FIELD TOXICITY OF NEW GENERATION INSECTICIDES TO BEE POLLINATORS

by

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THESIS

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DECLARATION

I, hereby declare that this thesis, entitled **"FIELD TOXICITY OF NEW GENERATION INSECTICIDES TO BEE POLLINATORS"** 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.

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Certified that this thesis, entitled **"FIELD TOXICITY OF NEW GENERATION INSECTICIDES TO BEE POLLINATORS"** is a record of bonafide research work done independently by **Ms. RAEESA P. (2016-11-052)** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to her.

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EXTERNAL EXAMINER

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RAEESA P.

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LIST OF ABBREVIATIONS AND SYMBOLS USED

a. i.	Active ingredient
et al.	And other co workers
@	At the rate of
cm	Centimetre
CD	Critical difference
DAS	Days after Spraying
^{o}C	Degree Celsius
Fig.	Figure
g	Gram
ha	Hectare
kg	Kilogram
µg	Microgram
ng	Nanogram
LD_{50}	Lethal dose
LC_{50}	Lethal concentration
m	Metre
mm	Millimetre
viz.	Namely
NS	Non significant
No.	Number
%	Per cent
ha^{-1}	Per hectare
CRD	Completely Randomized Block Design
RBD	Randomised Block Design
RH	Relative humidity
SE	Standard error
sp. or spp.	Species (Singular and Plural)

mL	Milliliter
m^2	Square metre
i.e.	That is
WP	Wettable powder
L	Litre
WG	Wettable granules
SG	Soluble granules
SC	Suspension concentrates
EC	Emulsifiable concentrates
HAT	Hours after treatment
ppm	Parts per million
ppb	Parts per billion
GC	Gas chromatography
LC	Liquid chromatography
rpm	Rotations per minute

INTRODUCTION

1. INTRODUCTION

Bees popularly called as "Angels of Agriculture" are essentially recognized as the most important insects in the world and are the primary insect pollinators of most of the cross pollinated crops (Deodikar and Suryanarayana, 1977). Honey bees contribute to 73 percentage of pollination in cross pollinated plants. Most of the horticultural crops are either pollinated or benefited by the service of pollination through bees (Thapa, 2006). Bee pollination assures increased quality and quantity of the produce in different crop plants. In brief, bee pollination is an essential component to maintain diet diversity, biodiversity and natural resources (Gallai *et al.*, 2013) and their conservation is vital for crop pollination and there by agricultural production.

Pest management is crucial in all productive agriculture systems across the world. Regular monitoring of the pest problems and judicious use of pesticides in agriculture leads to improved crop yield. Though the crop losses due to pest attack is more in India, the intensity of the pesticide consumption in the country is one among the lowest (Devi *et al.*, 2017). But, India is having a production system which is still supporting the manufacturing and using of several hazardous pesticides which are banned elsewhere. There is overwhelming evidence that some of these chemicals do pose potential risks to pollinators, particularly, honey bees.

While taking managerial decisions for sustaining crop productivity by employing insecticides against pests, bees' safety must be ensured. The exposure of honey bees to pesticides may occur through contaminated pollen/nectar or by their direct contact on the sprayed field crops (Jaycox, 1964). In particular, honey bees are exposed to lethal and sub lethal doses of pesticides during foraging leading to direct mortality of the bee population. Also, their indirect effect has led to weakening of the colonies which in turn, predisposes the population to other factors and results in a large scale decline (Fairbrother *et al.*, 2014).

Crops requiring cross pollination is an area of prime concern as insecticide use in such crops results in high bee mortality. Sometimes it exceeds 90 per cent in some apiaries (Wedberg and Erickson, 1986). Protection of honey bees from pesticidal hazards has been a challenging task. Higher levels of toxicity of the insecticides towards non target organisms and resistance development in the pest population have led to the replacement of the conventional insecticides with newer molecules; the insecticides with novel structures and their combination products with targeted action against pests become an integral part of IPM in the vegetable ecosystem.

With the increasing focus on the ecosystem health, the public is more concerned about the pesticide usage and residue contamination. The specific modes of actions of the new generation insecticides in the insect body, low dose requirement with high efficacy and low toxicity to non-target organisms made their use extensive in the agro ecosystems. But many of these insecticides are reported to have toxicity towards pollinators particularly, honey bees.

Although poorly studied, a harmonious compromise between pest management and honey bee pollination of crops in India is important. Information on safety of different insecticides to honey bees is scanty, hence, there is a need to evaluate the toxicity of these newer insecticides that entered recently in the market. It has been established that laboratory studies are specific and can be used in deciding the hazards of field application of insecticides (Atkins *et al.* 1973). Keeping these facts in view the present investigations have been carried out with the following objectives:

- To evaluate the field toxicity of new generation insecticides to major pollinators namely, *Apis cerana indica* Fab. and *Tetragonula iridipennis* Smith.
- To assess the insecticide residues in the flowers of culinary melon

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

Since the beginning of Agriculture, around 10,000 years ago, farmers have had to compete with harmful organisms – pests in their crop fields. For the prevention and control of crop losses due to these pests, crop protection has been developed which includes cultural, physical, biological, and chemical measures among which, the final one is more preferred by the farmers. Injudicious use of synthetic organic insecticides in agro ecosystems results in resistance development and their side effects on non-target organisms and environment which in turn, lead to their replacement with a group of molecules having novel mode of action, selectivity, higher bio efficacy in pest control in a comparatively safer manner to the environment.

Recently, several new generation insecticides *viz.,* thiamethoxam, dinotefuran, diafenthiuron, novaluron, cyantraniliprole and some combination products like thiamethoxam + chlorantraniliprole and flubendiamide + thiacloprid were proved to be effective against pests in several agro ecosystems but their toxicity towards honey bees and other pollinators are less studied.

Honey bees, which stand for around 77 per cent of the total pollinators are highly exposed to these agrochemicals sprayed in the crop field. Apart from causing direct mortality, they also come across with certain sub lethal effects that impair their communication mechanism, learning behaviour, memory capacity, foraging, etc. The literature pertaining to insecticide toxicity, foraging by the bee on flowers and pesticide residues of these new generation insecticides are reviewed here under.

2.1 INSECT FAUNA IN CULINARY MELON

Culinary melon is a highly cross pollinated crop and insects are designated as the major pollinating agents in them. Srivastava (1991) reported 23 species of insects visiting cucurbitaceous crop which belonged to the orders Hymenoptera,

Hemiptera, Thysanoptera, Lepidoptera, Diptera and Coleoptera in India. These insect visitors can be pests, natural enemies, pollinators or neutrals.

2.1.1 Pests

Ghule *et al.* (2014) reported that the major pests associated with *Cucumis sativus* are *Bactrocera cucurbitae* Coq., *Henosepilachna septima* Dieke and *Aulacophora foveicollis* Lucas and their visit on the crop was sequential. Also, culinary melon is designated as an important alternate host for cotton leaf hopper, *Amrasca devastans* Dist. (Saeed *et al.*, 2015).

The major defoliators of *Cucumis melo* L., pumpkin beetles, *Aulacophora* spp., were observed most abundantly in the month of December and least in August in Tripura and the feeding rate on the leaf was found to be 76.5 mm^2 day^{-1} (Roy and Pande, 1991). Blackmer and Byrne (1999) reported that amino acids developed during the active growing phase of the plant are the attractant for whiteflies, *Bemisia tabaci* Genn. on the leaf surface. The population dynamics of pumpkin caterpillar, *Diaphania indica* Saun. in culinary melon was studied by Peter and David (2008) and reported that lowest incidence of pest occurs in the period November to February and highest in April to September in Padappai, Tamil nadu. Fruit fly is another pest causing high economic losses and biological and chemical management of the pest is very difficult (Haldhar *et al.*, 2013).

2.1.2 Pollinators

In a study conducted in Punjab, it was found that the most abundant pollinator of musk melon was *Apis florea* Fab. and solitary bees. Apart from these, a few number of *Apis dorsata* Fab. and *Apis mellifera* L. were also visited the crop but not *A. cerana indica* (Grewal and Sindhu, 1978). The pollinating insects that visited watermelon were honey bees (91.26 %), solitary bees and a few dipterans (Rao and Suryanarayana, 1988). *A. dorsata*, *Xylocopa chlorina* Cock., *Xylocopa philippinensis* Smith. and *Megachile atrata* Smith. are the major

flower visitors in culinary melon and were most abundant from 1000 h to 1100 h (Cervancia and Bergonia, 1991). Later, Nogueira-Cautao and Calmona (1993) reported that Honey bees (*A. mellifera*) constituted 82.60 per cent of visitors of cucumber flower.

Eswarappa (2001) reported that a total of 24 insect species were visiting the chow-chow flowers among which, 14 belonged to Hymenoptera, four each to Diptera, Lepidoptera and Coleoptera. More than 80 per cent of the pollination was carried out by *A. florea*, *A. cerana*, *A. dorsata* and *T. iridipennis*. In Karnataka, Pateel and Sattagi (2010) reported that the most abundant pollinator in rabi crop of cucumber is *A. florea*, *A. dorsata* and *A. cerana indica*. The studies revealed that cucumber flowers attracted wide varieties of insects belonging to 4 orders, 12 families, 17 genera and 21 species. Of all these insects, honey bees were the most predominant and comprised more than 74 per cent of the total flower visiting insects. The abundance followed the order *A. mellifera*> *A. cerana*> *A. dorsata*> *A. florea* (Dorjay *et al.*, 2017).

2.2 FORAGING BEHAVIOUR OF POLLINATORS

Pollination is most effective when viable pollen is transferred to a receptive stigma. In case of bee pollination, the bee visits always coincide with these conditions which make the pollination most effective. In the course of evolution, flowers are adapted to produce nectar and pollen in order to attract these pollinators to aid in their pollination.

2.2.1 *Relative Abundance of Bee Pollinators*

Jangaiah (2007), on insect community analysis in cucurbitaceous vegetables found that bees are the predominant pollinators, where *A. cerana indica* and *Tetragonula* sp. had the highest foraging activity in oriental pickling melon compared to bitter gourd and snake gourd in Kerala.

2.2.1.1 *A. cerana indica*

In Bangalore, in the month of February, *A. cerana indica* began foraging on summer squash at 0600 h in the morning (Girish, 1981). Fakuda (1987) reported that the bees are visiting male flowers of water melon more frequently than the female flowers and the maximum abundance was from 0800 h to 1000 h in Egypt. Rao and Suryanarayana (1988) reported that, *A. cerana indica* is the principal pollinator in water melon and the maximum bee activity in the crop was reported at 0900 h. Viraktamath (1990) conducted a study on foraging profile of the bee and observed that major pollen gathering (80 %) was before noon with a major peak during 0600 h to 1100 h and minor peak during 1600 h to1800 h. More pollen and nectar foragers were observed during August to February and August to March respectively.

Sattigi *et al.* (1996) reported that in general, the foraging population of *A. cerana indica* was observed throughout the day with a peak between 0800 h to 1100 h in winter, 0600 h to 1100 h in summer and 0800 h to 1200 h in monsoon irrespective of the crop in the transitional area of Dharwad. Jyothi (2003) reported that the peak foraging activity of *A. cerana indica* was at 1300 h with an average bee population of 24.3 to 26.70 bees and lowest population (0.00 bees) at 1800 h. Two distinct peaks of pollinator abundance and activity was observed in Niger i.e., between 1000 h to 1200 h and 1600 h to1800 h in Bangalore. In cucumber flowers, the foragers started visiting by 0600 h in the morning and the activity was at a peak during 1000 h to 1100 h with a bee population of 6 bees m^{-2} 5 $min.^{-1}$.

2.2.1.2 *T. iridipennis*

Devanesan *et al.* (2002) reported that under Kerala condition, the foraging activity of *T. iridipennis* started by 0700 h in the morning and an increase in activity was observed till 1300 h and reached its peak by 1500 h. No activity was observed at 1800 h. Bennet *et al.* (2003) reported that the foraging behaviour of *T. iridipennis* differ significantly than that from *Apis* spp. The number of

incoming pollen foragers in a particular colony ranged between 0.70 to 2.92 min.$^{-1}$ while, that of non-foragers was around 0.34 to 6.94 min.$^{-1}$ (Prasad and Chand, 2003). Maximum foraging activity was observed during February to July where the activity started by the morning and reached its peak by 1000 h.

2.2.2 *Foraging Rate of the Bee Pollinators*

Chandel *et al.* (2004) reported that on onion seed crop, *A. dorsata* has maximum foraging period (6.30 to18.55 h) followed by *A. cerana indica* (6.45 to 18.30 h) while, *A. mellifera* had the least foraging period (0.65 to 18.20 h). Neupane *et al.* (2006) reported that peak activity of the rock bees is at 0730 h and 1100 h in Nepal. Kalmesh (2012) reported that maximum number of pollen foragers was noticed in morning hours at 1000 h with 19.60 foragers 5 min.$^{-1}$ and the peak foraging activities of outgoing and incoming bees were observed at 1100 h with 44.4 foragers 5 min.$^{-1}$ and 43.8 foragers 5 min.$^{-1}$.

Singh *et al.* (2006) reported that early stage of the mustard crop was more preferred by *Apis* spp. for foraging. Soni *et al.* (2010) studied the activity of different insect pollinators in different hours of the day on pepino flowers and reported that an average number of 2.35 and 2.36 bees m^{-2}10 min.$^{-1}$ visited the flowers in the morning and evening hours respectively.

2.2.3 *Time Spent by Bee Pollinators on Flowers*

In caged and open plots of chow-chow, different honey bee species were reported and the maximum time was spent by *A. florea* (14.63 sec.) followed by *A. dorsata* (5.77 sec.) (Eswarappa, 2001), whereas, studies by Sharma *et al.* (2001) revealed that the time spent by *A. florea* is 37.99 sec. on onion flower. Foraging time of *A. dorsata* was estimated by Gulati *et al.* (2015) and they reported the speed to be around 58 sec. flower^{-1} at 0700 h to 0800 h in cotton flowers.

2.2.3.1 *A. cerana indica*

Rao and Suryanarayana (1988) reported that *A. cerana* was the principal pollinating agent in water melon and they spent 1.40 to 6.90 sec. on each staminate flower. The time spent by the bees was lesser in the early morning hours and increased upto 1100 h and there after the pollen availability is decreased. In Bangalore, the time spent by *A. cerana indica* was 7.59 sec. in open and caged chow-chow plants (Eswarappa, 2001). Prakash (2002) reported that on an average, the bee spent 38.12 sec. and 35.31 sec. on staminate and pistillate flowers respectively. On onion flowers, the time spent by the bees varied from 8.50 to 21.00 sec. for pollen and 11.40 to 23.00 sec. for nectar (Mupade and Kulkarni, 2010).

