg; (B) and (C) Nymphal stages; (D) Pupa (E) Adult



B

Plate 4. Solanum whitefly, A. trachoides; (A) Egg; (B) (C) and (D) Nymphal stages; (E) Pupa (F) Adult



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(Psidium guajava) (f) Guava

(g) Cera rubber

(Morus rubra) (e) Mulberry















(Capsicum annum)

(b) Chilli

(Solanum lycopersicum) (a) Tomato





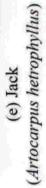


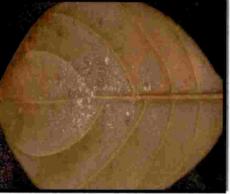




(d) Papaya (*Carica papaya*)

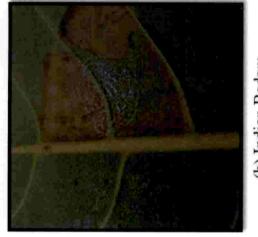






(d) Teak (Tectona grandis)





(b) Indian Badam (Terminalia catappa)



(c) Coconut (Coccus nucifera)





(a) Banana (Musa sp.)



(b) Tomato (Solanum lycopersicum)





(a) Chilli (*Capsicum annum*) value of 106.78 ppm by population of Kalliyoor with upper and lower fiducial limits, 121.45 and 91.91 ppm respectively.

The resistance ratio of LC_{50} values of whitefly population collected from Vellayani was 2.60 while in Kalliyoor population it was 1.14. Similarly, the resistance ratio calculated using LC_{90} , gave values *viz*. 2.02 and 1.03 from Vellayani and Kalliyoor respectively. Based on the LC _{50/90} values, Vellayani population were observed to be resistant.

4.2.2. Fenvalerate

The toxicity of fenvalerate to the populations of *A. dispersus* are shown in the Table 12. The lowest value was shown by the population from Sreekaryam (8.01ppm) which had upper fiducial limit 13.76 ppm and lower 1.68 ppm. The highest LC_{50} value (23.23 ppm) was shown by the population collected from Vellayani with upper and lower fiducial limits of 27.52 and 19.81 ppm respectively, followed by Kalliyoor population with LC_{50} value 12.94 ppm and fiducial limits, 15.62 ppm as upper and 10.13 ppm as lower fiducial limits.

The lowest LC₉₀ value of 48.08 ppm was recorded by Sreekaryam population having fiducial limits 82.04 ppm as upper and 39.62 ppm as lower. Toxicity to fenvalerate with respect to LC₉₀ value was also calculated where whitefly population of Vellayani had the highest LC₉₀ value of 66.97 ppm with upper fiducial limit 86.46 ppm and lower fiducial limit 56.68 ppm. Whitefly population collected from Kalliyoor recorded LC₉₀ value of 52.03 ppm with upper fiducial limit, 57.09 ppm and lower fiducial limit, 41.93 ppm.

Using these LC_{50} and LC_{90} values resistance ratio was calculated. While considering LC_{50} values, a resistance ratio of 2.90 was observed in case of population from Vellayani and 1.62 for Kalliyoor population. Population from Sreekaryam recorded a resistance ratio of 1. However, resistance ratio obtained using LC_{90} values were 1.39 for population from Vellayani, 1.08 for population from Kalliyoor and 1 for Sreekaryam population. Based on the LC _{50/90} values, Vellayani population showed more resistance to fenvalerate.

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| | LC_{50} | Fiducia | Fiducial limits | LC_{90} | Fiducia | Fiducial limits | <u></u> | | Resistance Ratio | ce Ratio |
|------------|-----------|---------|-----------------|-----------|---------|-----------------|---------|------------------|------------------|------------------|
| | (mdd) | Lower | Upper | (mqq) | Lower | Upper | X | - TO F adore | LC ₅₀ | LC ₉₀ |
| Sreekaryam | 35.39 | 27.53 | 41.13 | 103.31 | 93.42 | 129.52 | 0.18 | 7.17±0.003 | 1 | - |
| Vellayani | 92.11 | 77.49 | 126.99 | 208.74 | 159.62 | 336.32 | 0.88 | 4.38 ± 0.003 | 2.60 | 2.02 |
| Kalliyoor | 40.65 | 34.85 | 45.43 | 106.78 | 91.91 | 121.45 | 2.19 | 8.11±0.002 | 1.14 | 1.03 |

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| | LC ₅₀ | Fiducial | ll limits | LC_{90} | Fiducial limits | 1 limits | χ^2 | Slope \pm SE | Resistar | Resistance Ratio |
|--|------------------|---------------------|---------------|-----------|-----------------|----------|----------|----------------|------------------|------------------|
| | | Lower | Upper | - | Lower | Upper | | | LC ₅₀ | LC ₉₀ |
| Sreekaryam | 8.01 | 1.68 | 13.76 | 48.08 | 39.62 | 82.04 | 8.79 | 7.84±0.004 | 1 | 1 |
| Vellayani | 23.23 | 19.81 | 27.52 | 66.97 | 56.68 | 86.46 | 4.19 | 8.18±0.004 | 2.90 | 1.39 |
| Kalliyoor | 12.94 | 10.13 | 15.62 | 52.03 | 41.93 | 57.09 | 7.48 | 9.69±0.004 | 1.62 | 1.08 |
| χ^2 table value at 5 df = 11.07, χ^2 is non- significant at: p< 0.05 | df = 11.07, | χ^2 is non- si | gnificant at: | p< 0.05 | | | | | | |

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4.2.3. Imidacloprid

LC₅₀ values were in the order 3.54 ppm for Sreekaryam population, 6.54 ppm for population from Vellayani and 4.53 ppm for Kalliyoor population. Whitefly population from Sreekaryam had fiducial limits as 4.11 ppm upper and 2.75 ppm as lower. Population from Vellayani had fiducial limits of 7.54 ppm as upper limit and 5.86 ppm as lower limit. While Kalliyoor population recorded 5.08 ppm as upper limit and 3.91 ppm as lower limit (Table 13).

Sreekaryam population had LC₉₀ value of 10.67 ppm with upper fiducial limit, 12.95 ppm and lower fiducial limit, 9.34 ppm. LC₉₀ value of Vellayani population was 14.99 ppm with upper fiducial limit 19.67 ppm and lower fiducial limit 12.54 ppm while Kalliyoor population recorded 11.95 ppm as LC₉₀ value and upper fiducial limit 14.73 ppm and lower fiducial limit 10.34 ppm.

The resistance ratio calculated based on LC_{50} values were 1.85, 1.28 and 1 for Vellayani, Kalliyoor and Sreekaryam populations respectively. While LC_{90} values based on resistance ratio were observed as 1.40, 1.11 and 1 for population from Vellayani, Kalliyoor and Sreekaryam respectively. Based on the $LC_{50/90}$ values whiteflies collected from Vellayani were the resistant population.

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

The results showing the percentage mortality of resistant population against the new generation insecticides are given in the Table 14. Thiamethoxam 12.6% +lambda cyhalothrin 9.5% ZC @ 33+15.75 g a.i ha⁻¹ recorded the highest mortality of 45 per cent after 0.25 hours of treatment which was found to be significantly different from all other treatments, followed by clothianidin 50% WDG @ 20 g a.i ha⁻¹ with a mortality percentage of 26.67. Flonicamid 50% WG @ 50 g a.i ha⁻¹, cynantraniliprole 10.26% OD @ 90 g a.i ha⁻¹ and buprofezin 25% SC @ 75 g a.i ha⁻¹ with mortality percentages of 6.67, 3.33 and 1.67 respectively and found to be on par with each other while flonicamid 50% WG @ 50 g a.i ha⁻¹ (6.67) was

| | LC ₅₀ | Fiducial 1 | l limits | LC ₉₀ (ppm) | Fiduc | Fiducial limits | χ^2 | Slope \pm SE | Resistance Ratio | ce Ratio |
|--|------------------|----------------------|-----------------|---------------------------|-------|-----------------|----------|----------------|------------------|-----------|
| | | Lower | Upper | | Lower | Upper | | | LC_{50} | LC_{90} |
| Sreekaryam | 3.54 | 2.75 | 4.11 | 10.67 | 9.34 | 12.95 | 0.179 | 7.174±0.028 | 1 | 1 |
| Vellayani | 6.54 | 5.86 | 7.54 | 14.99 | 12.54 | 19.67 | 0.409 | 6.16±0.025 | 1.85 | 1.40 |
| Kalliyoor | 4.53 | 3.91 | 5.08 | 11.95 | 10.34 | 14.73 | 0.036 | 7.016±0.024 | 1.28 | 1.11 |
| χ^2 table value at 5 df = 11.07, χ^2 is non- significant at: p< 0.05 | df = 11.07, | χ^2 is non- sig | nificant at: p- | < 0.05 | | | | | | |

Table 13: Toxicity of imidacloprid to A. dispersus collected from three different locations at 0.8 hours after spraying

Table 14: Mortality of resistant population of A. dispersus treated with new generation insecticides in laboratory condition

| Treatment | | | E. | Mortality (%)* HAT |)* HAT | | |
|--|--------------------|--------------------|--------------------|---------------------|---------------------|---------------------|---------------------|
| | 0.25 | 0.5 | 0.75 | 1 | 1.25 | 1.5 | 1.75 |
| Bunrofezin 25% SC @ 75 v a i ha-l | 1.67 ^{cd} | 5.00 ^d | 25.00€ | 28.33° | 33.33° | 58.33 ^b | 100.00ª |
| | (4.72) | (10.66) | (29.92) | (32.01) | (35.16) | (49.83) | (89.37) |
| Clothianidin 50% WDG @ 20 e a i ha-l | 26.67 ^b | 65.00 ^a | 80.00 ^b | 100.00 ^a | 100.00^{a} | 100.00^{a} | 100.00 ^a |
| | (31.07) | (53.76) | (63.54) | (89.37) | (89.37) | (89.37) | (89.37) |
| Cynantraniliprole 10.26% OD $@$ 90 σ a i ha ⁻¹ | 3.33 ^{cd} | 35.00 ^b | 41.67 ^d | 61.67° | 100.00 ^a | 100.00^{a} | 100.00^{a} |
| | (6.56) | (36.23) | (40.19) | (51.80) | (89.37) | (89.37) | (89.37) |
| Dinotefuran 20% SG (m) 25 σ a i ha ⁻¹ | 0.00 ^d | 16.67° | 35.00 ^d | 68.33° | 100.00^{a} | 100.00^{a} | 100.00^{a} |
| | (0.62) | (26.45) | (36.24) | (55.77) | (89.37) | (89.37) | (89.37) |
| Flonicamid 50% WG @ 75 v a i ha-l | 6.67° | 36.67 ^b | 66.67° | 78.33 ^b | 100.00^{a} | 100.00 ^a | 100.00^{a} |
| | (12.12) | (37.20) | (54.83) | (62.29) | (89.37) | (89.37) | (89.37) |
| Thiamethoxam 25%WG @ 50 v a i ha-l | 0.00 ^d | 1.67 ^{de} | 41.67 ^d | 45.00 ^d | 75.00 ^b | 100.00 ^a | 100.00 ^a |
| | (0.62) | (4.72) | (40.19) | (42.13) | (60.07) | (89.37) | (89.37) |
| Thiamethoxam 12 6% + [amhda cyhalothrin 9 5% ZC @ $33+15$ 75 σ 3 i ha-l | 45.00 ^a | 66.67 ^a | 100.00^{a} | 100.00^{a} | 100.00^{a} | 100.00 ^a | 100.00 ^a |
| | (42.12) | (54.83) | (89.37) | (89.38) | (89.38) | (89.37) | (89.37) |
| Control (Water) | 0.00 ^d | 0.00€ | 0.00 ^f | 0.00 ^f | 0.00 ^d | 0.00° | 0.00 ^b |
| | (0.62) | (0.62) | (0.62) | (0.62) | (0.62) | (0.62) | (0.62) |
| CD (0.05) | (10.445) | (8.811) | (4.777) | (4.769) | (3.526) | (2.716) | (2.335) |
| Figures in parenthesis are angular transformed values; HAT - Hours after treatment *Mean of 3 replications | t t | | | | | | |

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found to be significantly different from dinotefuran 20% SG @ 25 g a.i ha⁻¹ and thiamethoxam 25% WG @ 50 g a.i ha⁻¹ with no mortality.