2.2.3.2 *T. iridipennis*

Eswarappa (2001) reported that in open and caged chow- chow plants, the time spent by *T. iridipennis* was 12.89 sec. for the collection of pollen. The time spent by the bees on the staminate and pistillate flowers was observed as 928.61 sec. and 271.99 sec. respectively (Prakash, 2002). Mupade and Kulkarni (2010) reported that the time spent by the stingless bees on onion flower for pollen collection varied from 39.00 to 55.00 sec. while for nectar collection, it was 39.00 sec. to 59.00 sec.

2.2.4 *Foraging Speed of Bee Pollinators*

Choudhari *et al.* (2006) studied the foraging speed of *A. cerana indica* and found that the speed was lesser in the early morning and evening hours. Foraging speed of *A. dorsata* was estimated by Gulati *et al.* (2015) and they reported the speed to be around 8 flowers $min.^{-1}$ between 0700 h to 0800 h on cotton flowers.

2.2.5 *Foraging Activities of Hived Bees*

Holi (1997) reported that the peak activity of *A. cerana indica* colonies were the same in both winter and monsoon period with peak activity of outgoing foragers during 1100 h to 1300 h in Dharwad. A major outgoing and pollen foragers occurred between 0700 h to 0800 h and minor peak between 1700 h to 1800 h. Foraging activities had positive correlation with temperature and negative correlation with rainfall and RH. Manghanvi *et al.* (2012) reported that peak foraging activities of outgoing and incoming bees (*A. cerana indica*) were observed between 1000 h to 1100 h with 43.8 and 19.6 foragers 5 $min.^{-1}$ respectively in Karnataka.

2.3 TOXICITY OF NEW GENERATION INSECTICIDES TO BEE POLLINATORS

2.3.1 Thiamethoxam

Thiamethoxam is an insecticide widely recommended against the sucking pests in vegetable ecosystem. Among the group neonicotinoids, this compound is having highest toxicity towards bees. In an experiment conducted under laboratory condition, acute indirect contact toxicity of thiamethoxam was tested against *A. mellifera* and the LD_{50} value of the compound was found to be 0.03 µg bee^{-1} (Iwasa *et al.,* 2004). Pastagia and Patel (2007) evaluated the toxicity of thiamethoxam to *A. cerana indica* by dry film technique. The mortality percentage was recorded as 85.67 at 24 h after exposure. Contact toxicity of thiamethoxam against different strains of *A. mellifera* was tested by Laurino *et al.* (2013) and the values of LC_{50} fluctuated between 3.53 to 3.75 ppm for different strains. Later, Stanley *et al.* (2015) conducted laboratory bioassay of the insecticide in *A. cerana indica* and *A. mellifera* where they observed cent per cent mortality of the two species within 48 h of treatment. In contact LD_{50} tests of the insecticide, the value for *A. cerana* was found to be 0.0024 µg bee^{-1} (Yasuda *et al.*, 2017).

Very few studies are being conducted in risk assessment of pesticides towards stingless bees in our country. In Brazilian stingless bees, *Scaptotrigona aff. Depilis*, the survival and development of the larvae was affected by consuming the food contaminated with thiamethoxam under laboratory condition (Rosa *et al.*, 2016).

Henry *et al.* (2012) studied the effect of thiamethoxam in foraging activity of *A. mellifera* colony and the intoxicated bees when tracked by RFID (Radio Frequency Identification) tracking mechanism, significant reduction was observed in number of bees returning after foraging. Sublethal exposure to the insecticide affects the memory and thereby the foraging activity of bumble bee *Bombus terrestris* Linn. (Stanley *et al.*, 2015) and honey bees, *A. mellifera* (Shi *et al.*, 2017). Acute consumption of thiamethoxam by *A. mellifera* along with the sugar syrup resulted in reduced motor activity, impaired locomotion and their physical ability to fly (Tosi *et al.*, 2017). Exposure of the stingless bees to sub lethal doses of thiamethoxam resulted in some physiological and morphological changes in their body which in turn affected their normal foraging activities (Moreira *et al.*, 2018).

2.3.2 Dinotefuran

Dinotefuran is another neonicotinoid with contact action and slight trans-laminar action. On toxicity evaluation of field concentration of neonicotinoids on *A. mellifera*, the nitro-substituted compound dinotefuran was found to be highly toxic with LD_{50} value for contact toxicity as 0.0075 µg bee^{-1} (Iwasa *et al.*, 2004) and for *A. cerana*, it was found to be 0.0024 µg bee^{-1} (Yasuda *et al.*, 2017).

Decourtye and Devillers (2010) reported significant reduction in proboscis extension in dinotefuran affected bees which could affect their foraging activity and an exposure to one half of the LD_{50} value caused significant reduction in successful homing flights in them (Matsumoto, 2013). Dinotefuran administered

at its sub lethal doses along with the sugar syrup in an *A. mellifera* colony resulted in extinction of the colony within 26 days (Yamada *et al.*, 2015)

2.3.3 Diafenthiuron

Diafenthiuron is one of the important insecticides widely used in the cardamom plantations but it was found to be slightly harmful to *A. cerana, A. florea* and *A. dorsata* and moderately harmful to *T. iridipennis* (Stanley *et al.*, 2009). The concentration of the insecticide, which caused 90 per cent mortality in *Conogethes punctiferalis* Guenee. caused cent per cent mortality in Indian bee, *A. cerana indica* (Stanley *et al.*, 2010), also the lowest concentration of the insecticide recorded 70 per cent mortality in *A. florea* and *T. iridipennis* under laboratory conditions (Aravind and Samiayyan, 2014).

In Pakistan, studies on field mustard showed that diafenthiuron is not having any field toxicity towards *A. mellifera* (Perveen *et al.*, 2000). Stanley *et al.* (2010) also reported similar results in cardamom that there is no significant reduction in number of bees visited in the treated plants at 3 h, 6 h and 12 h after spraying. Rape seed mustard sprayed with the formulation, diafenthiuron 50 EC resulted in a reduction of 61.25 per cent in the foraging of *A. mellifera* population (Dutta *et al.*, 2016). In Kerala with the cardamom cultivar, Njellani (Green gold), diafenthiuron is very effective against the pests and it is not found to have impact on pollinator and natural enemy diversity in cardamom ecosystem in terms of species richness, diversity and evenness (Aravind *et al.*, 2018).

2.3.4 Novaluron

Novaluron is a growth regulator and has a safe profile against the honey bees under laboratory conditions. Yu *et al.* (2015) conducted acute spray toxicity evaluation of the commercial formulation of the insecticide on caged *A. mellifera* and the results illustrated the same and the LC_{50} value of the compound against the species was found to be 4.03 mg bee^{-1} which is relatively safer.

In case of wild pollinators, the insecticide affected the development of the larvae. Hodgeson *et al.* (2011) exposed the leaf cutting bee *M. rotundata* larvae to the field realistic concentrations of novaluron and high larval mortality was observed in the laboratory.

Bumble bee colonies fed with pollen from flowers sprayed with novaluron reduced the life span of the worker bees (Malone *et al.*, 2007) but didn't affect the foraging adult bees such as *Bombus impatiens* Cresson, *M. rotundata* and *Osmia lignaria* Say (Scott-Dupree *et al.*, 2009). Being an insect growth regulator, its exposure to the adult bee during field application has no observable effect on them, but it resulted in strong immature mortality effects such as dead eggs, dead pupae, etc. in *M. rotundata* (Pitts-Singer and Barbour, 2016).

2.3.5 Cyantraniliprole

Dinter and Samel (2014) tested both oral and contact toxicity of the commercial formulation of the insecticide under laboratory conditions in which the LD_{50} values were found to be 0.39 and 0.63 µg $bee^{-1}$for oral and contact toxicity tests respectively. O' Neill *et al.* (2014) demonstrated laboratory bioassay of cyantraniliprole 20 SC on pollinators and the results revealed that the formulation poses no potential risks to the pollinators under laboratory condition.

In green house grown tomatoes, drip application or foliar spray of cyantraniliprole didn't affect the pollinating bumble bees, *B. terrestris* which open the possibility of pest management in effective pollination in protected cultivation (Dinter and Samel, 2014).

2.3.6 Chlorantraniliprole

Chlorantraniliprole, an anthranilic diamide with a novel and very specific mode of action has an outstanding profile of safety to beneficial arthropods, centipedes, millipedes and other non-target organisms. In a study conducted in Denmark, it was demonstrated a low intrinsic toxicity of the insecticide in both

contact and oral tests against *A. mellifera*. They also reported that chlorantraniliprole at 0.05 µg bee^{-1} had low toxicity towards *B. terrestris* (Dinter *et al.*, 2009). Boli (2013) in his contact toxicity evaluation of new generation insecticides towards the domesticated bees of Kerala, *A. cerana indica*, *A. mellifera* and *T. irridipennis*, found that among the insecticides tested, chlorantraniliprole is having lowest toxicity in all the three species. The contact toxicity evaluation of the insecticide against two native stingless bees of Northern America, *Partamona helleri* Friese. and *Scaptotrigona xanthotrica* Moure. proved that it exhibits relatively no mortality in both the species (Tome *et al.*, 2015).

As chlorantraniliprole posed no significant reduction in number of honey bees or bumble bees foraging on wild canola flowers (Scott- Dupree *et al.*, 2009) it can be designated as a safer insecticide under field condition. Similarly, green house application of the insecticide didn't affect the supplementary pollinators, *B. terrestris* inside (Gradish *et al.*, 2010).

2.3.7 Flubendiamide

Flubendiamide is widely recommended for pest management nowadays because of its specific mode of action. Still, many studies on the insecticide proved that it is highly toxic to the natural enemy population and the pollinators (Chakraborti and Sarkar, 2011). Gradish *et al.* (2012) evaluated the toxicity of the insecticide to the bumble bees, the dominant pollinators of blue berry orchard in the laboratory and showed that flubendiamide didn't cause any mortality even at double its recommended label rate. The result was the same in oral toxicity test and there were no other sublethal effects observed. Boli (2013) reported that cent per cent mortality was observed on dry film technique with the insecticide in the major pollinators in vegetable ecosystems of Kerala *viz., A. cerana indica*, *A. mellifera* and *T. iridipennis*, within 6 hours of exposure.

In Australia, the side effects of foliar spray of flubendiamide was assessed by National Registration Authority for Agricultural and Veterinary Chemicals (NRAVC, 2009) and found that it is having no effect on the pollinating bumble

bees (*B. terrestris*). Similarly, when the pollen reserves of the alfalfa leaf cutting bee, *M. rotundata* was contaminated with flubendiamide, the larval survivorship or foraging behaviour of adult bees was not affected (Gradish *et al.*, 2012).

2.3.8 Thiacloprid

Horvat (2001) reported that thiacloprid is relatively safe to the honey bees. The LD_{50} value for the contact toxicity of the insecticide to *A. mellifera* workers was found to be in the range of 10.00 to 40.00 µg bee^{-1} (Jeschke *et al.*, 2001). Rabia *et al.* (2005) conducted laboratory bioassay of thiacloprid in *A. cerana indica* and reported high mortality within 48 h of exposure, but when the *A. mellifera* workers were exposed to the insecticide, the mortality counts were not significant both in oral and contact methods (Laurino *et al.*, 2011).

Significant reduction in activity of the bees was observed by Elbert *et al.* (2000) upon foliar application of thiacloprid. Exposure to thiacloprid spray resulted in reduced navigation memory which in turn affected the normal flight mechanism and successful foraging activity (Fischer *et al.*, 2014).

2.3.9 Dimethoate

The contact and oral toxicities of dimethoate was tested by Gough *et al.* (1994) in worker bees of *A. mellifera* and the LD_{50} values ranged between 0.11 to 0.26 µg bee^{-1} (contact) and 0.11 to 0.33 µg bee^{-1} (oral). Intoxication of four days old *A. mellifera* larvae with dimethoate caused significant larval mortality, pupal mortality and reduced/abnormal adult emergence in the laboratory and the LD_{50} value for the larvae was recorded as 1.9 µg $larva^{-1}$ (Aupinel *et al.*, 2007).

Foraging on a source contaminated with 1ppm dimethoate lead to reduced pollinator effectiveness in *A. mellifera* workers (Waller *et al.*, 1979). Waller *et al.* (1984) reported that when a lemon orchard in USA was sprayed with dimethoate, the bees stopped visiting the flowers from the day of spraying. When a new colony was introduced to the orchard, the bee mortality was observed in the

colony in a range of more than 1000 bees per day and this was continued upto a week. Rana and Goyal (1996) reported that it causes toxic effect on the foraging of *A. cerana* population on *Brassica chinensis* Linn. whereas the repelling effect of dimethoate on bees have resulted in lesser number of bee visits in sprayed fields of Himachal Pradesh (Mall and Rathore, 2003).

2.4 RESIDUES OF NEW GENERATION INSECTICIDES

2.4.1 Thiamethoxam

Dively and Kamel (2012) reported that the amount of thiamethoxam residue detected from the nectar is 73.80 to 88.80 per cent less than that in pollen reserves of melon flowers. The pollen and nectar samples of Squash, *Cucurbita pepo* L. were analysed using the standard procedures and found that the level of thiamethoxam in them was around 11ppb (Stoner and Eitzer, 2012).

When the pollen collected by the bees from a field treated with thiamethoxam were analysed for the residues, the level of the insecticide was found to be in the range of 1 to 7 $\mu g\ kg^{-1}$ (Pilling *et al.*, 2013). Seed treatment with the systemic insecticide, thiamethoxam caused negative effects on beneficial insects (Gontijo *et al.*, 2014).

2.4.2 Dinotefuran

Trace amounts of dinotefuran was detected from the pollen and nectar samples of different flowers which might not cause any risk to the consuming nurse bees (Blacquiere *et al.*, 2012). Stoner and Eitzer (2012) reported that the residues of dinotefuran in squash flowers as around 10 ppb which was greater than that found in canola and sunflower raised from seeds treated with the same insecticide.