After 0.5 hours of treatment, thiamethoxam 12.6% + lambda cyhalothrin 9.5% ZC @ 33+15.75 g a.i ha⁻¹ showed a mortality of 66.67 per cent which was on par with clothianidin 50% WDG @ 20 g a.i ha⁻¹ (65%) which were significantly different from others. Mortality percentage of 36.67 was observed in case of flonicamid 50% WG @ 50 g a.i ha⁻¹ which was on par with cynantraniliprole 10.26% OD @ 90 g a.i ha⁻¹ (35%). Dinotefuran 20% SG @ 25 g a.i ha⁻¹ recorded 16.67 per cent mortality which was significantly different from buprofezin 25% SC @ 75 g a.i ha⁻¹ (5%) and thiamethoxam 25% WG @ 50 g a.i ha⁻¹ (1.67%), which were on par. The control treatment recorded no mortality which was on par with thiamethoxam 25% WG @ 50 g a.i ha⁻¹ (1.67%).

Thiamethoxam 12.6% + lambda cyhalothrin 9.5% ZC @ 33 + 15.75 g a.i ha⁻¹ recorded cent percent mortality after 0.75 hours of treatment followed by clothianidin 50% WDG @ 20 g a.i ha⁻¹ (80%) and flonicamid 50% WG @ 50 g a.i ha⁻¹ (66.67%). Cynantraniliprole 10.26% OD @ 90 g a.i ha⁻¹ and thiamethoxam 25% WG @ 50 g a.i ha⁻¹ had similar mortality percentage of 41.67 which were on par with dinotefuran 20% SG @ 25 g a.i ha⁻¹ (35%). Buprofezin 25% SC @ 75 g a.i ha⁻¹ recorded the lowest mortality (25%) which was significantly different from other treatments and superior to control.

After one hour of treatment both thiamethoxam 12.6% + lambda cyhalothrin 9.5% ZC @ 33+15.75 g a.i ha⁻¹ and clothianidin 50% WDG @ 20 g a.i ha⁻¹ recorded cent per cent mortality followed by flonicamid 50% WG @ 50 g a.i ha⁻¹(78.33%), dinotefuran 20% SG @ 25 g a.i ha⁻¹ (68.33%), cynantraniliprole 10.26% OD @ 90 g a.i ha⁻¹ (61.67%), thiamethoxam 25% WG @ 50 g a.i ha⁻¹(45%) and buprofezin 25% SC @ 75 g a.i ha⁻¹(28.33%).

Thiamethoxam 12.6% + lambda cyhalothrin 9.5% ZC @ 33+15.75 g a.i ha⁻¹, clothianidin 50% WDG @ 20 g a.i ha⁻¹, flonicamid 50% WG @ 50 g a.i ha⁻¹, dinotefuran 20% SG @ 25 g a.i ha⁻¹ and cynantraniliprole 10.26% OD

(a) 90 g a.i ha⁻¹ recorded hundred percent mortality after 1.25 hours of treatment followed by thiamethoxam 25% WG (a) 50 g a.i ha⁻¹ (75%) and buprofezin 25% SC (a) 75 g a.i ha⁻¹ (33.33%). After 1.5 hours all the treatments showed cent per cent mortality except buprofezin 25% SC (a) 75 g a.i ha⁻¹, which recorded only 58.33 per cent. All treatments were superior to the control which recorded no mortality. After 1.75 hour all treatments showed cent percent mortality whereas control recorded no mortality.

4.4. FIELD EVALUATION OF SELECTED NEW GENERATION INSECTICIDES AGAINST RESISTANT POPULATION OF WHITEFLIES.

4.4.1. A. dispersus

The results on the field evaluation of selected new generation insecticides against the resistant population of *A. dispersus* are presented in the Table 15. No significant difference was observed in the spiralling whitefly population before spraying among the treatments.

After 0.8 hours of treatment thiamethoxam 12.6% + lambda cyhalothrin 9.5% ZC @ 33+15.75 g a.i ha⁻¹ recorded the lowest number (5.20) of adults of spiralling whitefly and was significantly different from other treatments. Clothianidin 50% WDG @ 20 g a.i ha⁻¹ and flonicamid 50% WG @ 50 g a.i ha⁻¹ recorded 12.60 and 13.80 adult whiteflies per plant respectively and was statistically on par. The highest number of whiteflies was seen in control (21.80).

No whitefly were observed in treatment with thiamethoxam 12.6% + lambda cyhalothrin 9.5% ZC @ 33+15.75 g a.i ha⁻¹ after one day of spraying. While clothianidin 50% WDG @ 20 g a.i ha⁻¹ recorded 9.20 and flonicamid 50% WG @ 50 g a.i ha⁻¹ 11.80 whitefly adults per plant which was significantly different from the control treatment. Similar trend was seen in the second day where thiamethoxam 12.6% + lambda cyhalothrin 9.5% ZC @ 33+15.75 g a.i ha⁻¹ recorded no population and clothianidin 50% WDG @ 20 g a.i ha⁻¹ (5.00) and flonicamid 50% WG @ 50 g a.i ha⁻¹ showed lesser number of adult whiteflies (9.40) than the control (22.20).

Table 15 .Mean number of resistant population of A. dispersus treated with new generation insecticides under field conditions

| Treatment | | Mean | number of | f whitefly a | idults per p | Mean number of whitefly adults per plant after spraying (DAS)* | praying (D | AS)* | |
|---|--------------|------------------------------|------------------------------|------------------------------|------------------------------|--|------------------------------|------------------------------|------------------------------|
| | Pre count | 0.08 | 1 | 2 | 3 | 4 | 5 | 9 | 7 |
| Thiamethoxam 12.6% + lambda cyhalothrin 9.5% ZC $@$ 33+15.75 g a.i ha ⁻¹ | 24.60 | 5.20° (2.24) | 0.00 ^d (0.70) | 0.00 ^d (0.70) | 0.00 ^c (0.70) | 0.00 ^b (0.70) | 0.00 ^b (0.70) | 0.00 ^b (0.70) | 0.00 ^b (0.70) |
| Clothianidin 50% WDG @ 20 g a.i ha ^{-l} | 23.61 | 12.60 ^b (3.54) | 9.20° (3.11) | 5.00° (2.29) | 0.00° (0.70) | 0.00 ^b (0.70) | 0.00 ^b (0.70) | 0.00 ^b (0.70) | 0.00 ^b (0.70) |
| Flonicamid 50% WG @ 75 g a.i ha ^{-l} | 24.40 | 13.80 ^b (3.72) | 11.80 ^b (3.50) | 9.40 ^b (3.13) | 1.40 ^b (1.28) | 0.00 ^b (0.70) | 0.00 ^b (0.70) | 0.00 ^b (0.70) | 0.00 ^b (0.70) |
| Untreated Control | 24.00 | 21.80 ^a (4.66) | 21.80 ^a (4.73) | 22.20 ^a (4.78) | 22.60 ^a (4.80) | 22.80 ^a (4.82) | 23.00 ^a (4.84) | 23.00 ^a (4.84) | 23.00 ^a (4.84) |
| CD (0.05) | NS | (0.326) | (0.175) | (0.421) | (0.373) | (0.055) | (0.052) | (0.052) | (0.095) |
| Figures in parenthesis are square root transformed values; DAS- Days after spraying | ues; DAS- | Days after | spraying | | | | | | |

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*Mean of 5 replications

Thiamethoxam 12.6% + lambda cyhalothrin 9.5% ZC @ 33+15.75 g a.i ha⁻¹ and clothianidin 50% WDG @ 20 g a.i ha⁻¹ recorded no population at third day of spraying, while flonicamid 50% WG @ 50 g a.i ha⁻¹ treated plants recorded 1.40 whiteflies per plant. No whitefly population was observed after third day in all three treatments except control and it retained up to 7 days after spraying and all treatments were found to be non- significant.

4.4.2. A. trachoides

Whitefly species found along with *A. dispersus* was solanum whitefly *A. trachoides*. The results showing the evaluation of selected new generation insecticides against *A. trachoides* are given in the Table 16. Number of whiteflies before spraying was found to be non- significant.

After two hours of spraying thiamethoxam 12.6%+ lambda cyhalothrin 9.5% ZC @ 33+15.75 g a.i ha⁻¹ recorded the lowest number of adult whitefly (3.20) followed by clothianidin 50% WDG @ 20 g a.i ha⁻¹ (7.00) and flonicamid 50% WG @ 50 g a.i ha⁻¹ (10.20). Water spray as control recorded the highest whitefly population of 13.80.

No whitefly population was observed in plants treated with thiamethoxam 12.6% + 1 lambda cyhalothrin 9.5% ZC @ 33+15.75 g a.i ha⁻¹ after first day of spraying. Clothianidin 50% WDG @ 20 g a.i ha⁻¹ recorded whitefly population of 1.20 and flonicamid 50% WG @ 50 g a.i ha⁻¹ recorded 4.20. All treatments were significantly different from each other and also from control (14.80).

After two days of spraying there was no population of whitefly in thiamethoxam 12.6% + lambda cyhalothrin 9.5% ZC @ 33+15.75 g a.i ha⁻¹ and clothianidin 50% WDG @ 20 g a.i ha⁻¹ and there was no significant difference between them. Flonicamid 50% WG @ 50 g a.i ha⁻¹(0.60) showed higher population than the above two treatments and lower than the control treatment (15.60).

No whitefly population was observed in thiamethoxam 12.6%+ lambda cyhalothrin 9.5% ZC @ 33+15.75 g a.i ha⁻¹, clothianidin 50% WDG @ 20 g a.i

Table 16.Mean number of A. trachoides treated with new generation insecticides under field conditions

| | Mean nur | Mean number of whitefly adults per plant after spraying $(DAS)^*$ | hitefly ad | ults per pl | ant after | spraying (| DAS)* | | |
|---|--------------|---|-----------------------------|---|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
| Treatments | Pre count | 0.08 | 1 | 2 | 3 | 4 | 5 | 9 | 7 |
| Thiamethoxam 12.6% + lambda cyhalothrin 9.5% ZC @ 33+15.75 g a.i ha ⁻¹ | 14.60 | 3.20 ^d (1.75) | 0.00 ^d (0.70) | 0.00 ^b (0.70) | 0.00 ^b (0.70) | 0.00 ^b (0.70) | 0.00 ^b (0.70) | 0.00 ^b (0.70) | 0.00 ^b (0.70) |
| Clothianidin 50% WDG @ 20 g a.i ha ^{-l} | 14.20 | 7.00° (2.64) | 1.20° (1.21) | 0.00 ^b (0.70) | 0.00 ^b (0.70) | 0.00 ^b (0.70) | 0.00 ^b (0.70) | 0.00 ^b (0.70) | 0.00 ^b (0.70) |
| Flonicamid 50% WG @ 75 g a.i ha ⁻¹ | 14.00 | 10.20 ^b (3.19) | 4.20 ^b (2.17) | 0.60 ^b (0.98) | 0.00 ^b (0.70) |
| Control (Water) | 14.55 | 13.80 ^a (3.71) | 14.80^{a} (3.91) | 15.60^{a} (4.01) | 15.60^{a} (4.010) | 15.40^{a} (3.98) | 15.80^{a} (4.03) | 15.80^{a} (4.03) | 15.80^{a} (4.03) |
| CD (0.05) | SN | (0.263) | (0.387) | (0.263) (0.387) (0.281) (0.041) (0.041) (0.035) (0.035) (0.035) | (0.041) | (0.041) | (0.035) | (0.035) | (0.071) |
| Figures in parenthesis are square root transformed values; DAS- Days after spraying *Mean of 5 replications | S- Days af | ter sprayir | പ്പ | | | | | | |

ha⁻¹ and flonicamid 50% WG @ 50 g a.i ha⁻¹ three days after the spraying. After three to seven days of spraying no population of whitefly was noticed in all the treatments except control.

4.5. ESTIMATION INSECTICIDE RESIDUE IN TOMATO FRUITS.

4.5.1. Validation of methods for Pesticide Residue Analysis

Results of the validation for the estimation of selected insecticides, lambda cyhalothrin, thiamethoxam, clothianidin and flonicamid in tomato showed satisfactory recovery for the compounds which were fortified. Validation of the method was accomplished with good linearity (0.01-1 μ g ml⁻¹) and recovery which was within the acceptable range of 70-120 per cent at three levels of fortification (0.05, 0.10,0.25 μ g ml⁻¹). Repeatability of the recovery results as shown by the relative standard deviations (RSD) was below 20 per cent, thereby established that the method was sufficiently reliable for pesticide residue analysis and the results are presented in tables 17- 20.

In lambda cyhalothrin, the mean per cent recovery was 100.00, 114.46 and 108.09 per cent at respective fortification levels of 0.05, 0.10 and $0.25\mu g$ ml⁻¹ with relative standard deviation of 2.30, 1.42 and 3.55 per cent respectively. The percentage recovery of thiamethoxam was 87.93, 117.67 and 117.46 with relative standard deviation 4.94, 1.06 and 1.53 percentage at three levels of fortification. Mean recovery of clothianidin was calculated as 91.00, 101.33 and 118.13 per cent at three levels of fortification 0.05, 0.10 and 0.25 per cent respectively with relative standard deviations 4.06, 2.03 and 1.08 per cent.

In flonicamid per cent recovery of 93.00, 76.90 and 86.80 were obtained at 0.05, 0.1 and 0.25 levels of fortification respectively with relative standard deviations 2.02, 4.78 and 0.38 per cent.

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 new generation insecticides are presented in Table 21.

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| Table 17. Per cent recovery of thiamethoxam fortified at different levels | / of thiamethoxam fo | ortified at different lev | vels | | | |
|--|----------------------------------|---------------------------|----------------|---------------|------|--|
| 1 000 Lord 2000 L | | Recovery (%) | | Mean Recovery | RSD | |
| LUX (IIII KG) | R ₁ | \mathbb{R}_2 | R ₃ | $(\%) \pm SD$ | (%) | |
| 0.05 | 91.40 | 90.60 | 81.80 | 87.93±4.35 | 4.94 | |
| 0.10 | 118.00 | 119.00 | 116.00 | 117.67±1.25 | 1.06 | |
| 0.25 | 120.00 | 116.40 | 116.00 | 117.46±1.79 | 1.53 | |
| Limit of quantification (LOQ)- 0.05 mg kg ⁻¹ , RSD- Relative standard deviation | Q)- 0.05 mg kg ⁻¹ , R | SD- Relative standar | d deviation | | | |
| Table 18. Per cent recovery of lambda cyhalothrin fortified at different levels | ' of lambda cyhaloth | nrin fortified at differe | nt levels | | | |
| | | Recovery (%) | | Mean Recovery | USA | |
| LOQ (mg kg ⁻¹) | \mathbb{R}_{I} | \mathbb{R}_2 | \mathbb{R}_3 | $(\%) \pm SD$ | (%) | |

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| | g kg |
| | 5 mg |
| | .05 |
| |) - (|
| | LOQ |
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| | |

51

2.30

 100 ± 2.30

96.00

104.00

100.00

0.05

0.10

0.25

1.42

 114.46 ± 1.62

112.90

113.80

116.70

3.55

 108.09 ± 3.84

107.68

113.00

103.60

| ()()() | | Recovery (%) | | Mean Recovery | RSD |
|--|-----------------------------------|------------------------------|----------------|-------------------|------|
| LUQ (IIIg Kg) | R | \mathbb{R}_2 | \mathbb{R}_3 | $(\%) \pm SD$ | (%) |
| 0.05 | 92.20 | 94.80 | 86.00 | 91.00±3.69 | 4.06 |
| 0.10 | 101.00 | 104.00 | 99.00 | 101.33 ± 2.05 | 2.03 |
| 0.25 | 119.40 | 118.60 | 116.40 | 118.13±1.27 | 1.08 |
| Limit of quantification (LOQ)- 0.05 mg kg ⁻¹ , RSD- Relative standard deviation | 00)- 0.05 mg kg ⁻¹ , F | tSD- Relative standar | rd deviation | | |

Table 19.Per cent recovery of clothianidin fortified at different levels

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| f |
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| Table |

| 1 00 (mm [mm]) | | Recovery (%) | | Mean Recovery | RSD |
|--|-----------------------------------|----------------------------|----------------|---------------|------|
| LUQ (IIII Kg) | R_1 | \mathbb{R}_2 | R ₃ | $(\%) \pm SD$ | (%) |
| 0.05 | 92.20 | 95.60 | 91.20 | 93.00±1.88 | 2.02 |
| 0.10 | 76.90 | 72.40 | 81.40 | 76.90±3.67 | 4.78 |
| 0.25 | 86.40 | 86.80 | 87.20 | 86.80±0.33 | 0.38 |
| Limit of quantification (LOO)- 0.05 mg kg ⁻¹ . RSD- Relative standard deviation | OO)- 0.05 mg kg ⁻¹ . F | SD- Relative standa | rd deviation | | |

LIMIT OF QUARTICATION (LOU)- 0.00 mg kg , KSD- Relative standard deviation

| | | | | | - | | | _ | | | | 1 |
|--|--------------------------|--|---------------------------|-------|-------|-------|-------|-------|-----|-------------|----------------------|--|
| Flonicamid 50 % WG | | Dissipation (%) | | 7.14 | 14.28 | 21.42 | 50.00 | | | | 7.82 | |
| Floring | | Mean residue* (mg kg ⁻¹) | 0.14 | 0.13 | 0.12 | 0.11 | 0.07 | ГОО | | ĸ | 7. | |
| Clothionidin 5002 WDG | | Dissipation (%) | | 20.00 | 44.00 | 48.00 | 52.00 | 56.00 | | | 8.92 | |
| Clothionidi | | Mean residue* (mg kg ⁻¹) | 0.25 | 0.20 | 0.14 | 0.13 | 0.12 | 0.11 | Год | x | ∞ | |
| n 9.5% ZC | Thiamethoxam 12.6% | Dissipation (%) | r | 14.28 | | | | | | | 4.05 | 0.05 mg kg ⁻¹ |
| bda cyhalothri | Thiametho | Mean residue (mg kg ⁻¹) | 0.07 | 0.06 | ГОО | ı | t. | | ĩ | ı | 4. | ation (LOO)- |
| Thiamethoxam 12.6% +lambda cyhalothrin 9.5% ZC | othrin 9.5 % | Dissipation (%) | t; | 16.66 | | | | | | | 5 | nit of quantific |
| Thiamethoxa | Lambda cyhalothrin 9.5 % | Mean residue* (mg kg ⁻¹) | 0.06 | 0.05 | LOQ | ĩ | Ĩ. | | . 1 | 1 ,- | 3.42 | *Mean of three replications. Limit of quantification (LOO)- 0.05 mg kg |
| | Days after | spraying (DAS) | 0 (2 h after spraying) | 1 | 6 | 5 | 2 | 10 | 15 | 30 | Half- life (Days) | *Mean of three |

Table 21. Persistance and dissipation of insecticides in tomato

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4.5.2.1. Thiamethoxam 12.6% + lambda cyhalothrin 9.5% ZC (Insecticide mixture)

Thiamethoxam 12.6%

The mean initial deposit of thiamethoxam after two hours of spraying was found to be 0.07 mg kg^{-1} which dissipated to 0.06 mg kg^{-1} on first day after spraying with dissipation percentage of 14.28. On third day the residue reached limit of quantification with half- life of 3.42 days.

Lambda cyhalothrin 9.5%

The mean initial deposit of lambda cyhalothrin was found to be 0.06 mg kg^{-1} after two hours of spraying which dissipated to 0.05 mg kg^{-1} with dissipation percentage of 16.66 on one day after spraying. On the third day, residues got dissipated to below the limit of quantification and half -life calculated was 4.05 days.

4.5.2.2. Clothianidin 50% WDG

The mean initial deposit of 0.25 mg kg⁻¹ was observed on tomato fruit after two hours of spraying and it got dissipated to 0.20 mg kg⁻¹ on first day with a dissipation percentage of 20.00. On third day after spraying the residue dissipated to 0.14 mg kg⁻¹ with dissipation percentage of 44.00 then dissipated to 0.13 mg kg⁻¹ on fifth day after spraying with dissipation percentage of 48.00. On seventh day after spraying 0.12 mg kg⁻¹ residue was obtained and dissipation percentage was 52.00. On tenth day after spraying residue was 0.11 mg kg⁻¹ and dissipation percentage was 56.00 after which residue reached limit of quantification and the half-life calculated was 8.92 days.