2.4.3 Novaluron

Malone *et al.* (2007) assessed the risks posed by the residual novaluron in bumble bees of New Zealand and found that there was no significant difference between the control and treated bees. But, one week old residues of novaluron in canola plants affected the normal reproduction of alfalfa leaf cutting bee, *M. rotundata* (Pitts- Singer and Barbour, 2016).

2.4.4 Cyantraniliprole

When the bees were exposed to residues of cyantraniliprole of different days old cotton leaves, no adverse effect was seen on the bees and the residue levels in the pollen, nectar and the bee matrices were not significant (O' Neill *et al.*, 2014).

2.4.5 Chlorantraniliprole

Dinter *et al.* (2009) assessed the nectar, pollen and bee wax of the *A. mellifera* colony foraging near *Phacelia* flowers (soil treated with chlorantraniliprole) and found that there were quantifiable levels of the insecticides and metabolites in them.

2.4.6 Flubendiamide

Gradish *et al.* (2012) reported that flubendiamide is relatively safe for bumble bees, but when they feed the young ones with pollen and nectar with residues of this insecticide, it affected their growth and development.

2.4.7 Thiacloprid

In a study conducted in apple orchard, it was found that the pollen loads and bee bread contain detectable levels of thiacloprid upto one day after spraying (DAS) (Skerl *et al.*, 2009).

2.4.8 Dimethoate

Pollen and nectar of Alfalfa sprayed with dimethoate was subjected to pesticide residue analysis and observed that the pollen had only 0.5 ppm residue one DAS, but the nectar retained 3 ppm even after one week. In *A. mellifera* fed with 1 ppm dimethoate in sugar syrup the cholinergic activity was stopped and the survival was affected (Barker *et al.*, 1980). Nectar collected from dimethoate treated lemon flowers has the residue of 0.1 ppm concentration upto 8 days of treatment (Waller *et al.*, 1984).

MATERIALS AND METHODS

3. MATERIALS AND METHODS

The field toxicity of new generation insecticides to bee pollinators were evaluated both in laboratory and field conditions under All India Coordinated Research Project (AICRP) on honey bees and pollinators, Department of Agricultural Entomology, College of Agriculture, Vellayani during the period 2016-18. The materials used and the methods followed during the thesis work are depicted in this chapter.

3.1 LABORATORY EVALUATION OF INSECTICIDE TOXICITY TO HONEY BEES

The experiment was laid out in completely randomized design with fifteen treatments and four replications in two species of honey bees that are commonly domesticated under Kerala condition *viz., A. cerana indica* and *T. iridipennis*. The details of the insecticides used in the laboratory for their toxicity evaluation towards the bees are given in Table 1.

3.1.1 Preparation of the Spray Fluid for the Laboratory Evaluation of Toxicity in Honey Bees

The spray solutions of the following new generation insecticides were prepared in the laboratory at their recommended field concentration and half of the field concentration as mentioned below:

Thiamethoxam

The solutions were prepared by dissolving 0.3g and 0.15 g of Actara 25 % WG in 1000 mL tap water to get the concentrations of 0.03 % and 0.015 % respectively.

Table 1. Details of the insecticides used in the laboratory for their toxicity evaluation in honey bees

Chemical name	Trade name	Manufacturer	Recommended field dose (g a. i. ha^{-1})	Concentrations tested (g or mL L^{-1})
Thiamethoxam	Actara 25 WG	Syngenta	25	3.00
				1.50
Dinotefuran	Token 20 SG	Indofil Industries Ltd.	30	3.00
				1.50
Novaluron	Rimon 10 EC	Indofil Industries Ltd.	75	2.00
				1.00
Cyantraniliprole	Verimark 20 SC	EI DuPont	90	1.20
				0.60
Diafenthiuron	Pegasus 50 WP	Syngenta	300	1.20
				0.60
Thiamethoxam (17.5 %) + Chlorantraniliprole (8.8 %)	Voliam flexi 300 SC	Syngenta	150	0.40
				0.20
Flubendiamide (19.92 %) + Thiacloprid (19.92 %)	Belt Expert 480 SC	Bayer Crop Science	60+60	0.40
				0.20
Dimethoate	Rogor 30 EC	Tata Rallis Ltd.	200	0.50
				0.25

Dinotefuran

Field concentration of the insecticide was prepared by dissolving 0.3 g of Token 20 % SG in 1000 mL of water and for getting half of the field concentration, 0.15 g was dissolved in 1000 mL water.

Cyantraniliprole

1.2 mL of Verimark 20 % SC was taken with a micropipette and was dissolved in 1000 mL of water to get the field concentration. Similarly, 0.6 mL of the same was dissolved in 1000 mL water to get half of the field concentration.

Novaluron

For obtaining the concentrations of 0.002 % and 0.001 % of the solution, 2 mL and 1 mL of Rimon 10 % EC was taken in a micropipette respectively and were dissolved in 1000 mL of tap water.

Diafenthiuron

Pegasus 50 % WP weighed using an electronic balance to a quantity of 1.2 g and 0.6 g, were dissolved in two beakers with 1000 mL water to get the field and half the field concentrations.

Thiamethoxam (17.5%) + Chlorantraniliprole (8.8%)

For preparing the solutions of field concentration and half the field concentration, 0.4 mL and 0.2 mL of voliam flexi was measured using a micropipette and dissolved in 1000 mL tap water.

Flubendiamide (19.92%) + Thiacloprid (19.92%)

From the combination product, Belt Expert, 0.4 mL and 0.2 mL were taken using a micropipette and dissolved in 1000 mL of tap water.

3.1.2 Preparation of the Containers for the Experiment

For Indian bees, round aquarium glass bowls of 12 cm diameter were used. One jar served as one replication. The glass jars were washed thoroughly and dried. From the prepared spray solution, 3 mL was pipetted and poured to each of the container. Container with 3 mL of tap water served as the control. The bowls were rotated till the inner surfaces of them became completely covered with the spray solution. Then they were allowed to dry in shade to get a thin film of insecticide in the glass jar (Beevi *et al.*, 2004). An OHP strip of dimension 2.5 cm x 5 cm attached at the edge of the bowl using a gem clip served as the platform for providing 50 per cent honey solution as a food source.

For the toxicity evaluation in stingless bees, large test tubes of 2.5 cm diameter were used. Washed and dried tubes were added with 2 µL of the insecticide solution and rotated till the test tube get completely covered with the insecticide solution and then shade dried to get a thin film of insecticide inside the tube. OHP strip of dimension 1 cm x 4 cm was attached with the mouth of the tube for the provision of honey solution.

3.1.3 Collection of Honey Bees for the Laboratory Experiment

Active colonies of both *A. cerana indica* and *T. iridipennis* were maintained in the apiary unit. Bees used throughout the experiment were collected from a single hive. Foraging bees collected from the hive entrance were used in the experiment.

For the collection of *A. cerana indica*, transparent polythene bags of convenient size were kept open in the hive entrance so as to collect the bees emerging for foraging early morning just after sunrise. In each bag required number of bees were collected and were tied using a rubber band and were carried to the laboratory.

Stingless bees were collected in a transparent container of narrow mouth. The mouth of the container was placed at the hive entrance and gentle tapping were given above the entrance. When required number of bees gets collected in the container, it was closed with a lid and carried to the laboratory to continue with the experiment.

3.1.4 Laboratory Evaluation of Toxicity of New Generation Insecticides to Honey Bees

The collected Indian bees were freeze anesthetized by placing them in the refrigerator for 2 minutes and 10 bees were transferred to the bowls from the polythene bags. After the transfer, the mouth of the container was closed using a

muslin cloth and were tied with a rubber band (Plate 1). Three drops of 50 per cent honey solution was provided on the OHP strip.

The lid of the bottle containing stingless bees was provided with a small hole and the mouth of the test tube was kept over the hole without leaving any gap. Rest of the portion of the bottle was covered with a black cloth in order to attract them towards the light. When 10 numbers of them entered the tube, the hole was closed and the mouth of the test tube was covered with a muslin cloth and tied with a rubber band (Plate 2). Three small drops of honey solution were placed over the OHP strip which served as the food source for them.

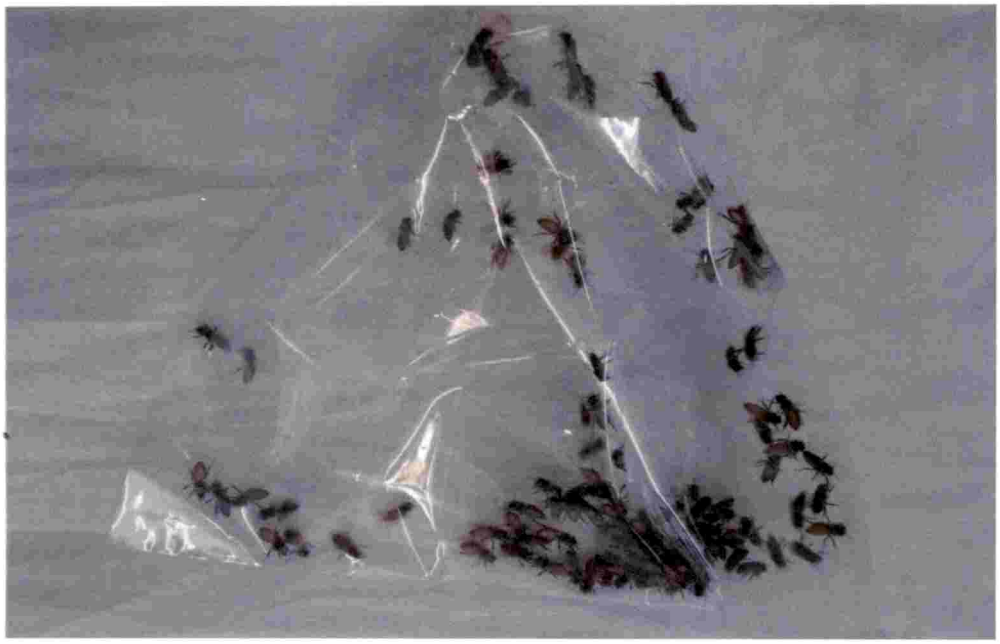
Bowls and tubes treated with water served as the control for the experiment. When the honey solution got exhausted, it was served again over the strip using a syringe and needle piercing through the muslin cloth covering. Mortality counts of the bees were taken at hourly intervals by visual observation.

3.1.5 Statistical Analysis

The mortality percentage obtained is adjusted using the Abbott's formula (Abbott, 1925) and the data generated were subjected to arc sine transformation followed by statistical analysis. Wherever the results were found to be significant, the critical differences were calculated at five per cent probability.

3.2 FIELD EVALUATION OF SAFETY/ TOXICITY OF INSECTICIDES TO BEE POLLINATORS

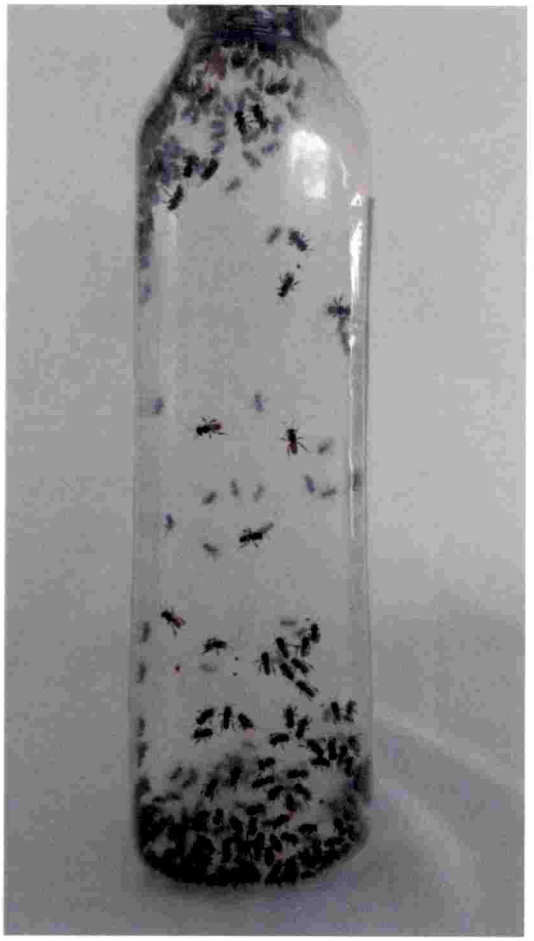
Two insecticides *viz.*, cyantraniliprole 20 SC and novaluron 10 EC that were found safe towards Indian bees and stingless bees in the laboratory bioassay were evaluated to find out their effect on their foraging activities under field condition.



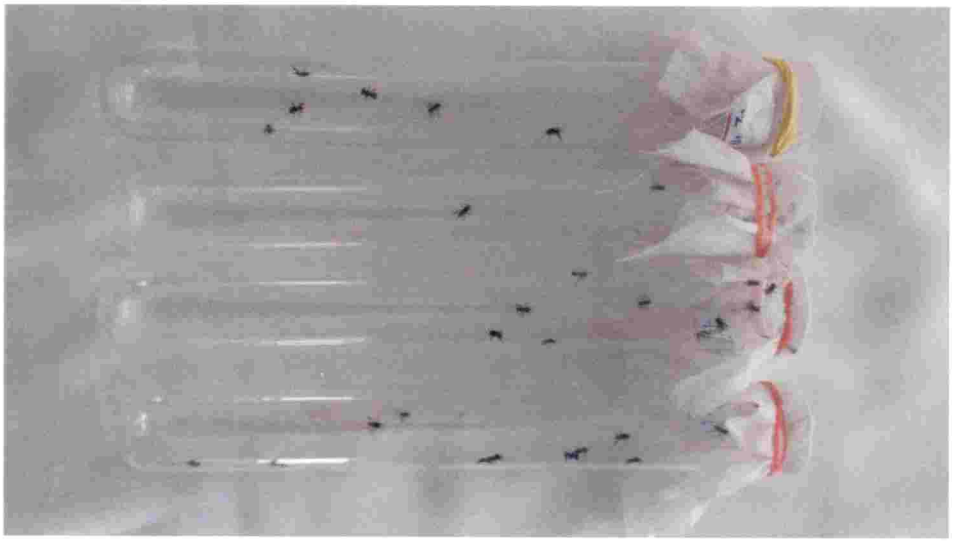
1 a. *A. cerana indica* workers collected from hive entrance

1 b. *A. cerana indica* workers inside treated bowls

Plate 1. Toxicity evaluation in *A. cerana indica*



2 a. *T. iridipennis* workers collected from hive entrance



2 b. *T. iridipennis* workers inside treated test tubes

Plate 2. Toxicity evaluation of insecticides in *T. iridipennis*

3.2.1 Lay out of the experiment

The field evaluation of new generation insecticides to test their effect on foraging activity of honey bees was carried out during the month of February to April of 2018 in the Instructional Farm, College of Agriculture, Vellayani (Plate 3).