4.5.2.3. Flonicamid 50% WG

The mean initial deposit of flonicamid was observed to be 0.14 mg kg⁻¹ which dissipated to 0.13 mg kg⁻¹ one day after spraying with dissipation percentage of 7.14. On third day after spraying, the residue was 0.12 mg kg⁻¹ with dissipation percentage of 14.28 which dissipated to 0.11 mg kg⁻¹ on fifth day after spraying and

dissipation percentage was calculated as 21.42. On seventh day after spraying residue was 0.07 mg kg^{-1} with dissipation percentage 50.00. On 10^{th} day after spraying, the residue dissipated to below the limit of quantification with half –life of 7.82 days.

4.5.3. Estimation of harvest time residue

The harvest time residues are shown in the table 19. Mean residues of thiamethoxam 12.6% + lambda cyhalothrin 9.5% ZC, clothianidin 50% WDG and flonicamid 50% WG in tomato fruit at the harvest time was found to be below the limit of quantification.

| | | Mean residue (| mg kg ⁻¹) | | |
|-----------------|---|---|---|----------------------|--|
| Harvest time | Thiamethoxam 1 cyhalothrin | | Clothianidin | Flonicamid 50% WG | |
| residue | Thiamethoxam | Lambda cyhalothrin | 50% WDG | | |
| | <loq< td=""><td><loq< td=""><td><loq< td=""><td><loq< td=""></loq<></td></loq<></td></loq<></td></loq<> | <loq< td=""><td><loq< td=""><td><loq< td=""></loq<></td></loq<></td></loq<> | <loq< td=""><td><loq< td=""></loq<></td></loq<> | <loq< td=""></loq<> | |

Table 22. Harvest time residues of insecticides in tomato

4.5.4. Risk Assessment of Insecticides in Tomato

Risk assessment of insecticides in tomato was calculated by taking average body weight of a human in India as 60 kg and daily consumption of tomato as 50 g per day and is expressed in Tables 23-25.

4.5.4.1. Thiamethoxam 12.6% + Lambda cyhalothrin 9.5% ZC (Insecticide mixture)

The ADI values of thiamethoxan and lambda cyhalothrin were 0.08 and 0.02 mg kg⁻¹ bw d⁻¹ respectively. The mean residue in case of lambda cyhalothrin were 0.06 and 0.05 mg kg⁻¹ for 0th and first day after spraying respectively, while mean residue for thiamethoxam were 0.07 for 0th day and 0.06 mg kg⁻¹ for first day. MPI

| TMRC (μg person ⁻¹ d ⁻¹) | Thiamethoxam 12.6% | 3.50 | 3.00 | cimum residue |
|--|---|------|------|---|
| TN | Lambda cyhalothri n 9.5% | 3.00 | 2.50 | retical may |
| Mean residue (μg g ⁻¹) | Thiamethoxam 12.6% | 0.07 | 0.06 | - TMRC- Theo |
| Mea (µ | Lambda cyhalothri n 9.5% | 0.06 | 0.05 | sible intake |
| MPI (μg person ⁻¹ d ⁻¹)* | Thiamethoxam 12.6% | 4800 | 4800 | compared and with the Maximum nermissible intake. TMRC- Theoretical maximum residue |
| N | Lambda cyhalothr in 9.5% | 1200 | 1200 | MPI- M |
| ÷ | Daily consumption (gd ⁻¹) | 50 | 50 | la daily intak |
| | DAS | 0 | 1 | hentah |
| Average | body weight (kg ⁻¹) | 60 | 60 | · ADI- A |
| ADI (mg kg ⁻¹ bw d ⁻¹) | Thiamethoxam 12.6% | 0.08 | 0.08 | DAC Dave after contriner ADI A |
| (mg k | Lambda cyhalothri n 9.5% | 0.02 | 0.02 | DAC Dave |

Table 23.Risk assessment of thiamethoxam 12.6% + lambda cyhalothrin 9.5% ZC in tomato

DAS- Days after spraying; ADI- Acceptable daily intake; MPI- Maximum permissible intake; 1 MKC contribution

*MPI= ADI× Average body weight×1000

Table 24. Risk assessment of clothianidin 50% WDG in tomato

| _ | person ⁻¹ d ⁻¹) | 12.5 | 10.00 | 7.00 | 6.50 | 6.00 | 5.50 | I maximum residue |
|------------------------------------|---|------|-------|------|------|------|------|--|
| Ň | $(\mu g g^{-1})$ | 0.25 | 0.20 | 0.14 | 0.13 | 0.12 | 0.11 | C_ Theoretica |
| MPI (µg | person ⁻¹ d ⁻¹)* | 6000 | 6000 | 6000 | 6000 | 6000 | 6000 | ble intabe. TMB |
| Daily consumption | (gd^{-1}) | 50 | 50 | 50 | 50 | 50 | 50 | Athe definition of the manifestible interest TMPC. Theoretical maximum residue |
| | CAU | 0 | - | ю | 5 | 7 | 10 | Polos NA |
| A-main the desired and the desired | Average bouy weight (kg) | 60 | 60 | 60 | 60 | 60 | 60 | in A DI A second she had a second sec |
| ADI | (mg kg ⁻¹ bw d ⁻¹) | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | |

DAS- Days after spraying; ADI- Acceptable daily intake; MPI- Maximum permissible intake; TMRC- Theoretical maximum residue contribution

*MPI= ADI× Average body weight×1000

| | | | | | 1 | 1 |
|--|-------|-------|-------|-------|-------|---|
| TMRC (µg person ⁻¹ d ⁻¹) | 7.00 | 6.50 | 6.00 | 5.50 | 3.50 | um residue |
| Mean residue $(\mu g g^{-1})$ | 0.14 | 0.13 | 0.12 | 0.11 | 0:07 | Cheoretical maxim |
| MPI (µg person ⁻¹ d ⁻¹)* | 1500 | 1500 | 1500 | 1500 | 1500 | iissible intake; TMRC- 7 |
| Daily consumption (gd ⁻¹) | 50 | 50 | 50 | 50 | 50 | ¹ - Maximum pern |
| DAS | 0 | | 3 | 5 | 7 | ake; MI |
| Average body weight (kg ⁻¹) | 60 | 60 | 60 | 60 | 60 | DAS- Days after spraying; ADI- Acceptable daily intake; MPI- Maximum permissible intake; TMRC- Theoretical maximum residue contribution |
| ADI (mg kg ^{-l} bw d ^{-l}) | 0.025 | 0.025 | 0.025 | 0.025 | 0.025 | DAS- Days after spray contribution |

Table 25. Risk assessment of flonicamid 50% WG in tomato

H

*MPI= ADI× Average body weight×1000

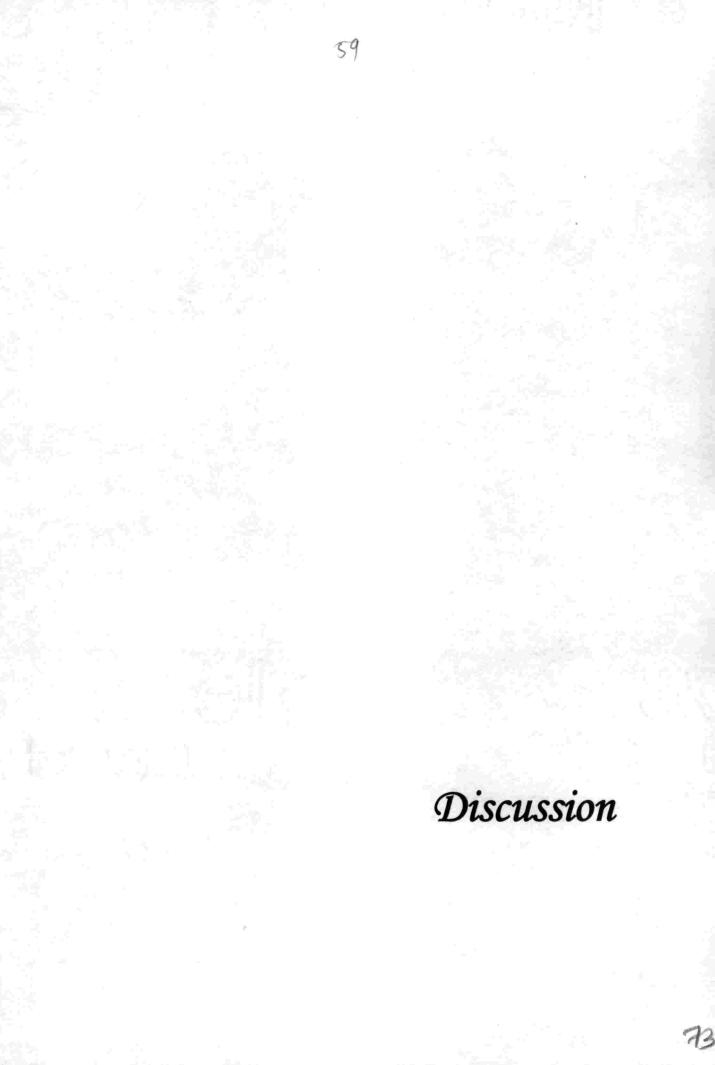
values calculated were 1200 and 4800 μ g person⁻¹ d⁻¹ for thiamethoxam and lambda cyhalothrin respectively. TMRC values for lambda cyhalothrin were 3.00 and 2.50 μ g person⁻¹ d⁻¹ for 0th and first day respectively and for thiamethoxam it was 3.50 and 3.00 μ g person⁻¹ d⁻¹ respectively and these values were lower than the MPI values of for thiamethoxam and lambda cyhalothrin

4.5.4.2. Clothianidin 50% WDG

ADI of clothianidin was 0.1 mg kg⁻¹ bw d⁻¹. Mean residues from 0th to 10th day after spraying were 0.25,0.20,0.14,0.13,0.12 and 0.11 mg kg⁻¹ respectively. MPI was 6000 μ g person⁻¹ d⁻¹. From 0th to 5th day after spraying TMRC values were 12.50, 10.00, 7.00, 6.50, 6.00 and 5.50 μ g person⁻¹ d⁻¹ respectively which was lower than MPI value (6000 μ g person⁻¹ d⁻¹).

4.5.4.3. Flonicamid 50% WG

ADI of flonicamid was 0.025 mg kg⁻¹ bw d⁻¹. Mean residues from 0th to 7th day after spraying were 0.14, 0.13, 0.12, 0.11 and 0.07 mg kg⁻¹ respectively. MPI was 1500 μ g person⁻¹ d⁻¹. TMRC values 0th to 7th day after spraying were 7.00, 6.50, 6.00, 5.55 and 3.50 μ g person⁻¹ d⁻¹ respectively which were lower than the MPI value (1500 μ g person⁻¹ d⁻¹).