Seeds of local variety of culinary melon (*C. melo*) vernacularly known as 'Vellari' were purchased from the Instructional Farm, Vellayani. The crop husbandry practices were done as mentioned in the package of practices recommendations of Kerala Agricultural University (KAU, 2016). The details of the experiment are given below.

Design: RBD

Plot size: 5 m x 3 m

Spacing: 1.5 m x 2 m

No. of plants per pit: 2

No. of observational plants per plot: 4

No. of replications: 5

No. of treatments: 4

A separation of 1m was given between the treatments within a block to avoid drift while spraying the insecticides. At 10 per cent flowering, strong colonies of *A. cerana indica* and *T. iridipennis* were placed 5 m away from the experimental site (Plate 4).

Spraying of cyantraniliprole 20 SC @ 1.2 mL L^{-1} and novaluron 10 EC @ 2 mL L^{-1} in the field were done at the peak flowering stage of the crop, i.e., 45 days after sowing. Hand compression sprayer was used for the application and the spraying was commenced by 6 am in the morning. In order to avoid cross

Plate 3. General view of the experimental field

4 a. Indian bee hive

4 b. Stingless bee hive

Plate 4. Hives placed near the field

contamination due to drifting, the treatments were separated using polythene sheets at the time of spraying.

3.2.2 Effect of New Generation Insecticides on Foraging Behaviour of Bee Pollinators

Pre-treatment count of pests, flower visitors and pollinators were recorded at the beginning of the experiment. The foraging activity of different pollinators like bees, wasps, butterflies, beetles, etc. were observed before and after spraying of insecticides. Most abundant pollinators in the field were recognized as the major pollinators of the crop. Peak periods of activity of each pollinator species was evaluated and further observations were taken at these periods.

In order to ensure sufficient number of pollinators in the field, one hive each of Indian and stingless bees were placed 5 m away from the field. The density of the pollinators in the field were evaluated before and after the installation of hives in the field. Foraging activity in terms of relative abundance, foraging rate and the time spent by the pollinator species were also noted before and after spraying.

3.2.2.1 *Relative Abundance of Pollinators*

Each plot in the experimental site was marked with an area of one m^2 on random basis. The observations were taken on five days before spraying and one, three, five, seven and fifteen DAS at 2 h intervals from 6 am to 6 pm. The peak time of foraging activity was recorded for each species. The number of flower visitors or pollinators was recorded from the marked area for a period of five minutes at their peak period of activity. Relative abundance of a particular pollinator species is given by the formula;

$$\text{Relative Abundance (\%)} = \frac{\text{No. of pollinator sp. } m^{-2}\ 5\ \text{min.}^{-1}}{\text{Total no. of pollinators } m^{-2}\ 5\ \text{min.}^{-1}} \times 100$$

3.2.2.2 *Foraging Rate of Bee Pollinators*

The number of bee pollinators per minute was recorded from one m^2 area in the plot and expressed as number of bees visited per unit time at their peak period of activity. The observations were taken before spraying and one, three, five, seven and fifteen DAS.

3.2.2.3 *Time Spent by the Pollinators on Flower of Culinary Melon*

Time spent by a pollinator in seconds was recorded using a stop watch at their peak period of activity. The time spent by each pollinator species was noted before spraying and one, three, five, seven and fifteen DAS.

3.2.2.4 *Foraging Speed*

Each of the flower visitor was observed for a period of one minute and the average number of the flowers visited in a single plot was recorded and expressed as the number of flowers visited per unit time.

3.2.3 Effect of New Generation Insecticides on Foraging Activity of Hived Bees

The numbers of foraging bees returning to the hives with pollen loads on their legs were recorded before insecticide application in field and after one, three, five, seven and fifteen DAS and were expressed as number of returning foragers per 5 minutes.

3.3 PERSISTENCE OF INSECTICIDES ON THE FLOWERS OF CULINARY MELON

The flowers in the field sprayed with the insecticides were subjected to pesticide residue analysis in the Pesticide Residue Research Analytical Lab (PRRAL), All India Network Project on Pesticide Residues, College of Agriculture, Vellayani. The flowers collected on zero (2 h after application), one, three, five, seven, ten and fifteen DAS were analyzed for pesticide residues.

3.3.1 Estimation of Persistence and Degradation of Residues of Insecticides

Flowers collected from each plot at each occasion were homogenized, sub-sampled and extracted following the QuEChERS method. The estimation of residues of novaluron was done using LC-MS/MS and the estimation of dimethoate was done using GC-ECD and confirmed in GC-MS/ MS.

3.3.1.1 *Chemicals and Reagents*

Acetonitrile, n- hexane, water, methanol (HPLC grade), sodium chloride, anhydrous sodium sulphate and magnesium sulphate were supplied from Merck, Germany. Certified Reference Materials (CRM) of novaluron and dimethoate were purchased from Sigma. Primary Secondary Amine (PSA) was procured from Agilent technologies, USA. Sodium chloride, anhydrous sodium sulphate and magnesium sulphate were activated in a muffle furnace at a temperature of 350 °C for 4 h and kept in desiccators. Commercial formulations of the insecticides were purchased from agro chemical shops.

3.3.1.2 *Preparation of Standards*

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

3.3.1.3 *Recovery Studies*

Recovery studies were conducted by spiking different concentrations (0.05, 0.25, 0.50 $\mu g\ kg^{-1}$) of analytical standards of novaluron and dimethoate in untreated culinary melon flowers. Five replicates were analysed at each spiking level and accuracy of analytical method was determined based on repeatability and relative standard deviation which is mandatory for residue validation.

3.3.1.4 *Extraction and Clean up*

QuEChERS method was adopted for residue extraction and clean-up in cucumber flowers. A well homogenized flower sample of 10 g was taken into 250 mL centrifuge bottle. The analyte was extracted by the addition of 20 mL acetonitrile of HPLC grade. The centrifuge bottles were closed tightly and homogenized with a high speed tissue homogenizer (Heidolph Silent Crusher-M) at 14000 rpm for 3 minutes, to which 4 g of activated sodium chloride was added and vortexed for 2 minutes to achieve good separation of acetonitrile layer. The homogenized mixture was centrifuged at 2500 rpm for 5 minutes. The extract of 12 mL was carefully transferred to a 50 mL centrifuge tube containing 6 g pre activated sodium sulphate. Vortexed for 2 minutes and the extracts were cleaned up by dispersive solid phase extraction (DSPE). From this, 8 mL of supernatant was transferred to 15 mL centrifuge tube containing 0.20 g PSA and 1.20 g magnesium sulphate and vortexed for 2 minutes. The vortexed mixture was centrifuged at 2500 rpm for 5 minutes from which 4 mL of supernatant liquid was transferred to turbo tube and evaporated to dryness under a gentle steam of nitrogen using a turbovap set at 40 °C and 7.5 psi nitrogen flow. The residues were reconstituted in 2 mL of methanol and filtered through a 0.2 micron PVDF filter prior to estimation in LC-MS/MS. A 3 ml of the extract was evaporated in a turbovap and made up to 1.5 ml using n-hexane for GC-ECD analysis.

3.3.1.4 *Instrumentation*

3.3.1.4.1 *LC-MS/MS*

Residues of novaluron were estimated using LC-MS/MS. The chromatographic separation was achieved using Waters Acquity UPLC system equipped with a reversed phase Atlantis d C-18 (100 ×2.1 mm, 5 µm particle size) column. A gradient system involving the following two eluent components: (A) 10 % methanol in water + 0.1 % formic acid + 5 mM ammonium acetate; (B) 10 % water in methanol + 0.1 % formic acid + 5 mM ammonium acetate was used as mobile phase for the separation of residues. The gradient elution was done as

follows: 0 min isocratic 20 % B, increased to 90 % in 4 min, then raised to 95 % with 5 min and increased to 100 % B in 9 minutes, decreased to the initial composition of 20 % B in 10 minutes and hold to 12 minutes for re-equilibration. The flow rate remains constant at 0.8 mL min^{-1} and injection volume was 10 µL. The column temperature was maintained at 40 °C. The effluent from the LC system was introduced into triple quadrupole API 3200 MS/MS system equipped with an electrospray ionization interface (ESI), operating in the positive ion mode. The source parameters were temperature 600 °C, ion gas (GSI) 50 psi, ion gas (GS2) 60 psi, ion spray voltage 5,500 V, curtain gas 13 psi.

3.3.1.4.2 *GC-ECD and GC-MS*

Estimation of residues of dimethoate was performed using Gas Chromatograph (Shimadzu 2010 AT) equipped with Electron Capture Detector (ECD). Operating conditions of GC are Column, DB- 5 capillary (0.25 µm film thickness x 0.25 mm x 30 m), carrier gas- Nitrogen, column flow- 0.79 mL/min., injector temperature -250 °C and detector temperature used was 300 °C. The residues of dimethoate was confirmed in GC-MS (Shimadzu GC- MS QP 2010 Plus) with retention time of 50.25 minutes.

Helium was used as carrier gas in GC-MS operated with Electron Impact Ionization (70 eV). In GC-MS, injector temperature, column, column flow was similar to that of GC-ECD. The MS/MS conditions were optimized 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 quantitation ion chromatogram and for qualitative purpose. The ion source temperature was 550 °C with ion spray voltage of 5500 V. Chromatographic elution zones were divided into appropriate number of time segments. In each segment corresponding MS/MS transitions were monitored using multiple reactions – monitoring (MRM) mode.

3.3.2 *Residue quantification*

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

Pesticide residue ($\mu g\ g^{-1}$) = (Concentration of the peak obtained from chromatogram) × Dilution factor

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

RESULTS

4. RESULTS

The results of the investigation on the toxicity evaluation of new generation insecticides to bee pollinators conducted at All India Co-ordinated Research Project (AICRP) on Honey bees and Pollinators, Department of Agricultural Entomology, College of Agriculture, Vellayani are presented in this chapter.

4.1 LABORATORY EVALUATION OF TOXICITY OF THE INSECTICIDES TO HONEY BEES

The mortality percentages of adult bees of two different species of honey bees *viz., A. cerana indica* and *T. iridipennis* at two concentrations (recommended field dosage and half of the field dosage) are presented in Table 2 and 3.

4.1.1 Toxicity of New Generation Insecticides to Indian Bees, *A. cerana indica*

4.1.1.1 *Mortality of the Bees at One HAT*

In *A. cerana indica*, no mortality was observed for novaluron 10 EC (2 mL L^{-1} and 1 mL L^{-1}) and cyantraniliprole 20 SC (1.2 mL L^{-1} and 0.6 mL L^{-1}) and dimethoate 30 EC (0.5 mL L^{-1} and 0.25 mL L^{-1}) at one HAT (Table 2). Diafenthiuron 50 WP @ 0.6 g L^{-1} recorded 35 per cent mortality which was on par with that of dinotefuran 20 SG @ 0.15 g L^{-1} (40 % mortality). While, 42.50 per cent mortality in Indian bees was recorded with diafenthiuron 50 WP @ 1.2 g L^{-1} which was on par with the combination product thiamethoxam (17.5%) + chlorantraniliprole (8.8%) 300 SC of concentration 0.2 mL L^{-1} (45 % mortality). Flubendiamide (19.92 %) + thiacloprid (19.92 %) 480 SC of concentration 0.2 mL L^{-1} and dinotefuran 20 SG @ 0.3 g L^{-1} were observed with 47.50 and 52.50 percentages of mortalities respectively and these two treatments were on par. Field doses of the insecticide mixtures, flubendiamide (19.92 %) + thiacloprid (19.92 %) 480 SC and thiamethoxam (17.50 %) + chlorantraniliprole (8.80 %) 300 SC treated bees recorded mortality percentages of 60.00 and 71.94

Table 2. Percentage mortality of *A. cerana indica* after application of new generation insecticides

Treatments	Dosage (g or mL L^{-1})	*Percentage Mortality HAT			
		1	3	6	12
Thiamethoxam 25 WG	0.30	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
Thiamethoxam 25 WG	0.15	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
Dinotefuran 20 SG	0.30	52.50 (46.42)	74.16 (59.57)	90.00 (76.70)	100.00 (90.00)
Dinotefuran 20 SG	0.15	40.00 (39.09)	55.00 (47.86)	68.33 (55.89)	100.00 (90.00)
Cyantraniliprole 20 SC	1.20	0.00 (0.00)	0.00 (0.00)	5.00 (9.21)	32.50 (34.40)
Cyantraniliprole 20 SC	0.60	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	15.56 (22.88)
Novaluron 10 EC	2.00	0.00 (0.00)	0.00 (0.00)	15.00 (19.92)	44.72 (41.88)
Novaluron 10 EC	1.00	0.00 (0.00)	0.00 (0.00)	2.50 (4.61)	28.61 (32.16)
Diafenthiuron 50 WP	1.20	42.50 (40.59)	52.50 (46.42)	71.66 (57.91)	88.88 (75.92)
Diafenthiuron 50WP	0.60	35 (36.21)	43.61 (41.30)	57.78 (49.52)	83.88 (66.69)
Thiamethoxam (17.5%) + Chlorantraniliprole (8.8%) 300 SC	0.40	71.94 (58.03)	97.22 (85.12)	100.00 (90.00)	100.00 (90.00)
Thiamethoxam (17.5%) + Chlorantraniliprole (8.8%) 300 SC	0.20	45.00 (42.10)	72.50 (58.43)	84.44 (67.32)	100.00 (90.00)
Flubendiamide (19.92%) + Thiacloprid (19.92%) 480 SC	0.40	60 (50.81)	66.38 (54.62)	91.94 (75.63)	100.00 (90.00)
Flubendiamide (19.92%) + Thiacloprid (19.92%) 480 SC	0.20	47.50 (43.54)	62.50 (52.25)	81.66 (65.30)	100.00 (90.00)
Dimethoate 30 EC (insecticidal check)	0.50	0.00 (0.00)	0.00 (0.00)	33.33 (35.22)	68.88 (56.30)
Dimethoate 30 EC (insecticidal check)	0.25	0.00 (0.00)	0.00 (0.00)	23.61 (28.99)	51.38 (45.82)
S. E. (m)		2.778	2.707	1.771	4.414
CD (0.05)		7.199	6.126	10.103	11.787

* Mean of four replications; Figures in parenthesis- Angular transformed; HAT: Hours after Treatment

respectively. Among the insecticides tested, the bee population exposed to thiamethoxam 25 WG (0.3 g L^{-1} and 0.15 g L^{-1}) showed cent per cent mortality within one HAT.

4.1.1.2 *Mortality of the Bees at Three HAT*

As in the case of one HAT, no mortality was observed for the insecticides novaluron 10 EC (2 mL L^{-1} and 1 mL L^{-1}) and cyantraniliprole 20 SC (1.2 mL L^{-1} and 0.6 mL L^{-1}) and dimethoate 30 EC (0.5 mL L^{-1} and 0.25 mL L^{-1}) at three HAT. Diafenthiuron 50 WP @ 0.6 g L^{-1} which recorded 43.61 per cent mortality in Indian bees, while, 52.50 per cent mortality was observed with diafenthiuron 50 WP @ 1.2 g L^{-1}, which was on par with dinotefuran 20 SG @ 0.15 g L^{-1} that recorded 55 per cent mortality. The mortality of *A. cerana indica* treated with the combination product, flubendiamide (19.92 %) + thiacloprid (19.92 %) 480 EC (0.4 mL L^{-1} and 0.2 mL L^{-1}) were statistically on par (66.38 per cent and 62.5 per cent respectively). The treatments, thiamethoxam (17.5 %) + chlorantraniliprole (8.8 %) 300 SC of concentration 0.2 mL L^{-1} and dinotefuran 20 SG @ 0.3 g L^{-1} were statistically on par, and the mortality percentages associated with them were 72.50 and 74.16 respectively. Higher mortality percentage of 97.22 was associated with thiamethoxam (17.5 %) + chlorantraniliprole (8.8 %) 300 SC of concentration 0.4 mL L^{-1} at three HAT. Thiamethoxam 25 WG (0.3 g L^{-1} and 0.15 g L^{-1}) showed cent per cent mortality within three HAT.

4.1.1.3 *Mortality of the Bees at Six HAT*

No significant difference was observed among cyantraniliprole 20 SC (0.6 mL L^{-1} and 1.2 mL L^{-1}) and novaluron 10 EC @ 1 mL L^{-1} at six HAT, the mortality percentages being 0.00, 2.50 and 5.00 respectively. Novaluron 10 EC @ 2 mL L^{-1} recorded 15.00 per cent mortality which was on par with that of dimethoate 30 EC (0.25 mL L^{-1} and 0.5 mL L^{-1}), which recorded percentage mortalities of 23.61 and 33.33 respectively. Diafenthiuron 50 WP @ 0.6 g L^{-1} recorded 57.78 per cent mortality in Indian bees and this was followed by

dinotefuran 20 SG @ 0.15 g L^{-1} which recorded 68.33 per cent mortality in them. While, 71.66 per cent mortality was observed in the population treated with diafenthiuron 50 WP @ 1.2 g L^{-1}, this was statistically on par with flubendiamide (19.92%) + thiacloprid (19.92%) 480 SC @ 0.2 mL L^{-1} (81.66 %). At 6 HAT, cent per cent mortality was observed in thiamethoxam 25 WG (0.3 gL^{-1} and 0.15 gL^{-1}) and the combination insecticide, thiamethoxam (17.5 %) + chlorantraniliprole (8.80%) 300 SC @ 0.4 mL L^{-1}. This was on par with, flubendiamide (19.92 %) + thiacloprid (19.92 %) 480 SC @ 0.4 mL L^{-1} and dinotefuran 20 SG @ 0.3 g L^{-1}, the mortality percentages being 91.94 and 90.00 respectively.

4.1.1.4 *Mortality of the Bees at Twelve HAT*

A similar trend as that of 6 HAT was observed at 12 HAT where cyantraniliprole 20 SC @ 0.6 mL L^{-1} (15.56 % mortality) recorded the lower mortality of the bees. Novaluron 10 EC (1 mL L^{-1} and 2 mL L^{-1}) and cyantraniliprole 20 SC @ 1.2 mL L^{-1} were statistically on par, the mortalities being 28.61 per cent, 44.72 per cent and 32.50 per cent respectively. Dimethoate 30 EC @ 0.25 mL L^{-1} (51.68 %) and 0.5 mL L^{-1} (68.88 %) were statistically on par at 12 HAT. The treatments which recorded higher bee mortality were dinotefuran 20 SG (0.15 g L^{-1} and 0.3 g L^{-1}), thiamethoxam (17.5 %) + chlorantraniliprole (8.8 %) 300 SC (0.2 mLL^{-1}), flubendiamide (19.92%) + thiacloprid (19.92 %) 480 SC (0.2 mL L^{-1} and 0.4 mL L^{-1}), diafenthiuron 50 WP (0.6 g L^{-1}) and diafenthiuron 50 WP (1.2 g L^{-1}) and these treatments were statistically on par the mortality percentages being 100, 100, 100, 100, 100, 83.88 and 88.88 respectively at 12 HAT.

4.1.2 Toxicity of New Generation Insecticides to Stingless Bees, *T. iridipennis*

4.1.2.1 *Mortality of the Bees at One HAT*

In the case of *T. iridipennis*, no mortality was observed in the case of novaluron 10 EC (1 mL L^{-1} and 2 mL L^{-1}) and cyantraniliprole 20 SC (0.6 mL L^{-1}

and 1.2 mL L^{-1}) and dimethoate 30 EC (0.25 mL L^{-1} and 0.5 mL L^{-1}) at one HAT (Table 3). This was followed by diafenthiuron 50 WP @ 0.6 g L^{-1} which recorded a mortality of 15 per cent. The two concentrations of the combination product, flubendiamide (19.92%) + thiacloprid (19.92 %) 480 SC (0.2 mL L^{-1} and 0.4 mL L^{-1}) were statistically on par, the mortality percentages being 17.50 and 20.00 respectively. Diafenthiuron 50 WP @ 1.2 g L^{-1} recorded with 30 per cent bee mortality and was statistically on par with that of dinotefuran 20 SG @ 0.15 g L^{-1}(32.50 %). While the combination product, thiamethoxam (17.5 %) + chlorantraniliprole (8.8 %) (0.2 mL L^{-1}) treated bees recorded a mortality percentage of 42.50. This was followed by thiamethoxam (17.5 %) + chlorantraniliprole (8.8%) 300 SC @ 0.4 mL L^{-1} which was statistically on par with thiamethoxam 25 WG @ 0.15 g L^{-1}, the mortality percentages being 62.50 in both the treatments. The treatments thiamethoxam 25 WG @ 0.3 g L^{-1} and dinotefuran 20 SG @ 0.3 g L^{-1} recorded higher stingless bee mortality, as the population exposed to the treatment showed cent per cent mortality within one HAT.

4.1.2.2 *Mortality of the Bees at Three HAT*

At three HAT, the treatments, novaluron 10 EC (2 mL L^{-1} and 1 mL L^{-1}), cyantraniliprole 20 SC (1.2 mL L^{-1} and 0.6 mL L^{-1}) and dimethoate 30 EC (0.5 mL L^{-1} and 0.25 mL L^{-1}) were found to have no mortality in stingless bees. This was followed by diafenthiuron 50 WP @ 0.6 g L^{-1} which recorded 27.50 per cent mortality in the bees. The combination product, flubendiamide (19.92 %) + thiacloprid (19.92 %) 480 SC (0.2 mL L^{-1} and 0.4 mL L^{-1}) and diafenthiuron 50 WP (1.2 g L^{-1}) were found to have no significant difference, as the mortality percentages associated with the treatments were 30.00, 32.50 and 37.50 respectively. Dinotefuran 20 SG @ 0.15 g L^{-1} recorded 47.50 per cent mortality which was followed by thiamethoxam (17.5 %) + chlorantraniliprole (8.8 %) 300 SC @ 0.2 mL L^{-1} concentration and dinotefuran 20 SG @ 0.3 g L^{-1} the mortality percentages were 65.00 and 85.00 respectively.

Table 3. Percentage mortality of *T. iridipennis* after application of new generation insecticides

Treatments	Dosage (g or mL L^{-1})	*Percentage mortality HAT			
		1	3	6	12
Thiamethoxam 25 WG	0.30	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
Thiamethoxam 25 WG	0.15	62.50 (52.25)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
Dinotefuran 20 SG	0.30	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
Dinotefuran 20 SG	0.15	32.50 (34.70)	47.50 (43.54)	63.89 (53.11)	100.00 (90.00)
Cyantraniliprole 20 SC	1.20	0.00 (0.00)	0.00 (0.00)	2.50 (4.61)	30.56 (33.43)
Cyantraniliprole 20 SC	0.60	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	25.56 (30.27)
Novaluron 10 EC	2.00	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	30.55 (32.94)
Novaluron 10 EC	1.00	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	19.44 (25.57)
Diafenthiuron 50 WP	1.20	30.00(33.04)	37.50 (37.65)	72.22 (58.85)	100.00 (90.00)
Diafenthiuron 50WP	0.60	15.00 (22.49)	27.50 (31.54)	46.11 (42.74)	91.94 (78.34)
Thiamethoxam (17.5%) + Chlorantraniliprole (8.8%) 300 SC	0.40	62.50 (52.32)	85.00 (67.47)	100.00 (90.00)	100.00 (90.00)
Thiamethoxam (17.5%) + Chlorantraniliprole (8.8%) 300 SC	0.20	42.50 (40.66)	65.00 (53.76)	100.00 (90.00)	100.00 (90.00)
Flubendiamide (19.92%) + Thiacloprid (19.92%) 480 SC	0.40	20.00 (26.18)	32.50 (34.55)	45.83 (42.55)	100.00 (90.00)
Flubendiamide (19.92%) + Thiacloprid (19.92%) 480 SC	0.20	17.50 (24.52)	30.00 (33.04)	38.05 (37.82)	66.66 (54.75)
Dimethoate 30 EC (insecticidal check)	0.50	0.00 (0.00)	7.50 (13.82)	20.56 (26.94)	69.44 (56.49)
Dimethoate 30 EC (insecticidal check)	0.25	0.00 (0.00)	2.50 (4.61)	15.56 (22.88)	56.39 (48.66)
SE. m.		1.936	2.476	2.234	3.321
CD (0.05)		4.513	8.036	8.916	8.556

* Mean of four replications; Figures in parenthesis- Angular transformed; HAT: Hours after Treatment

Cent per cent mortality was observed in the population treated with thiamethoxam 25 WG @ 0.15 g L^{-1} at three HAT.

4.1.2.3 *Mortality of the Bees at Six HAT*

Among the treatments, novaluron 10 EC (1 mL L^{-1} and 2 mL L^{-1}) and cyantraniliprole 20 SC (0.6 mL L^{-1} and 1.2 mL L^{-1}) were observed to have lower stingless bee mortality (0.00, 0.00, 0.00 and 2.50 respectively) and they were statistically on par. The two concentrations of dimethoate 30 EC (0.25 mL L^{-1} and 0.5 mL L^{-1}) had mortality percentages of 15.56 and 20.56 respectively. The combination insecticide, flubendiamide (19.92 %) + thiacloprid (19.92 %) 480 SC of concentration 0.2 mL L^{-1} and 0.4 mL L^{-1} recorded a mortality of 38.05 per cent and 45.83 per cent which were on par with diafenthiuron 50 WP @ 0.6 g L^{-1} (46.11 %). While, dinotefuran 20 SG @ 0.15 g L^{-1} was statistically on par with diafenthiuron 50 WP @ 1.2 g L^{-1}, the mortality percentages being 63.89 and 72.22. At six HAT, higher bee mortality (100 %) was observed with the insecticide thiamethoxam (17.50 %) + chlorantraniliprole (8.8 %) 300 SC of concentrations 0.2 mL $L^{-1}$and 0.4 mL L^{-1}.

4.1.2.4 *Mortality of the Bees at Twelve HAT*

Lower mortality of the bees was observed with novaluron10 EC @ 1 mL L^{-1} at 12 HAT (19.44 %). A percentage mortality of 30.55 per cent was observed for the treatment, novaluron 10 EC (2 mL L^{-1}) which was statistically on par with that of cyantraniliprole 20 SC (1.2 mL $L^{-1}$and 0.6 mL L^{-1}) with mortality percentages of 30.56 and 25.56 per cent respectively. At 12 HAT, dimethoate 30 EC @ 0.25 mL L^{-1}recorded 56.39 percent mortality. No significant difference was observed between the combination product flubendiamide (19.92 %) + thiacloprid (19.92 %) 480 SC of concentration 0.2 mL L^{-1} (66.66 % mortality) and dimethoate 30 EC @ 0.5 mL L^{-1} (69.44 % mortality). The treatments diafenthiuron 50 WP (0.6 g L^{-1} and 1.2 g L^{-1}), flubendiamide (19.92 %) + thiacloprid (19.92 %) 480 SC @ 0.4 mL L^{-1}, dinotefuran 20 SG @ 0.15 g L^{-1}.

were statistically on par and the mortality percentages associated were 91.94, 100.00, 100.00 and 100.00 respectively at 12 HAT.

4.2 FIELD EVALUATION OF TOXICITY OF THE INSECTICIDES TO BEE POLLINATORS

4.2.1 Insect Fauna on Culinary Melon

The insect pollinators/ flower visitors, pests and natural enemies observed on culinary melon during the study are presented in Table 4.

4.2.1.1 *Insect Pests*

Seven different types of insect pests were recorded on culinary melon (Plate 5). Of these, two species comes under family Chrysomelidae of order Coleoptera. Dipterans in the field constituted one species each from families Tephritidae and Agromyzidae. Other pests in the field belong to the order Hemiptera, Lepidoptera and Orthoptera.

4.2.1.2 *Natural Enemies*

There were three species of predators found on culinary melon; two of them were from Odonata and one from Coleoptera. These included dragon flies, damsel flies and ground beetles (Plate 6).