5. DISCUSSION

The spiralling whitefly *A. dispersus* was a fortuitous introduction to Kerala with its first outbreak on cassava in dry seasons of 1993-94 (Palaniswamy *et al.*, 1995). Gradually they started to colonise other plants thus attaining a status of polyphagous pest. Being a threat to the farmers, the task of its management has paved way to the unrestricted use of insecticides which can ultimately result in resistance to these chemicals. However, the scientific reports on the development of insecticide resistance in spiralling whitefly *A. dispersus* is meagre. The results obtained from the present study "Insecticide resistance in spiralling whitefly *Aleurodicus dispersus* Russell and its management" are discussed below.

5.1. DOCUMENTATION OF WHITEFLIES IN DIFFERENT HOSTS.

Whitefly species recorded includes spiralling whitefly, *A. dispersus*, rugose spiralling whitefly, *A. rugioperculatus* and solanum whitefly, *A. trachoides*. The spiralling whitefly was observed from seven host plants belonging to six families, rugose whitefly was recorded from five host plants belonging to five families and the solanum whitefly, which was first reported in Kerala from College of Agriculture, Vellayani from tomato by Mohan (2017) was observed from two host plants belonging to same family (Table 10).

A. dispersus is a native of the Caribbean region and Central America, where it is known from a wide range of host plants, but not regarded as a pest (Waterhouse and Norris, 1989). It is more commonly known worldwide as 'spiralling whitefly' because it lays eggs in a typical spiral pattern. In India it was first reported from Kerala in 1993 on cassava (Palaniswamy *et al.*, 1995). It infests banana, guava, avocado, papaya, coconut, cucurbits, tomato, bell pepper (Srinivasa, 2000), mulberry (Kumari, 2011), coccinia (Vijayasree *et al.*, 2011), pepper (Nasruddhin *et al.*, 2014) and sweet potato (Mani, 2017).

Host plants of *A. dispersus* was documented by Rani (2004) from Instructional Farm, College of Agriculture, Vellayani and reported a total of 50 plant species of which 15 recorded high infestation. According to Shanas *et al.* (2016) seventeen host plants of rugose spiralling whitefly were reported from Kottayam district of Kerala which included the above-mentioned host plants with an addition of new host teak (*Tectona grandis*). A total of 118 host plants from 43 families were reported worldwide for rugose spiralling whitefly. Recent survey conducted by Mohan *et al.* (2017) reported 12 alternate host plants in coconut homesteads for rugose spiralling whitefly. Host plants of spiralling whitefly *A. dispersus* are prevalent in and around the homesteads of Kerala which facilitate the easy movement and spread of whiteflies from one plant to another. Being an invasive pest the initial spread will be quiet rampant due to the absence of natural enemies.

5.2. ASSESSMENT OF INSECTICIDE RESISTANCE IN FIELD POPULATION OF SPIRALLING WHITEFLY *A. dispersus* IN TOMATO.

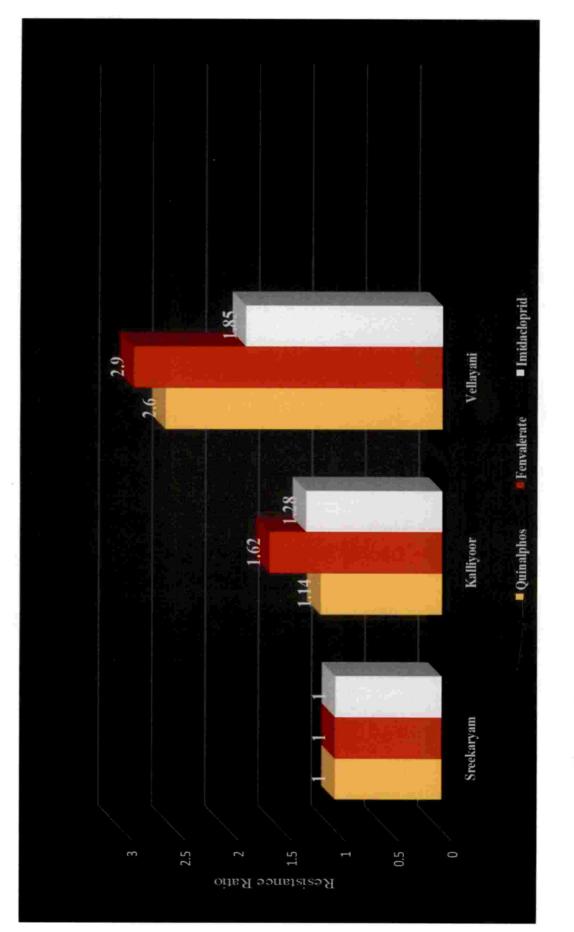
The present study was conducted to assess the development of insecticide resistance in three field populations of *A. dispersus* in tomato from Sreekaryam (no insecticide application), Vellayani (heavy application of insecticides and control failures reported) and Kalliyoor (no control failures reported after insecticide application).

Results publicised that population collected from location-I (Sreekaryam) was observed to be susceptible to insecticides with resistance ratio-1 for all three insecticides *viz.*, fenvalerate (sodium channel modulator) followed by imidacloprid (Neonicotinoid- nicotinic acetylcholine receptor (nAChR) competitive modulator) and quinalphos (organophosphate- Acetylcholine esterase (AChE) inhibitor) which was considered as reference strain. Population collected from location-II (Vellayani) showed higher resistance with resistance ratio of 2.60, 2.90 and 1.85 and population from location-III (Kalliyoor) was found to be moderately resistant with resistant ratio of 1.14, 1.62 and 1.28 against quinalphos, fenvalerate and imidacloprid respectively. Considering the resistance shown by whitefly population towards quinalphos and imidacloprid in location II (Vellayani), quinalphos showed higher resistance (2.6- folds) than imidacloprid (1.85- folds). However, in location III (Kalliyoor) a greater resistance of 1.28 was

shown by whitefly population against imidacloprid than quinalphos (1.14- folds). This shows the higher use of organophosphates in location II compared to location III. The resistance ratio shown by whiteflies collected from three different locations against three different insecticides are shown in the Fig 1.

Insecticide resistance developed and proliferated during the course of time has to be considered as a major challenge. The rapid upsurge of this phenomenon can be well justified if we ponder into the pest management strategies adopted world-wide using chemicals. Certainly, the unrestricted use of insecticides has flared-up this problem to a greater extent (Bhatia, 1986). Spiralling whitefly is a polyphagous sucking pest which extensively colonises on the abaxial surface of the leaves (Srinivasa, 2000). Recent management strategies are mainly focussed on insecticides belonging to organophosphates, carbamates and synthetic pyrethroids. Nevertheless, a high rate of control failure was reported in case of whiteflies, mainly B. tabaci. High resistance to organophosphates, carbamates, pyrethroides, chlorinated hydrocarbons and insect growth regulators are shown by them in many agriculture systems world-wide (Elbert and Nauen, 2000). A strong resistance was observed against oxydemeton methyl (RF 59-66) in B. tabaci in India (Kranthi et al., 2002). However, reports on insecticide resistance against spiralling whitefly in India is meagre and no studies have been carried out in Kerala. Hence, the study was carried out to assess the insecticide resistance in A. dispersus on tomato.

Similar research works on the development of insecticide resistance in various insects on different crops were carried out in Kerala Agricultural University from 2014 onwards. The spotted pod borer, *Maruca vitrata* showed 2.28 to 2.93 fold resistance against chlorpyriphos and 2.38 to 7.94 fold resistance against lambda cyhalothrin collected from cowpea grown in different areas of Thiruvananthapuram (Sreelekshmi, 2014). Another study was conducted in *Spodoptera litura*, where 1 to 6.14 folds resistance against chlorpyriphos, 1 to 1.79 times resistance to fenvalerate and 1 to 8.50 times resistance against lambda cyhalothrin was reported (Sreelekshmi, 2017). In 2018, Hampaiah studied the





development of resistance in cowpea aphid *Aphis craccivora* and he reported 1.67 - 1.71 fold resistance to quinalphos, 2.97-19.46 fold resistance against fenvalerate and 2.81-7.94 times resistance against imidacloprid. These studies supported the result of present study on the development of insecticide resistance in various pest affecting vegetables in homesteads of Kerala.

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In the present study population of spiralling whitefly collected from location II (Vellayani) and location III (Kalliyoor) has shown a higher resistance ratio of 2.9 and 1.62 respectively towards fenvalerate which makes evident the wide use of synthetic pyrethoids in these locality. Resistance build up against synthetic pyrethroids are much easier when compared to organophosphates and carbamates as they constitute as a single isomer, which may force the production of detoxifying enzyme resulting in rapid resistance development. However, in case of organophosphates and carbamates they do not exist as a stereo isomer so the insects has to develop several mechanisms, which need many enzyme systems for detoxifying the insecticides (Sreelekshmi, 2014). Pyrethroid resistance are increasing at an alarming rate in the recent past even though they have been in use for a limited period of time. Moderate to high level of resistance to all insecticides tested has been reported in *B.tabaci* in Pakistan of which 300 folds resistance was observed against cypermethrin by whiteflies collected from brinjal crop. This substantiates the heavy use of synthetic pyrethroids in fields of Pakistan by farmers (Dhaliwal and Koul, 2017). Mechanisms of pyrethroid resistance is derived from the studies on housefly. Gour and Sridevi (2017) proposed the reason for reduced susceptibility as decreased sensitivity of the nerve membrane and lower availability of pyrethroids at primary target site that is mediated by several mechanisms.

Comparing synthetic pyrethroids, organophosphates are one of the oldest insecticides which was introduced 50 years ago for the pest management. Insecticide resistance in organophosphates are described by mechanisms *viz.*, resistance mechanisms involving enhanced biotransformation and target site insensitivity. In Colorado potato beetle *Leptinotarsa decemlineata*, altered AChE

is a major mechanism resulting in azinphos-methyl resistance (Siegfried and Scharf, 2001). A resistance ratio of 4.74 was observed in cowpea aphid *A. craccivora* against malathion in Egypt (Mokbel, 2013). According to the study conducted by Sreelekshmi (2017) in *S. litura*, a resistance ratio of 10.41 fold against quinalphos was reported which was in congruence with the results obtained in the present study.

According to Cahill *et al.* (1995), pair wise comparison of different organophosphate insecticides for the development of insecticide resistance by *B. tabaci* showed positive result implying that whiteflies resistant to one organophosphate will show resistance to other OP compounds. *B. tabaci* in cotton showed 67 fold resistance to chlorpyriphos and 56 fold resistance to profenophos. Ahmed *et al.* (2002) reported a higher resistance ratio of 782 against dimethoate by *B. tabaci* in cotton. However Kranthi *et al.* (2002) has shown negligible resistance to chlorpyriphos by *B. tabaci* in cotton.

Along with the organophosphates and synthetic pyrethroids, resistance shown to neonicotinoid insecticides has also increased to an alarming rate. The first signs of imidacloprid resistance in whitefly *B. tabaci* was reported from Almeria region of southern Spain. *B. tabaci* collected from three different host's melon, lettuce and cole crops from Imperial valley, California were studied for its resistance development to Imidacloprid. Here the resistant development was at a slow pace, resistance developed at a rate of 4 folds at F4 generation, 34 folds at F16, 78 folds by F24 and the maximum was obtained as 82 fold at F27 (Prabhakar *et al.*, 1997). Mechanism of neonicotinoid resistance mainly involve enhanced activity of P450 through over expression or amplification (Li *et al.*, 2016). There are recent reports of development of resistance in brown plant hopper to imidacloprid in Asian countries such as China, India, Indonesia, Japan, Thailand and Vietnam (Dhaliwal and Koul, 2017).

The over-use or misuse of an insecticide against a pest species can result in resistance build-up and can consequently pave way to evolution of insects which has no effect by these chemicals (IRAC, 2018). Among all the different categories of pests, insects are known to exhibit resistance at alarming rates.

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

The result of previous experiment has revealed a higher resistance to the insecticides viz., quinalphos and fenvalerate in the population of whiteflies collected from Vellayani. For successfully managing this resistant population a laboratory experiment was conducted with different new generation insecticides viz., buprofezin 25% SC @ 75 g a.i ha⁻¹, clothianidin 50% WDG @ 20 g a.i ha⁻¹, cynantraniliprole 10.26% OD @ 90 g a.i ha⁻¹, dinotefuran 20% SG @ 25 g a.i ha⁻¹, flonicamid 50% WG @ 75 g a.i ha⁻¹, thiamethoxam 25% WG @ 50 g a.i ha⁻¹, thiamethoxam 12.6% +lambda cyhalothrin 9.5% ZC @ 33+15.75 g a.i ha⁻¹ and water as control. The results showed that higher mortality was observed in A. dispersus treated with combination product viz., thiamethoxam 12.6% +lambda cyhalothrin 9.5% ZC @ 33+15.75 g a.i ha⁻¹ (100%) followed by clothianidin 50% WDG @ 20 g a.i ha⁻¹ (80%) and flonicamid 50% WG @ 75 g a.i ha⁻¹ (66.67%). Similar results were reported by Hampaiah (2018) in Kerala where the highest mortality of cowpea aphids in laboratory condition was observed in 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.

The action of insecticide mixtures can be elaborated with the following reasons as explained by Das (2014). First, independent action of two different compounds showing similar effect when they are used as a mixture. Second, additive effect of two combined insecticide similar to sum of effect of each component given together. Third, synergistic effect where toxicity of the mixture is greater than sum of each components given alone. Finally, antagonistic effect where effect of one chemical is reduced due to the effect of the other. Synergism can be used to explain the success of insecticide mixtures. These mixtures have to be made by mixing insecticides with different modes of action or those affecting different biochemical processes so that the phenomenon of insecticide resistance can be subdued to a greater extent.

The three insecticides found efficient in managing whitefly population belong to neonicotinoid group (thiamethoxam and clothianidin), synthetic pyrethroid (lambda cyhalothrin) and flonicamid. Neonicotinoids are the fastest growing broad-spectrum insecticide against sucking and certain chewing pests. They selectively act on their target site of insect nicotinic acetyl choline receptor (Jeschke and Nauen, 2008). Their efficient mode of action rules out the possibility of cross resistance to conventional insecticides and also the upsurge of insecticidal resistance (Jeschke *et al.*, 2010).

However, reports of Nauen *et al.* (2003) stated thiamethoxam as the neonicotinoid precursor of clothianidin and called it as pro insecticide. Srinivasan *et al.* (2004) reported that whitefly population was significantly controlled by thiamethoxam when compared to imidacloprid treated plot. According to Rafee *et al.* (2004), clothianidin 50% WG @ 25 g a.i ha⁻¹ was found to be effective against whitefly population in cotton and it subdued its population (1.09 adults/leaf) at 3 days after spraying. Patnaik *et al.* (2010) also experimented the efficacy of clothianidin against whitefly infesting mulberry *Dialeuropora decempuncta* (Quaintance & Baker) and observed 99.07 per cent reduction by first spray itself.

Flonicamid is a novel insecticide with selective homoptera feeding blocker mechanism. It belongs to novel group of chemical pyridine carboxamides. Contradictory to the present study Babcock *et al.* (2011) reported flonicamid to be less effective against whiteflies with less than 50 per cent mortality at 200 mg L⁻¹. However, in another study it caused 95 per cent mortality of whitefly, *B. tabaci* 10 days after treatment and delayed population growth by one generation (Roditakis *et al.*, 2014). Similar result was obtained by Kodandaram *et al.* (2017), where three dosages of flonicamid 50 WG @ 50, 75 and 100 g a.i. ha⁻¹ and three standard check treatments (field recommended dosage), *viz.*, imidacloprid 200 SL @ 20 g a.i. ha⁻¹ , thiamethoxam 25 WG @ 25 g a.i. ha⁻¹ and dimethoate 30 EC @

600 g a.i. ha⁻¹ were taken and flonicamid 50 WG @ 100 g a.i. ha⁻¹ recorded the highest reduction in the whitefly population (95.3%) over the untreated control.

5.4 FIELD EVALUATION OF SELECTED NEW GENERATION INSECTICIDES AGAINST WHITEFLIES

Field experiment was carried out to evaluate the efficacy of three new generation insecticide selected from the laboratory viz., thiamethoxam 12.6% + lambda cyhalothrin 9.5% ZC @ 33+15.75 g a.i ha⁻¹, clothianidin 50% WDG @ 20 g a.i ha-1 and flonicamid 50% WG @ 75 g a.i ha-1 against the resistant population of spiralling whitefly. The results showed that all treatments were effective in controlling the whitefly A. dispersus when compared to control. After two days of treatment no spiralling whiteflies were observed from thiamethoxam 12.6% + lambda cyhalothrin 9.5% ZC, 5.00 adults per plant in clothianidin 50% WDG and 9.40 adults per plant in flonicamid 50% WG. Similar result was obtained in the case of the other whitefly A. trachoides that was present along with A. dispersus. The results from the present study shows the effect of insecticide mixtures in the pest control. Insecticide resistance can be successfully suppressed if the insecticide mixtures are used which includes different chemicals with different mode of action (Georghiou et al, 1983). In thiamethoxam + lambda cyhalothrin, thiamethoxam belongs to neonicotinoids with mode of action as nicotinic acetyl choline receptor (nAChR) competitive modulators and lambda cyhalothrin is a synthetic pyrethroid with sodium channel blocking activity (IRAC, 2018).

Use of insecticide mixtures for resistance management can be substantiated by the combination of insecticides with different mode of action. This results in the synergism where one insecticides enhances the action of other, which can be seen in case of synthetic pyrethroids and organophosphates where organophosphate binds to active site on esterase enzymes which detoxifies pyrethroid enzymes (Ahmad, 2004). The insecticide mixture indoxacarb + acetamiprid 100 g a.i.ha⁻¹ was found effective in managing resistant population of *M. vitrata* (Sreelekshmi *et al.*, 2016). The efficacy of thiamethoxam and lambda cyhalothrin was observed to be most effective in managing various pest in different crops *viz.*, tea (Samanta *et al.*, 2017), cotton (Borude *et al.*, 2018) and cowpea (Hampaiah, 2018). According to Reddy (2018) chlorantraniliprole 8.8% + thiamethoxam 17.5% SC @ 150 g a. i ha⁻¹ was effective against cowpea aphid *A. craccivora* and pod bug *Riptortus pedestris* (Fabricius).

5.5 ESTIMATION OF INSECTICIDE RESIDUE IN TOMATO FRUITS

Insecticides have played a pivotal role in the success of feeding a large number of starving communities. However, the sumptuous and irrational use of insecticides during the period has ultimately led to the accumulation of these chemicals in the environment (Malhat *et al.*, 2014). Thus, estimation of residue of insecticides after the spraying can be helpful in determining the fate of the insecticides that are being used and also can aid in choosing right chemicals that are comparatively safer for the human and the environment.

In the present study the best three insecticides selected from the laboratory and field studies along with the other insecticides were sprayed in the tomato plant at the time of fruit initiation and the fruit samples were analysed for the estimation of insecticide residue in it. Mean residue of each insecticide was determined at specified time intervals of 0, 1, 3, 5, 7, 10, 15 and 30 days after spraying and the data was used for further analysis. The results revealed that residues of single insecticide in the insecticide mixture thiamethoxam 12.6 + lambda cyhalothrin 9.5% ZC @ 33+15.75 g a.i ha⁻¹ dissipated within three days. However, clothianidin 50% WDG @ 20 g a.i ha⁻¹ and flonicamid 50% WG @ 75 g a.i ha⁻¹ dissipated within 15 and 10 days. The half-lives of these insecticides were calculated and thiamethoxam and lambda cyhalothrin in the insecticide mixture had a half-life of 4.05 and 3.42 respectively. Half -lives of clothianidin and flonicamid were 8.92 and 7.82 respectively. Barik *et al.* (2010) reported the initial residues of lambda cyhalothrin and thiamethoxam in paddy as 0.26 and 0.50 mg kg⁻¹after two hours of spraying which dissipated to below the limit of quantification within 20 days in case of thiamethoxam and within 5 days in case of lambda cyhalothrin. More or less similar results was obtained by Hampaiah (2018) in cowpea in Kerala. He reported that the initial residue of lambda cyhalothrin and thiamethoxam after two hours of spraying was 0.19 mg kg⁻¹ and 0.42 mg kg⁻¹ respectively which dissipated to 0.06 and 0.08 mg kg⁻¹ respectively in the first day after spraying reached below the limit of quantification by the third day. The half -lives observed were 0.31 days for lambda cyhalothrin and 0.37 days for thiamethoxam.

The present study was in accordance with the studies conducted by Li *et al.* (2012) where the half- life of clothianidin in tomato fruit was in the range of 7 to 11.9 days with a dissipation pattern fitting the first order kinetics. In contrast to the present study, half- life of clothianidin in made tea and green leaf tea was observed to be within the range of 3.71 to 4.07 days and 4.07 to 4.49 days respectively (Chowdhury *et al.*, 2012). In another study by Ramasubramanian (2013) on the dissipation and persistence of clothianidin in sandy loam soil of sugarcane, revealed that clothianidin is more persistent than imidacloprid and thiamethoxam in the soil with half- lives of 17.2 and 17.4 days at the single (50 g a. i ha⁻¹) and double doses (100 g a. i ha⁻¹) respectively.

The half-life obtained through the dissipation studies of flonicamid in cucumber was 3.0–4.9 days (Liu *et al.*, 2014). According to Kodandaram *et al.* (2017) residues of flonicamid in okra dissipated with half-lives of 3.0 and 3.5 days for the doses of 75 and 150 g a.i. ha⁻¹ respectively which was lower than the values observed in the present study. However, in the study of residue analysis of flonicamid in cotton by Chawla *et al.* (2018) half-life ranged between 4.6 to 7.0 days which was in congruence with the present study.