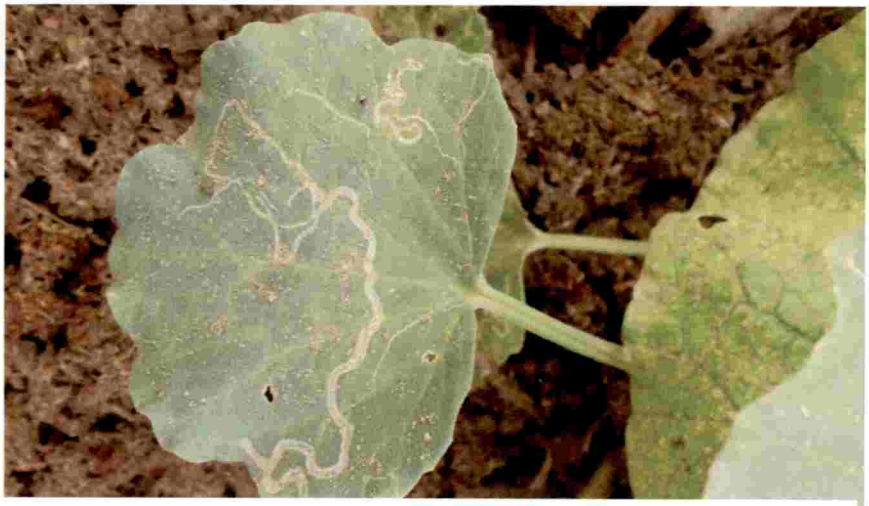
4.2.1.3 *Insect Pollinators/ Flower Visitors*

A total of 11 different species of insect pollinators/ flower visitors were recorded from the flowers of culinary melon (Plate 7). Of these, seven species belongs to Hymenoptera, four species belongs to Coleoptera and two species to Lepidoptera respectively. Among the Hymenopterans, three species were from the family Apidae, two from Halictidae and one each from the family Xylocopidae

Table 4. Insect fauna in culinary melon

Common name	Scientific name	Family	Order
Pumpkin beetles	*Aulacophora foveicollis* Lucas. *Aulacophora lewisii* Baly.	Chrysomelidae	Coleoptera
American serpentine leaf miner	*Liriomyza trifolii* Burgess.	Agromyzidae	Diptera
Pumpkin caterpillar	*Diaphania indica* Saunders.	Pyralidae	Lepidoptera
Melon fly	*Bactrocera cucurbitae* Coquillet.	Tephritidae	Diptera
Grass hopper	*Attractomorpha crenulata*	Acrididae	Orthoptera
Green shield bug	*Nezara viridula*	Pentatomidae	Hemiptera
Damsel fly	-	-	Odonata
Dragon fly	-	-	Odonata
Ground beetle	*Ophionea* sp.	Carabidae	Coleoptera
Indian bee	*Apis cerana indica* Fab.	Apidae	Hymenoptera
Rock bee	*Apis dorsata* Fab.	Apidae	Hymenoptera
Stingless bee	*Tetragonula iridipennis* Smith.	Apidae	Hymenoptera
Halictid bee	-	Halictidae	Hymenoptera
Halictid bee	-	Halictidae	Hymenoptera
Leaf cutter bee	*Megachile* sp.	Megachilidae	Hymenoptera
Carpenter bee	*Xylocopa* sp.	Xylocopidae	Hymenoptera
Flower beetle	*Luperomorpha* sp.	Chrysomelidae	Coleoptera
Blue butter fly	*Lampides* sp.	Lycaenidae	Lepidoptera

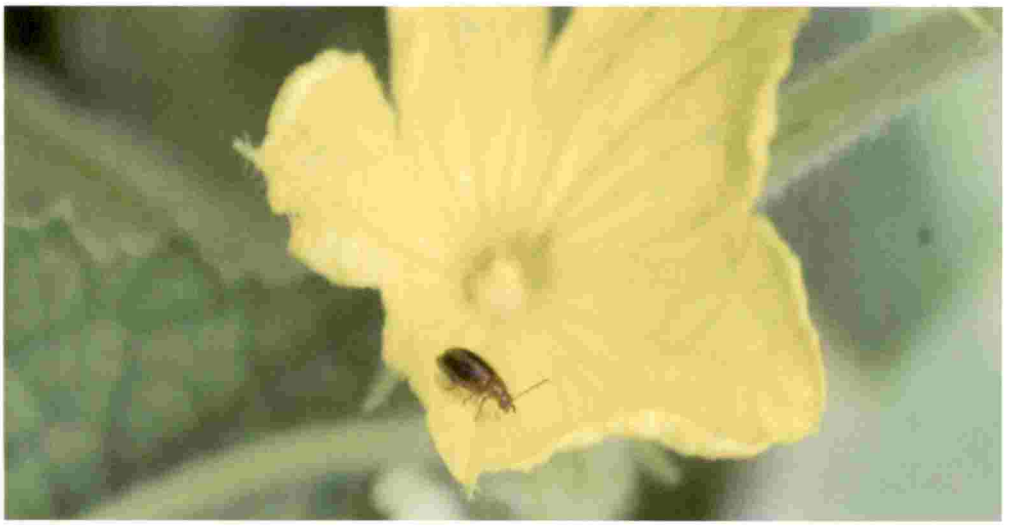
5 a. *Aulacophora foevicollis*



5 b. *Liriomyza trifolii*

5 c. *Diaphania indica*

Plate 5. Major pests of *Cucumis melo*



5 d. *Aulacophora lewesi*

5 e. Bactrocera cucurbitae attack on fruit

Plate 5. Major pests of *Cucumis melo*

6 a. Ground beetle

6 b. Damsel fly

Plate 6. Natural enemies observed in the field

7 a. *A. cerana indica*

7 b. *T. iridipennis*

7 c. Halictid bee

7 d. Halictid bee

7 e. *Aulacophora* sp.

7 f. *Luperomorpha* sp.

Plate 7. Pollinators observed in the field

and Megachilidae. The major pollinators were *A. cerana indica* and *T. iridipennis*, both belonging to the family Apidae under order Hymenoptera. The major coleopterans included *A. lewesi* and *A. foveicollis*. One Lepidoptera pollinator observed in the field was *Lampides* sp.

4.2.1.3.1 *Abundance of Insect Pollinators/ Flower Visitors under Pesticide Free Condition*

Density of insect pollinators / flower visitors *viz., A. cerana indica*, *A. dorsata*, *T. iridipennis*, *Aulacophora* sp., *Luperomorpha* sp. and Halictid bees visiting flowers of culinary melon at different hours of the day under pesticide free conditions are presented in Table 5. The population of the insect pollinators was observed 45 days after sowing (peak flowering stage). The population is expressed as mean number of pollinators m^{-2} 5 $min.^{-1}$.

A. cerana indica populations were observed in the field from 0600 h and were present throughout the observation period (6 am to 6 pm). The data presented in Table 5 revealed that there were significant variation in the population of *A. cerana indica* observed at different time periods. Highest population (7.67 bees m^{-2} 5 $min.^{-1}$) was recorded at time period of 1000 h to 1200 h which was significantly higher than all other time periods. This was followed by their population recorded between 0800 h to 1000 h (5.67 bees m^{-2} 5 $min.^{-1}$) and 1400 h to1600 h (5.33 bees m^{-2} 5 $min.^{-1}$) which were on par. The population of Indian bees in the field was recorded as 2.67 bees m^{-2} 5 $min.^{-1}$ in the period 1600 h to1800 h which was statistically on par with their abundance in the period 0600 h to 0800 h (2.00 bees m^{-2} 5 $min.^{-1}$). Significantly lower population was recorded at the time period of 1200 h to 1400 h, the population being 0.33 bees m^{-2} 5 $min.^{-1}$.

The rock bee, *Apis dorsata* was present in the field from 0600 h onwards with the maximum density in the period 1000 h to1200 h (3.67 bees m^{-2} 5 $min.^{-1}$) which was on par with that recorded in 0800 h to 1000 h period (3.00 bees m^{-2}

Table 5. Pollinator density in culinary melon under pesticide free condition at different hours of the day

Time period	*No. of pollinators m^{-2} 5 $min.^{-1}$					
	A. cerana indica	*A. dorsata*	*T. iridipennis*	Halictid bees	*Aulacophora* spp.	*Luperomorpha* sp.
0600 h- 0800 h	2.00	2.33	0.33	2.00	0.33	0.33
0800 h- 1000 h	5.67	3.00	9.33	2.00	0.00	0.00
1000 h- 1200 h	7.67	3.67	8.00	1.67	4.00	0.67
1200 h-1400 h	0.33	1.67	5.33	0.33	2.00	0.33
1400 h- 1600 h	5.33	1.00	5.67	0.00	1.33	0.66
1600 h-1800 h	2.67	1.67	2.33	0.66	2.33	0.33
CD (0.05)	1.505	1.569	1.738	1.357	1.985	NS

*Mean of three replications

5 min.$^{-1}$) and 0600 h to 0800 h (2.33 bees m^{-2} 5 min.$^{-1}$). Among the time period of 1200 h to 1400 h, 1600 h to 1800 h and 1400 h to 1600 h, the bee density being 1.67, 1.67 and 1.00 bees m^{-2} 5 min^{-1} respectively were on par.

The peak population of *T. iridipennis* was observed between 0800 h to 1000 h (9.33 bees m^{-2} 5 min.$^{-1}$), which was on par with the density during 1000 h to1200 h (8.00 bees m^{-2} 5 min.$^{-1}$). In the time period between 1400 h to 1600 h density of stingless bee was 5.67 bees m^{-2} 5 min.$^{-1}$ which was followed by that between 1200 h to 1400 h (5.33 bees m^{-2} 5 min.$^{-1}$). The density of stingless bees between 1600 h to 1800 h was observed to be 2.33 bees m^{-2} 5 min.$^{-1}$. Significantly lower density was recorded during 0600 h to 0800 h, the recorded population being 0.33 bees m^{-2} 5 min.$^{-1}$.

The population of Halictid bees observed during 0600 h to 0800 h, 0800 h to 1000 h and 1000 h to 1200 h in the field were statistically on par with the average population densities being 2.00, 2.00 and 1.67 bees m^{-2} 5 min.$^{-1}$ respectively. Similarly, no significant difference was recorded in the number of Halictid bees visiting the field in time period 1200 h to 1400 h, 1400 h to 1600 h and 1600 h to1800 h with their average densities being 0.33, 0.00 and 0.66 bees m^{-2} 5 min.$^{-1}$ respectively.

Higher density of *Aulacophora* spp. occurred between 1000 h to 1200 h (4.00 beetles m^{-2} 5 min.$^{-1}$) and this was statistically on par with that recorded between 1600 h to 1800 h (2.33 beetles m^{-2} 5 min.$^{-1}$). This was followed by the population in the period 1200 h to 1400 h (2.00 beetles m^{-2} 5 min.$^{-1}$) and 1400 h to1600 h (1.33 beetles m^{-2} 5 min.$^{-1}$). No *Aulacophora* sp. visited the field during 0600 h to 0800 h.

Though the flower visitor, *Luperomorpha* sp. were present in the field in the observation periods 0600 h to 0800 h, 1000 h to 1200 h, 1200 h to 1400 h, 1400 h to 1600 h and 1600 h to 1800 h, the differences in their densities were non-significant at different hours of the day.

4.2.1.3.2 *Abundance of A. cerana indica and T. iridipennis after Installation of Hives in the Experimental Field*

Abundance of *A. cerana indica* and *T. iridipennis* before and after installation of the hives were recorded from marked areas in the field for 5 minutes and was expressed as average number of bees m^{-2} 5 $min.^{-1}$. The data is depicted in Table 6.

After placing an Indian bee hive, the abundance of Indian bees in the field were found to be increased from 7.67 to 9.67 bees m^{-2} 5 $min.^{-1}$ in the time period 1000 h to 1200 h which was on par with that between 0800 h to 1000 h (8.33 bees m^{-2} 5 $min.^{-1}$). This was followed by their density at 1400 h to 1600 h (6.33 bees m^{-2} 5 $min.^{-1}$) after installation of hive. The average bee density in the field between 1600 h to 1800 h, 0600 h to 0800 h and 1200 h to 1400 h were increased up to 3.33, 3.67 and 4.67 bees m^{-2} 5 $min.^{-1}$ respectively and were found to be statistically on par.

The abundance of the bees was increased from 9.33 to 14.00 m^{-2} 5 $min.^{-1}$ after the installation of hive in the field between 0800 h and 1000 h. The abundance of the bees in the field in time periods 1000 h to 1200 h (9.33 bees m^{-2}5 $min.^{-1}$), 1200 h to 1400 h (9.33 bees m^{-2}5 $min.^{-1}$) and 1400 h to 1600 h (8.33 m^{-2} 5 $min.^{-1}$) didn't differ significantly. The density of the bees in the field was increased from 0.33 m^{-2} 5 min^{-1} to 1.33 m^{-2} 5 min^{-1}in the period 0600 h to 0800 h.

4.2.2 Effect of the Insecticides on the Foraging Activity of Bee Pollinators

The insecticides which were found to be safe under laboratory condition were evaluated under field condition. For this the effect of the insecticides on the foraging behaviour of the major pollinators of culinary melon *viz*, *A. cerana indica* and *T. iridipennis* were studied. The parameters such as relative abundance, foraging rate, time spent by the bees on flowers and foraging speed was recorded before the spraying and at 1, 3, 5, 7 and 15 DAS (Tables 7 - 16).

Table 6. Density of pollinators in the field after the installation of hives

Time period	* No. of bees m^{-2} 5 $min.^{-1}$			
	Apis cerana indica		*Tetragonula iridipennis*	
	Before installation	After installation	Before installation	After installation
0600h- 0800h	2.00	3.67	0.33	0.67
0800h- 1000h	5.67	8.33	9.33	14.00
1000h- 1200h	7.67	9.67	7.00	9.33
1200h-1400h	0.33	0.67	5.33	9.33
1400h- 1600h	5.33	7.33	5.67	8.33
1600h-1800h	2.67	3.33	2.33	4.33
CD (0.05)	1.505	2.283	1.738	1.967
t value	-4.309**		-4.259**	

* Mean of three replications

**Significant at both 0.05 and 0.01 level of significance

Apart from these, number of the returning foragers (with pollen load on their leg) to respective hives placed in the field was also estimated.

4.2.2.1 *Relative Abundance of Pollinators at Different Intervals of Insecticide Application*

Abundance of both *A. cerana indica* and *T. iridipennis* with respect to the total number of pollinators was recorded from the field and were expressed in percentage in Tables 7 and 8.

4.2.2.1.1 *Relative Abundance of A. cerana indica*

Studies on the relative abundance of *A. cerana indica* before insecticide spraying did not vary significantly at their peak period of activity and ranged from 25.37 to 29.81 per cent and (Table 7).

On the day of spraying, higher relative abundance of Indian bees was recorded from control plot (29.27 %), while all the insecticides significantly reduced the population. The plots treated with cyantraniliprole 20 SC @ 1.2 mL L^{-1}, novaluron 10 EC @ 2 mL L^{-1} and dimethoate 30 EC @ 0.5 mL L^{-1} recorded the relative abundance of 11.11, 5.00 and 3.33 per cent respectively and these treatments were statistically on par.

On one DAS, the relative abundance of bees in cyantraniliprole 20 SC @ 1.2 mL L^{-1} was on par with that of control, the values being 32.19 and 31.43 per cent respectively. Relative abundance of *A. cerana indica* was found to be 18.89 per cent and 11.83 per cent in case of dimethoate 30 EC @ 0.5 mL L^{-1} and novaluron 10 EC @ 2 mL L^{-1}.

A similar trend was recorded on three DAS, where the plots treated with cyantraniliprole 20 SC @ 1.2 mL L^{-1} (30.98 %) had maximum relative abundance of bees followed by control (29.56 %) which were on par. Novaluron 10 EC @ 2 mL L^{-1} and insecticidal check, dimethoate 30 EC @ 0.5 mL L^{-1} possessed a

significantly lower relative abundance of 20.50 per cent and 19.58 per cent which were on par.

Table 7. Relative abundance of *A. cerana indica* at different intervals of insecticide application

Treatment	Dosage (mL L^{-1})	*Relative abundance (%) DAS						
		Before spraying	0	1	3	5	7	15
Cyantraniliprole 20 SC	1.20	29.10	11.11	32.19	30.98	31.84	31.40	29.10
Novaluron 10 EC	2.00	25.37	5.00	18.89	20.50	36.97	35.52	25.37
Dimethoate 30 EC	0.50	29.81	3.33	11.83	19.58	26.50	30.23	29.81
Control (Untreated)	-	29.17	29.27	31.43	29.56	30.89	31.32	29.17
S.E. (m)	-	2.026	3.321	2.132	2.443	1.777	2.777	2.338
C.D. (0.05)	-	NS	10.346	6.643	7.612	5.537	NS	NS

* Mean of five replications

DAS: Days After Spraying

On five DAS, novaluron 10 EC @ 2 mL L^{-1} recorded highest relative abundance (36.97 %) which was on par with cyantraniliprole 20 SC @ 1.2 mL L^{-1} (31.84 %). However, the relative abundance observed in cyantraniliprole 20 SC @ 1.2 mL L^{-1} was on par with that observed in the insecticidal check dimethoate 30 EC @ 0.5 mL L^{-1} and the untreated control, the values being 31.44, 26.50 and 30.89 per cent respectively.

No significant variation was observed among the insecticides with control on seven and fifteen DAS.

4.2.2.1.2 *Relative Abundance of T. iridipennis*

Table 8 gives the relative abundance of stingless bees before spraying and on 0^{th}, 1^{st}, 3^{rd}, 5^{th}, 7^{th} and 15^{th} DAS. Before spraying the insecticides, the relative abundance of the bees in the field did not vary significantly and ranged from 38.17 per cent to 39.96 per cent.

On the day of spraying (0 DAS), maximum relative abundance of bees was observed with the control (36.18 %) which was significantly higher than the other insecticide treatments. The relative abundance of the bees in the plots treated with novaluron 10 EC @ 2 mL L^{-1}, cyantraniliprole 20 SC @ 1.2 mL L^{-1} and dimethoate 30 EC @ 0.5 mL L^{-1} were 17.52, 13.06 and 12.33 per cent which were statistically on par.

Lowest relative abundance in stingless bees was observed for cyantraniliprole 20 SC @ 1.2 mL L^{-1} (16.46 %) which was followed by dimethoate 30 EC @ 0.5 mL L^{-1} (10.00 %) and novaluron 10 EC @ 0.5 mL L^{-1} (24.33 %) on one DAS which were statistically on par. While the control plot had maximum relative abundance (30.25 %) which was significantly different from other treatments.

Table 8. Relative abundance of *T. iridipennis* at different intervals of insecticide application

Treatment	Dosage (mL L^{-1})	*Relative abundance (%) DAS						
		Before spraying	0	1	3	5	7	15
Cyantraniliprole 20 SC	1.20	39.68	13.06	16.46	18.04	34.43	36.84	37.23
Novaluron 10 EC	2.00	39.32	17.52	24.33	29.08	37.95	40.75	37.78
Dimethoate 30 EC	0.50	38.17	12.33	10.00	15.30	23.84	23.87	40.28
Control (Untreated)	-	38.96	36.18	30.25	42.83	34.37	39.00	39.22
S. E. (m)	-	2.155	3.172	2.967	2.190	2.244	2.642	2.998
C.D. (0.05)	-	NS	10.815	9.243	6.823	6.991	8.231	NS

* Mean of five replications

DAS: Days After Spraying

A similar trend was noticed on three DAS, with the control possessing significantly higher relative abundance (42.83 %) when compared to other treatments. Novaluron 10 EC @ 0.5 mL L^{-1} (15.30 %) and cyantraniliprole 20 SC @ 1.2 mL L^{-1} (18.04 %) were statistically on par with that of insecticidal check dimethoate 30 EC @ 0.5 mL L^{-1} (29.08 %).

Dimethoate 30 EC @ 0.5 mL L^{-1} lowered significantly from other three treatments on five DAS (23.84 %) and seven DAS (23.87 %) in the relative abundance of bees. While, relative abundance of bees in plots treated with new generation insecticides, cyantraniliprole 20 SC @ 1.2 mL $L^{-1}$and novaluron 10 EC @ 0.5 mL L^{-1} were on par with the control. Observations on relative abundance of stingless bees on 15 DAS, revealed that the differences among the treatments were non-significant.

4.2.2.2 *Foraging Rate of the Pollinators Before and After Application of Insecticides*

Foraging rate (number of bees visited per minute) of the major pollinators (*A. cerana indica* and *T. iridipennis*) in the field was studied before spraying and on 0, 1, 3, 5, 7 and 15 DAS of new generation insecticides (Tables 9 &10).

4.2.2.2.1 *Foraging Rate of A. cerana indica*

No significant difference in the foraging rate was observed on five DAS, 15 DAS and on the day before spraying (Table 9).

Among the treatments, foraging rate of *A. cerana indica* in control was recorded to be 2.80 bees min^{-1} which was statistically on par with that in cyantraniliprole 20 SC @ 1.2 mLL^{-1} (2.60 bees m^{-2} $min.^{-1}$) on the day of spraying. Novaluron 10 EC @ 2 mL L^{-1} and dimethoate 30 EC @ 0.50 mL L^{-1} were recorded with foraging rates of 1.00 and 0.20 bees m^{-2} $min.^{-1}$ respectively and these treatments were statistically on par.

Table 9. Foraging rate of *A. cerana indica* at different intervals of insecticide application

Treatment	Dosage (mL L^{-1})	*No. of bees m^{-2} $min.^{-1}$ DAS						
		Before spraying	0	1	3	5	7	15
Cyantraniliprole 20 SC	1.20	2.00	2.60	2.60	3.00	2.00	2.20	2.82
Novaluron 10 EC	2.00	2.41	1.00	1.60	1.80	1.40	2.30	3.00
Dimethoate 30 EC	0.50	2.28	0.20	0.20	0.80	1.20	1.80	1.40
Control (Untreated)	-	2.40	2.80	2.60	3.20	2.00	2.00	2.40
S. E. (m).	-	0.091	0.152	0.123	0.133	0.158	0.132	0.127
C.D. (0.05)	-	NS	0.285	0.472	0.384	NS	NS	NS

*mean of five replications

DAS: Days After Spraying

The observations on one DAS showed that the foraging rate associated with control (2.80 bees $min.^{-1}$) and cyantraniliprole 20 SC @ 1.2 mL L^{-1} (2.60 bees m^{-2} $min.^{-1}$) were statistically on par. Though, novaluron 10 EC @ 2 mL L^{-1} was recorded with foraging rate of 1.60 bees $min.^{-1}$, it was significantly lower than that of plots treated with dimethoate 30 EC @ 0.5 mL L^{-1} (0.20 bees m^{-2} $min.^{-1}$).

Three DAS, the foraging rate of *A. cerana indica* associated with the treatments cyantraniliprole 20 SC @ 1.2 mL L^{-1} and control were recorded as 3.00 and 3.20 bees m^{-2} $min.^{-1}$ respectively and these treatments didn't differ significantly. Though, the foraging rate recorded with novaluron 10 EC @ 2 mL L^{-1} was 1.80 bees m^{-2} $min.^{-1}$, it was lower than that of dimethoate 30 EC @ 0.5 mL L^{-1} (0.80 bees m^{-2} $min.^{-1}$).

4.2.2.2.2 *Foraging Rate of T. iridipennis*

In case of stingless bees, the foraging rates did not vary significantly among the treatments cyantraniliprole 20 SC @ 1.2 mL L^{-1}, novaluron 10 EC @ 2 mL L^{-1}, dimethoate 30 EC @ 0.5 mL $L^{-1}$and control plots, the values being 6.23, 5.82, 5.75 and 5.76 bees m^{-2} $min.^{-1}$ respectively (Table 10).

On the day of spraying, the foraging rate in untreated plots was observed as 6.80 bees m^{-2} $min.^{-1}$ which was statistically on par with novaluron 10 EC @ 2 mL L^{-1} (5.60 bees m^{-2} $min.^{-1}$). Though, the foraging rate of the bees in plots treated with cyantraniliprole 20 SC @ 1.2 mL L^{-1} was recorded as 4.60 bees m^{-2} min^{-1}, it was statistically on par with that of dimethoate 30 EC @ 0.5 mL L^{-1} (2.20 bees m^{-2} $min.^{-1}$).

Table10. Foraging rate of *T. iridipennis* at different intervals of insecticide application

Treatment	Dosage (mL L^{-1})	*No. of bees m^2 visited $min.^{-1}$ DAS						
		Before spraying	0	1	3	5	7	15
Cyantraniliprole 20 SC	1.20	6.23	2.60	4.50	4.60	5.40	5.20	5.60
Novaluron 10 EC	2.00	5.82	5.60	5.80	6.10	6.00	4.90	6.00
Dimethoate 30	0.50	5.75	2.20	4.60	4.40	6.60	5.40	5.20
Control (Untreated)	-	5.76	6.80	6.40	6.60	7.00	5.80	6.40
S. E. (m).	-	1.032	0.574	0.749	0.925	1.029	1.011	1.102
C.D. (0.05)	-	NS	1.322	1.469	1.544	NS	NS	NS

* Mean of five replications

DAS: Days After Spraying

One DAS, the foraging rate recorded with novaluron 10 EC @ 2 mL L^{-1} (5.80 bees m^{-2} $min.^{-1}$) was statistically on par with that of control (6.40 bees m^{-2} $min.^{-1}$). The foraging rate was significantly lower in cyantraniliprole 20 SC @ 1.2 mL L^{-1} (4.50 bees m^{-2} $min.^{-1}$) and the insecticidal check, dimethoate 30 EC @ 0.50 mL L^{-1} (4.60 bees m^{-2} $min.^{-1}$).

Three DAS, no significant reduction in foraging rate of *T. iridipennis* was observed in treatments cyantraniliprole 20 SC @ 1.2 mL L^{-1} (4.60 bees m^{-2} $min.^{-1}$) and dimethoate 30 EC @ 0.5 mL L^{-1} (4.40 bees m^{-2} $min.^{-1}$). However, the treatments novaluron 10 EC @ 2 mL L^{-1} and cyantraniliprole 20 SC @ 1.2 mL L^{-1} were on par. Novaluron 10 EC @ 2 mL L^{-1} (6.10 bees m^{-2} $min.^{-1}$) didn't affect the foraging rate of the bees, which was on par with that of control (6.60 bees m^{-2} $min.^{-1}$).

No significant reduction in foraging rates of *T. iridipennis* was observed on 5, 7 and 15 DAT among the insecticides with respect the untreated control.

4.2.2.3 *Time Spent by the Pollinators on Flowers of Culinary Melon at Different Intervals of Insecticide Application*

Foraging time is given by average time spent by the bees on flowers of culinary melon and was recorded in seconds.

4.2.2.3.1 *Time Spent by the A. cerana indica on Flowers of Culinary Melon*

Before spraying the insecticides, the time spent by Indian bees on male flowers did not vary significantly and ranged from 2.98 sec. to 3.85 sec. (Table 11).

On the day of spraying, it was found that the treatments cyantraniliprole 20 SC @ 1.2 mL L^{-1} (3.22 sec.) and novaluron 10 EC @ 2 mL L^{-1} (2.16 sec.) were statistically on par, but lesser than that of control (3.33 sec.). Significant

reduction in the time spent was observed in case of dimethoate 30 EC @ 0.5 mL L^{-1} (1.18 sec.)

One DAS, it was found that time spent by the bees on flowers in the plots treated with dimethoate 30 EC @ 0.5 mL L^{-1} was only 1.61 sec., while cyantraniliprole 20 SC @ 1.2 mL L^{-1} (2.80 sec.) and novaluron 10 EC @ 2 mL L^{-1} (2.49 sec.) were statistically on par with that of control (3.36 sec.)

No significant variation in time spent by Indian bees on male flowers was observed on 0, 3, 5, 7 and 15 DAS.

Table 11. Time spent by *A. cerana indica* on male flowers of culinary melon at different intervals of insecticide application

Treatment	Dosage (mL L^{-1})	*Time spent (sec.) /male flower DAS						
		Before spraying	0	1	3	5	7	15
Cyantraniliprole 20 SC	1.20	3.54	3.22	2.80	3.59	3.61	3.69	3.58
Novaluron 10 EC	2.00	3.85	2.16	2.49	3.11	3.43	3.52	3.42
Dimethoate 30 EC	0.50	2.98	1.18	1.61	3.79	3.23	3.32	3.87
Control (Untreated)	-	3.54	3.33	3.36	3.46	4.05	3.68	3.50
S.E. (m)	-	0.214	0.314	0.294	0.322	0.274	0.297	0.251
C.D. (0.05)	-	NS	0.979	0.919	NS	NS	NS	NS

* Mean of five replications

DAS: Days After Spraying

Similarly, on female flowers (Table 12), significant variation among the insecticides was recorded only on the day of spraying and one DAS.