The quantity of commodity consumed by a person is so important for predicting the risk caused by the particular pesticide. The safety of the product can only be concluded by considering the results of risk assessment studies. In order to assist the dissipation, risk assessment studies was also conducted to analyse the impact of insecticides on the health of the consumers. It is only a theoretical calculation by comparing the theoretical maximum residue contribution (TMRC) with maximum permissible intake (MPI). If values of TMRC were lower than the MPI values the concentration of insecticides does not cause any risk to the consumer. In the present study insecticides viz, thiamethoxam + lambda cyhalothrin, clothianidin and flonicamid were found to be safe as they do not pose any serious health risk for the consumers. The safety of ready to use mixtures towards the health of consumers are also reported by Parmar *et al.* (2016) and Bhattacharyya *et al.* (2017) of flubendiamide + thiacloprid in red gram and emamectin benzoate + fipronil in chilli respectively.

Studies of Hampaiah (2018) was in line with the present work where thiamethoxam + lambda cyhalothrin was safe for consumers even from first day after spraying. Risk assessment studies conducted by Padmanabhan (2018) revealed that in cabbage and cauliflower dimethoate and fipronil was found to be harmful for consumers while other insecticides *viz.*, chlorantraniliprole, flubendiamide, indoxacarb, emamectin benzoate, quinalphos, cypermethrin, acetamiprid and thiamethoxam were observed to be safe. Studies by Reddy (2018) revealed insecticide mixtures chlorantraniliprole 8.8 % + thiamethoxam 17.5 % SC, lambda cyhalothrin 4.6 % + chlorantraniliprole 9.3 % ZC and thiamethoxam 12.6 % + lambda cyhalothrin 9.5 % ZC were observed to be safe while comparing MPI and TMRC values.

A prevalent resistance management plan is the need of the hour for the successful management of whiteflies. Several research works have been carried out across the world on the insecticide resistance against *B. tabaci* (Cahill *et al.*, 1995). However no study on the insecticide resistance in spiralling whitefly *A. dispersus* has been carried out even though they are causing severe damage in many crops especially vegetables. Compared to old generation insecticides, new generation insecticides have high potential for managing insects as they are more selective with toxicity to target pests even at lower dose and often not as

persistent as conventional insecticides. The present study is a maiden attempt in assessing the development of insecticide resistance in the field populations of *A. dispersus* in tomato in Kerala. This investigation revealed the development of insecticide resistance in the field population of *A. dispersus* against quinalphos and fenvalerate. In order to manage the resistance build-up in spiralling whitefly thiamethoxam 12.6 + lambda cyhalothrin 9.5% ZC @ 33+15.75 g a.i ha⁻¹ is an effective insecticide followed by clothianidin 50% WDG @ 20 g a.i ha⁻¹ and flonicamid 50% WG @ 75 g a.i ha⁻¹. Residue estimation and risk assessment also revealed the safety of these insecticides to the consumers.



6. SUMMARY

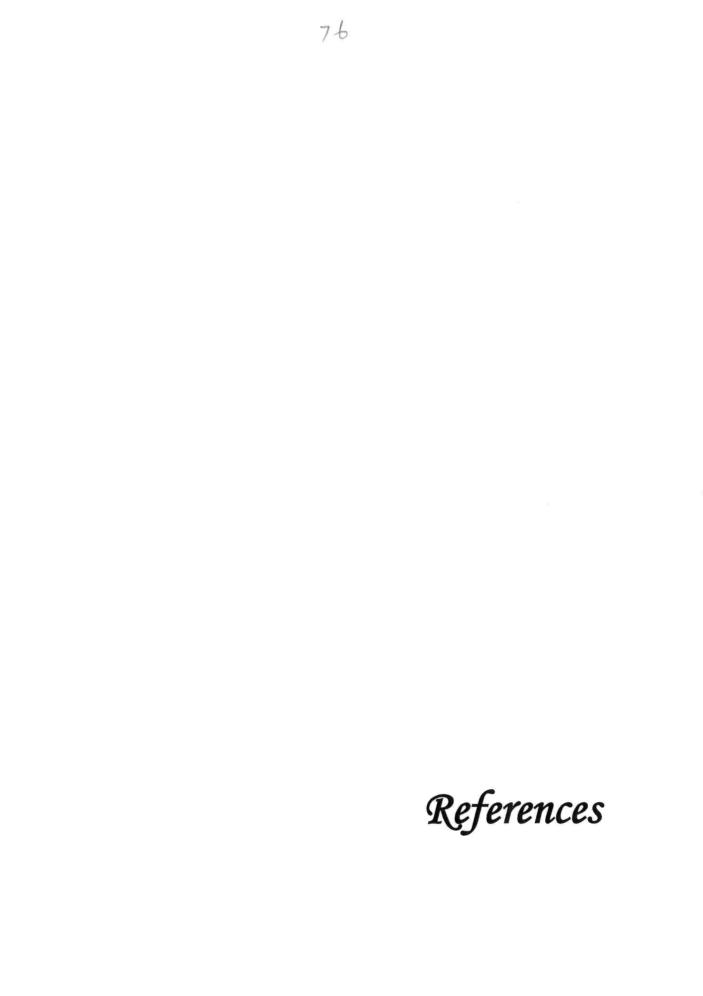
Extensive damage caused by insect pests to the agro ecosystem has forced the farmers to choose chemicals over other pest management strategies. However, indiscriminate and irrational use of insecticides for a long time has resulted in the buildup of resistance by these insect pests. Thus insecticide resistance against a frequently used chemical is a heritable change incurred by the insect which ultimately results in a flared up insect population. The present study was undertaken to assess insecticide resistance in the field population of spiralling whitefly, *A. dispersus*, to evaluate the efficacy of new generation insecticides against the resistant population of *A. dispersus* and to study the persistence and degradation of residues of insecticides in tomato. The results are summarized as:

- The whitefly species observed were spiralling whitefly, *A. dispersus*, rugose spiralling whitefly, *A. rugioperculatus* and solanum whitefly, *A. trachoides*.
- Bioassay was conducted to assess the insecticide resistance in the field population of *A. dispersus* against quinalphos, fenvalerate and imidacloprid from three different locations *viz.*, location I- field with no previous history of pesticide application (Sreekaryam), location II- field with pesticide application and control failure (Vellayani) and location III- field with pesticide application and no control failure (Kalliyoor). Population collected from location I was considered as the susceptible population with resistance ratio 1. Population gathered from Vellayani (location II) showed 2.9 times resistance to fenvalerate followed by quinalphos (2.6) and imidacloprid (1.84). Population from location III showed 1.62 times resistance to fenvalerate, 1.14 times resistance to quinalphos and 1.28 times resistance to imidacloprid.
- Laboratory experiment undertaken to evaluate the efficacy of new generation insecticides *viz.* buprofezin 25% SC @ 75 g a.i ha⁻¹, clothianidin 50% WDG @ 20 g a.i ha⁻¹, cynantraniliprole 10.26% OD @ 90 g a.i ha⁻¹, dinotefuran 20% SG @ 25 g a.i ha⁻¹, flonicamid 50% WG @ 75 g a.i ha⁻¹, thiamethoxam 25% WG @ 50 g a.i ha⁻¹ and thiamethoxam 12.6 % +

lambda cyhalothrin 9.5% ZC @ 33+15.75 g a.i ha⁻¹ against the resistant population of *A. dispersus* revealed that cent per cent mortality was observed in *A. dispersus* treated with thiamethoxam 12.6% +lambda cyhalothrin 9.5% ZC @33+15.75 g a.i ha⁻¹ followed by clothianidin 50% WDG @ 20 g a.i ha⁻¹ (80%) and flonicamid 50% WG @75 g a.i ha⁻¹ (66.67%).

- Results of field experiment with insecticides viz. thiamethoxam 12.6% +lambda cyhalothrin 9.5% ZC @33+15.75 g a.i ha⁻¹, clothianidin 50% WDG @ 20 g a.i ha⁻¹ and flonicamid 50% WG @ 75 g a.i ha⁻¹ against resistant population of *A. dispersus* showed no whiteflies after one days of treatment in thiamethoxam 12.6% +lambdacyhalothrin 9.5% ZC followed by clothianidin 50% WDG (9.20) and flonicamid 50% WG (11.80).
- Dissipation studies on the residues of selected insecticides viz. thiamethoxam 12.6% + lambda cyhalothrin 9.5% ZC @ 33+15.75 g a.i ha⁻¹, clothianidin 50% WDG @ 20 g a.i ha⁻¹, flonicamid 50% WG @ 75 g a.i ha⁻¹ were conducted by collecting insecticide treated tomato fruits at 0, 1, 3, 5, 7, 10, 15 and 30 days after spraying revealed that thiamethoxam 12.6% + lambda cyhalothrin 9.5% ZC @ 33+15.75 g a.i ha⁻¹ dissipated within three days, while clothianidin 50% WDG @ 20 g a.i ha⁻¹ and flonicamid 50% WG @ 75 g a.i ha⁻¹ dissipated within 15 and 10 days with half-lives of 4.05, 3.42, 8.92 and 7.82 respectively.
- Risk assessment studies were undertaken by using dissipation data to calculate and compare theoretical maximum residue contribution (TMRC) with maximum permissible intake (MPI). In all the three insecticides TMRC value was lower than MPI indicating the safety for consumption of fruits after insecticide application.





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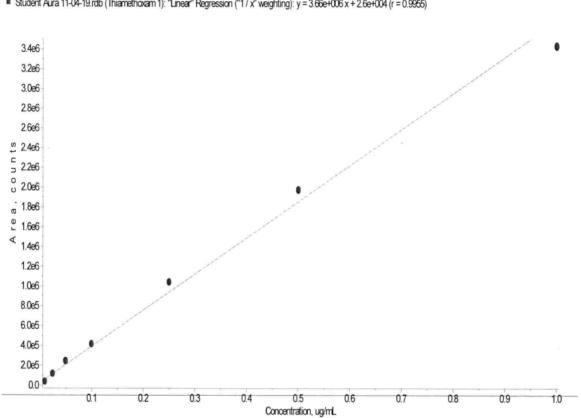
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Appendices

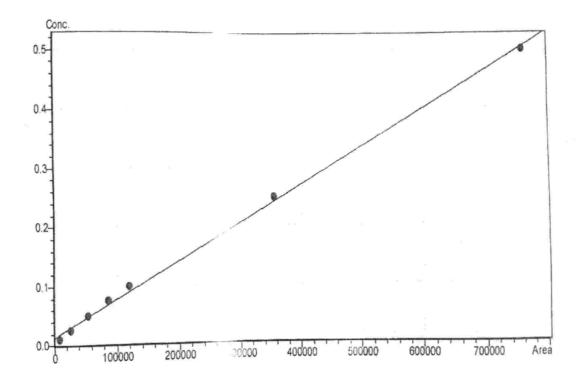




Student Aura 11-04-19.rdb (Thiamethoxam 1): "Linear" Regression ("1 / x" weighting): y = 3.66e+006 x + 2.6e+004 (r = 0.9955)

Calibration curve of thiamethoxam

APPENDIX- II

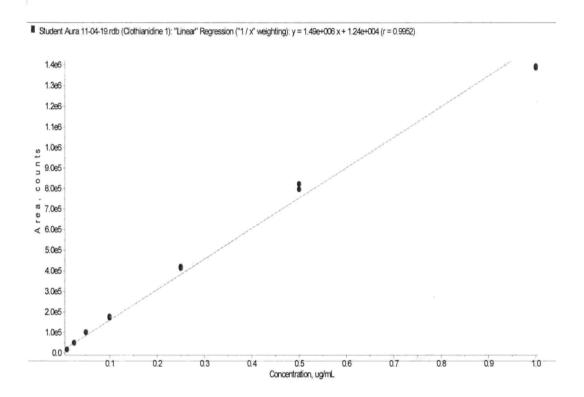


Y= aX+b, a= 6.453712e -007 and b= 1.401983e-002, R² = 0.9982105, R= 0.9991049, Mean RF: 8.492301e-007, RF SD : 1.293477e-007, RF % RSD : 15.23117

Calibration curve of lambda cyhalothrin

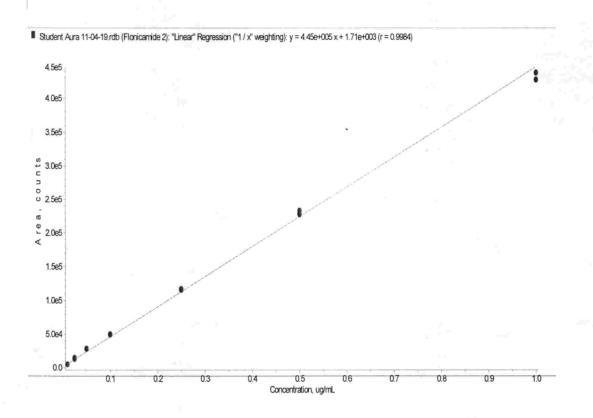
106





Calibration curve of clothianidin

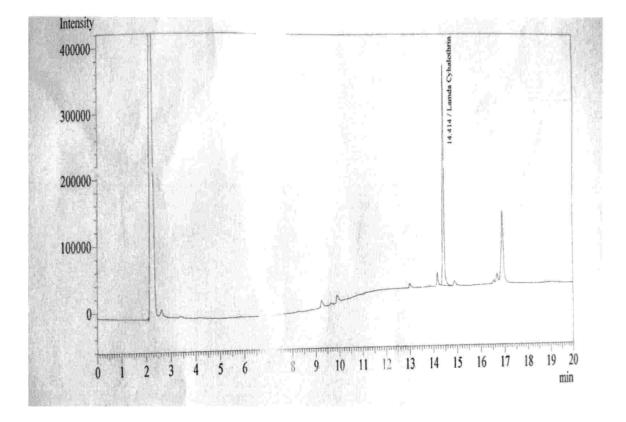




Calibration curve of flonicamid

APPENDIX- V

95

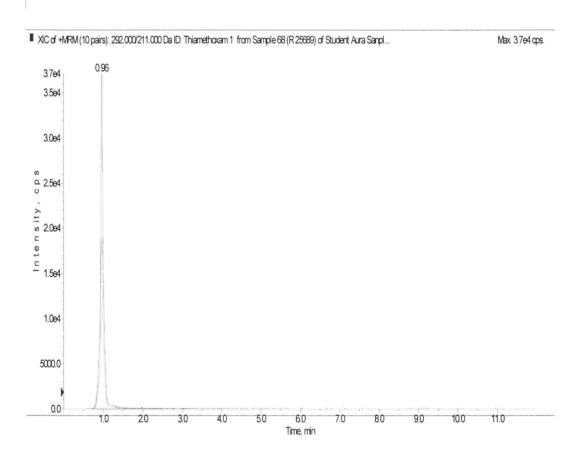


Retention time= 14.414; Area= 1335989; Height= 340242; Conc. = 0.522; Units= ppm

GC-ECD chromatogram of lambda cyhalothrin

109

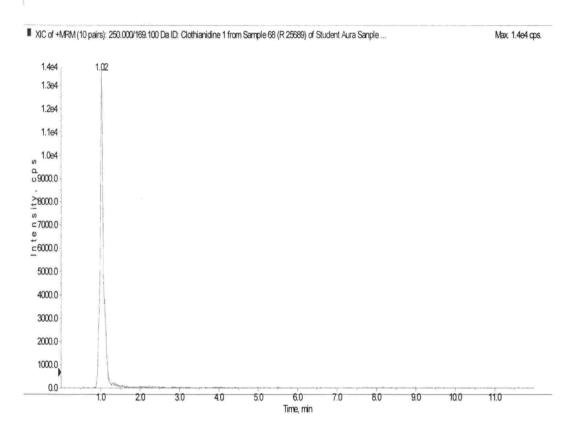




LC-MS/MS chromatogram of thiamethoxam



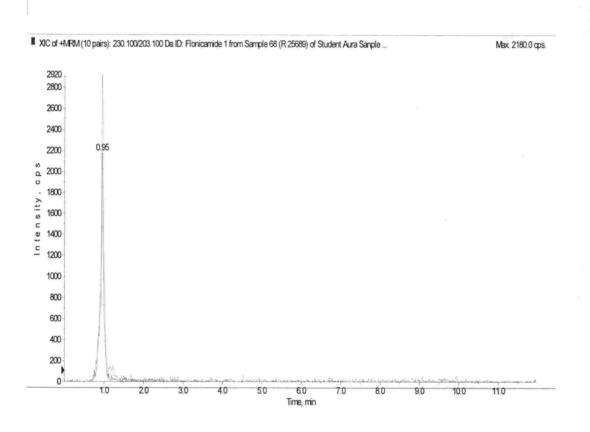
APPENDIX- VII



LC-MS/MS chromatogram of clothianidin

111





LC-MS/MS chromatogram of flonicamid

ala

INSECTICIDE RESISTANCE IN SPIRALLING WHITEFLY, Aleurodicus dispersus RUSSELL (HEMIPTERA: ALEYRODIDAE) AND ITS MANAGEMENT

by AURA SENSON

(2017-11-063)

Abstract of the thesis Submitted in partial fulfilment of the requirements for the degree of

MASTER OF SCIENCE IN AGRICULTURE

Faculty of Agriculture Kerala Agricultural University



DEPARTMENT OF AGRICULTURAL ENTOMOLOGY COLLEGE OF AGRICULTURE VELLAYANI, THIRUVANANTHAPURAM-695522 KERALA, INDIA

ABSTRACT

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A study on "Insecticide resistance in spiralling whitefly, *Aleurodicus dispersus* Russell (Hemiptera: Aleyrodidae) and its management" was done at College of Agriculture, Vellayani and farmer's field at Kalliyoor during 2018 to 2019. To assess the insecticide resistance in the field population of spiralling whitefly, *A. dispersus* and to evaluate the efficacy of new generation insecticides against resistant population of *A. dispersus* were the objectives of the study.

Bioassay was carried out to assess the insecticide resistance in field population of *A. dispersus* collected from three different locations (location I-Sreekaryam, location II-Vellayani and location III-Kalliyoor) based on the intensity of insecticide application. A series of concentrations of three insecticides *viz.*, quinalphos, fenvalerate and imidacloprid were prepared in aqueous solution and leaf dip bioassay was done using the field population of whiteflies collected from three locations. Results revealed that population collected from location-I (Sreekaryam) was found to be susceptible to insecticides with resistance ratio-1 for all three insecticides, which was considered as reference strain. Population collected from location-II (Vellayani) showed higher resistance with resistance ratio of 2.60, 2.90 and 1.85 and population from location-III (Kalliyoor) was found to be moderately resistant with resistance ratio of 1.14, 1.62 and 1.28 with respect to quinalphos, fenvalerate and imidacloprid respectively.

Laboratory experiments were conducted to evaluate the efficacy of new generation insecticides *viz.* buprofezin 25% SC @ 75 g a.i ha⁻¹, clothianidin 50% WDG @ 20 g a.i ha⁻¹, cynantraniliprole 10.26% OD @ 90 g a.i ha⁻¹, dinotefuran 20% SG @ 25 g a.i ha⁻¹, flonicamid 50% WG @ 75 g a.i ha⁻¹, thiamethoxam 25%WG @ 50 g a.i ha⁻¹ and thiamethoxam 12.6% + lambda cyhalothrin 9.5% ZC @ 33+15.75 g a.i ha⁻¹ against resistant population of *A. dispersus* in tomato plants. The results revealed that significantly higher mortality was observed in *A. dispersus* treated with thiamethoxam 12.6% + lambda cyhalothrin 9.5% ZC @ 33+15.75 g a.i ha⁻¹ (100%), followed by clothianidin 50% WDG @ 20 g a.i ha⁻¹

(80%) and flonicamid 50% WG @ 75 g a.i ha⁻¹ (66.67%) after 0.75 hours of treatment.

Field experiment was conducted by using tomato plants (var. Vellayani Vijay) at Vellayani from where resistant population was collected with three effective insecticides selected from laboratory along with control. No whiteflies were seen in thiamethoxam 12.6% + lambda cyhalothrin 9.5% ZC @ 33+15.75 g a.i ha⁻¹ treated plants followed by clothianidin 50% WDG @ 20 g a.i ha⁻¹ (9.20) and flonicamid 50% WG @ 75 g a.i ha⁻¹(11.80) after one day of spraying.

Studies on the dissipation of residues of effective insecticides *viz.*, thiamethoxam 12.6% + lambda cyhalothrin 9.5% ZC @ 33+15.75 g a.i ha⁻¹, clothianidin 50% WDG @ 20 g a.i ha⁻¹ and flonicamid 50% WG @ 75 g a.i ha⁻¹ were conducted in tomato plants at farmer's field at Kalliyoor. Tomato fruits collected at 0,1,3,5,7,10,15 and 30 days after application of insecticides at recommended dose and results showed that insecticides dissipated within 10 days with half- lives of 4.05, 3.42, 8.92 and 7.82 days respectively. The risk assessment studies also proved the safety of insecticides for the end users.

The present study revealed the development of insecticide resistance in the field population of *A. dispersus* against fenvalerate and quinalphos. Thiamethoxam 12.6% + lambda cyhalothrin 9.5% ZC @ 33+15.75 g a.i ha⁻¹ followed by clothianidin 50% WDG @ 20 g a.i ha⁻¹ and flonicamid 50% WG @ 75 g a.i ha⁻¹ could be recommended against the resistant population of *A. dispersus* in tomato. Dissipation and risk assessment studies also supported the result by establishing their safety to consumers. Further studies have to be taken up to develop and popularize Insecticide Resistant Management strategies against *A. dispersus* by developing Good Agricultural Practices on efficient use of insecticides and to conserve the ecosystem for sustainable pest management.