On the day of treatment, the time spent by the bees on female flowers were longer in treatments, cyantraniliprole 20 SC @ 1.2 mL L^{-1} (2.33 sec.) and untreated control (2.30 sec.) while it was lower in both novaluron 10 EC @ 2 mL L^{-1} (0.80 sec.) and dimethoate 30 EC @ 0.5 mL L^{-1} (0.87 sec.) which were statistically on par.

On one DAS, the time spent by the bees in control (2.20 sec.) and cyantraniliprole 20 SC @ 1.2 mL L^{-1} (2.16 sec.) didn't differ significantly. However, the time spent by the bees was significantly lower in treatment, novaluron 10 EC @ 2 mL L^{-1} (1.27 sec.) and dimethoate 30 EC @ 0.5 mL L^{-1} (0.54 sec.).

Table12. Time spent by *A. cerana indica* on female flowers of culinary melon at different intervals of insecticide application

Treatment	Dosage (mL L^{-1})	*Time spent (sec.) / female flower DAS						
		Before spraying	0	1	3	5	7	15
Cyantraniliprole 20 SC	1.20	2.23	2.33	2.16	1.98	2.00	2.29	2.27
Novaluron 10 EC	2.00	2.50	0.87	1.27	1.82	1.90	2.35	2.29
Dimethoate 30 EC	0.50	2.26	0.80	0.54	0.66	1.35	2.34	2.25
Control (Untreated)	-	2.43	2.30	2.20	1.74	1.91	2.11	2.37
S.E. (m)	-	0.158	0.313	0.123	0.322	0.199	0.151	0.147
C.D. (0.05)	-	NS	0.669	0.384	NS	NS	NS	NS

* Mean of five replication

DAS: Days After Spraying

4.2.2.3.2 *Time Spent by T. Iridipennis on Flowers of Culinary Melon*

Before treatment, the time spent by *T. iridipennis* on male flowers was non-significant with the values ranging from 38.24 sec. to 40.21 sec. (Table 13). After application of insecticides, the parameter under study was significant only up to five DAS. Thereafter, the differences among the treatments were non-significant.

On the day of treatment, time spent by stingless bees were lower in the treatments cyantraniliprole 20 SC @ 1.2 mL L^{-1} (15.51 sec.), novaluron 10 EC @ 2 mL L^{-1} (11.56 sec.) and dimethoate 30 EC @ 0.5 mL L^{-1} (14.22 sec.), which were on par, when compared with that of untreated control (40.04 sec.).

Table 13. Time spent by *T. iridipennis* on male flower of culinary melon at different intervals of insecticide application

Treatment	Dosage (mL L^{-1})	*Time spent by the bees (Sec.) / male flower DAS						
		Before spraying	0	1	3	5	7	15
Cyantraniliprole 20 SC	1.20	38.24	15.51	16.02	21.36	26.14	43.28	37.87
Novaluron 10 EC	2.00	40.21	11.56	21.15	36.96	36.22	38.35	37.47
Dimethoate 30 EC	0.50	39.24	14.22	14.65	15.03	16.99	39.51	34.93
Control (Untreated)	-	40.12	40.04	40.26	41.83	42.67	40.98	40.87
S.E.(m)	-	2.058	1.483	0.949	1.305	2.801	1.938	1.857
C.D. (0.05)	-	NS	4.619	2.956	4.067	8.722	NS	NS

*mean of five replications

DAS: Days After Spraying

One DAS also, time spent by the bees on male flowers was lower in cyantraniliprole 20 SC @ 1.2 mL L^{-1} (16.02 sec.) and dimethoate 30 EC @ 0.5 mL L^{-1} (14.65 sec.) which were statistically on par. This was followed by the time spent by bees on flowers of plots treated with novaluron 10 EC @ 2 mL L^{-1} (21.15 sec.) which was significantly lower when compared to that of control plot (40.26 sec.).

Three DAT, time spent by stingless bees was affected by dimethoate 30 EC @ 0.5 mL L^{-1} in which it recorded only 15.03 sec. on flower. This was followed by the time spent by the bees on flowers treated with cyantraniliprole 20 SC @ 1.2 mL L^{-1} (21.36 sec.) and novaluron 10 EC @ 2 mL L^{-1} (36.96 sec.) which differed significantly from each other but was lower than that of control (41.83 sec.).

Significant reduction in foraging time was recorded only in treatments, dimethoate 30 EC @ 0.5 mL $L^{-1}$and cyantraniliprole 20 SC @ 1.2 mL L^{-1} with the average time spent by the bee on the flower being 26.14 sec. and 16.99 sec. respectively on five DAS.

With regard to the female flowers, no significant variation in time spent by stingless bees was observed before treatment (Table 14). On the day of treatment, the time spent by stingless bees was significantly lower in cyantraniliprole 20 SC @ 1.2 mL L^{-1} (12.06 sec.), novaluron 10 EC @ 2 mL L^{-1} (16.42 sec.) and dimethoate 30 EC @ 0.5 mL L^{-1} (15.19 sec.), as compared to that of control which recorded a foraging time of 25.60 sec.

One DAS, dimethoate 30 EC @ 0.5 mL L^{-1} recorded a foraging time of 15.20 sec. lesser than all other treatments, which was followed by cyantraniliprole 20 SC @ 1.2 mL L^{-1} (17.34 sec.) and novaluron 10 EC @ 2 mL L^{-1} (17.47 sec.). The time spent by the stingless bees in these two treatments were statistically on par, but lower than that of control (24.81 sec.).

Table 14. Time spent by *T. iridipennis* on female flowers of culinary melon at different intervals of insecticide application

Treatment	Dosage (mL L^{-1})	*Time spent (sec.) /female flower DAS						
		Before spraying	0	1	3	5	7	15
Cyantraniliprole 20 SC	1.2	23.26	12.06	14.34	22.34	21.97	22.32	22.66
Novaluron 10 EC	2	21.21	16.42	17.47	21.47	20.61	24.49	21.23
Dimethoate 30 EC	0.5	22.41	15.19	15.20	15.39	16.85	22.76	23.05
Control (Untreated)	-	23.12	25.60	24.81	26.30	23.90	22.59	25.79
S.E.(m)	-	0.986	1.272	0.969	1.871	1.032	1.142	0.997
C.D. (0.05)	-	NS	3.962	3.024	5.828	3.186	NS	NS

*mean of five replications
DAS: Days After Spraying

Three DAS also, lowest time spent by the bees were recorded in treatment dimethoate 30 EC @ 0.5 mL L^{-1} (15.39 sec.). This was followed by novaluron 10 EC @ 2 mL L^{-1} (21.47 sec.) and cyantraniliprole 20 SC @ 1.2 mL L^{-1} (22.34 sec.) which were statistically on par with control (26.30 sec.).

Five DAS, significant reduction in time spent by the bees was observed only in case of dimethoate 30 EC @ 0.5 mL L^{-1} (16.85 sec.), whereas, cyantraniliprole 20 SC @ 1.2 mL L^{-1} (21.97 sec.) and novaluron 10 EC @ 2 mL L^{-1} (20.61 sec.) were on par with control (23.90 sec.).

No significant variation in the time spent by stingless bees on female flowers of culinary melon was observed on 7 and 15 DAS. On 7 DAS, the time spent by the bee on female flowers ranged from 22.32 to 24.49 sec., while on 15 DAS, the values ranged from 21.23 to 25.79 sec.

4.2.2.4 *Foraging Speed of the Pollinators at Different Intervals of Insecticide Application*

Foraging speed, number of flowers visited by a bee per minute in a single plot, were recorded before and after spraying insecticides and was expressed as average number of flowers $minute^{-1}$ (Table 15 & 16).

4.2.2.4.1 *Foraging Speed of A. cerana indica*

The foraging speed of *A. cerana indica* before spraying of insecticides did not vary significantly and ranged from 5.40 to 6.40 flowers min^{-1}. Significant difference in the foraging speed of *A. cerana indica* among the treatments was observed up to three DAS (Table 15).

On the day of spraying, the foraging speed of the bee was significantly reduced in plots treated with dimethoate 30 EC @ 0.5 mL L^{-1}, the speed being 2.00 flowers $min.^{-1}$ the foraging speed recorded in other two treatments were at par with the untreated control, the values being 4.60 flowers $min.^{-1}$, 5.00 flowers

min.$^{-1}$ and 5.40 flowers min.$^{-1}$ for novaluron 10 EC @ 2 mL L^{-1}, cyantraniliprole 20 SC @ 1.2 mL L^{-1} and control.

One DAS, the number of flowers visited was higher in treatments, cyantraniliprole 20 SC @ 1.2 mL L^{-1}, control and in novaluron 10 EC @ 2 mL L^{-1}the foraging speeds associated with them being 6.4, 5.8 and 4.2 flowers min.$^{-1}$ respectively which didn't differ significantly. The insecticidal check, dimethoate 30 EC @ 0.5 mL L^{-1} recorded significantly lower foraging speed of 1.60 flowers min.$^{-1}$.

Table 15. Foraging speed of *A. cerana indica* at different intervals of insecticide application

Treatment	Dosage (mL L^{-1})	*No. of flowers min^{-1} DAS						
		Before spraying	0	1	3	5	7	15
Cyantraniliprole 20 SC	1.2	6.00	5.00	6.40	6.20	6.00	6.20	5.80
Novaluron 10 EC	2	6.20	4.60	4.20	6.20	6.20	6.40	6.00
Dimethoate 30 EC	0.5	6.40	2.00	1.60	3.00	6.00	6.00	5.20
Control (Untreated)	-	5.40	5.40	5.80	5.80	5.80	6.20	5.60
S.E.(m)	-	0.478	0.847	0.615	0.552	0.557	0.678	0.685
C.D.	-	NS	1.725	1.856	1.852	NS	NS	NS

*Mean of five replications

DAS: Days After Spraying

On three DAS, the insecticides cyantraniliprole 20 SC @ 1.2 mL L^{-1} and novaluron 10 EC recorded a foraging speed of 6.2 flowers $min.^{-1}$ which was on par with that of untreated control (5.80 flowers $min.^{-1}$). Dimethoate 30 EC @ 0.5 mL L^{-1} significantly reduced the foraging speed of *A. cerana indica* on 3 DAS.

4.2.2.4.2 *Foraging Speed of T. iridipennis*

The foraging speed of *T. iridipennis* before spraying insecticides ranged from 9.90 to 11.20 flowers min^{-1} (Table 16).

All the insecticides significantly reduced the foraging speed of *T. iridipennis* on the day of spraying. It was the lowest in insecticidal check, dimethoate 30 EC @ 0.5 mL L^{-1} (1.80 flowers $min.^{-1}$) followed by cyantraniliprole 20 SC @ 1.2 mL L^{-1} (4.40 flowers $min.^{-1}$) and novaluron 10 EC @ 2 mL L^{-1} (5.00 flowers $min.^{-1}$).

Table 16. Foraging speed of *T. iridipennis* at different intervals of insecticide application

Treatment	Dosage (mL L^{-1})	*No. flowers min^{-1} DAS						
		Before spraying	0	1	3	5	7	15
Cyantraniliprole 20 SC	1.20	10.60	4.40	5.40	10.10	9.40	9.70	10.20
Novaluron 10 EC	2.00	11.20	5.00	8.20	10.20	9.90	9.80	10.80
Dimethoate 30 EC	0.50	10.40	1.80	5.80	10.00	10.30	10.20	10.30
Control (Untreated)	-	9.90	9.60	11.00	10.30	10.80	9.40	10.40
S.E.(m).	-	0.512	0.596	0.594	0.668	0.608	0.506	0.426
C.D. (0.05)	-	NS	2.637	1.916	NS	NS	NS	NS

*Mean of five replications

DAS: Days After Spraying

Three DAS also the treatments cyantraniliprole 20 SC @ 1.2 mL L^{-1} (5.40flowers $min.^{-1}$), dimethoate 30 EC @ 0.5 mL L^{-1} (5.80 flowers $min.^{-1}$) and novaluron 10 EC @ 2 mL L^{-1} (8.20 flowers $min.^{-1}$) were significantly lower than that in control (11.00 flowers $min.^{-1}$). Five DAS and seven DAS, foraging rates associated with all the treatments were non-significant.

4.2.3 Effect of Insecticide Application on Foraging Behaviour of the Colonies Placed Near the Field

The number of Indian and stingless bee returning foragers getting into their respective hives with pollen load in their leg was estimated at 0, 1, 3, 5, 7 and 15 DAS and expressed as percentage reduction in number of successful foragers per 5 minutes (Table 17).

In case of *A. cerana indica*, number of foragers getting into the hive was recorded to be 19 in five minutes which significantly differed from that on 0, 1 and 3 DAS where the average number of foragers getting in with pollen load being 11.33, 11.67 and 12.00 bees 5 $min.^{-1}$. No significant variation was observed on five days after spraying.

The number of stingless bees getting into their hive before insecticides spraying was 32.67 foragers 5 $min.^{-1}$ which was on par with seven DAS (29.67 foragers 5 $min.^{-1}$). The number of foragers was found to be reduced to 13.00 and 14.33 bees5 min^{-1} on the day of spraying and one DAS respectively. While the number of foragers on three DAS (17.67 foragers 5 $min.^{-1}$) and five DAS (23.00 foragers 5 $min.^{-1}$) were also on par.

Table 17 Average number of returning foragers before and after application of insecticides in the field

Days after spraying	*No. of Foragers 5 min.$^{-1}$	
	A. cerana indica	*T. iridipennis*
Before spraying	19.00	32.67
0	11.33	13.00
1	11.67	14.33
3	12.00	17.67
5	18.00	23.00
7	17.67	29.67
S.E.(m).	1.452	1.750
CD (0.05)	5.586	4.282

*Mean of five replications

DAS: Days After Spraying

4.3 PERSISTENCE OF INSECTICIDES ON THE FLOWERS OF CULINARY MELON

Pesticide residues on cucumber flowers were evaluated on 0, 1, 3, 5, 7, 10 and 15 DAS in the field following standard procedures of pesticide residue analysis and were expressed in mg kg^{-1} (Table 18).

Recovery studies revealed that percentage recovery of the insecticides *viz.*, dimethoate and novaluron were within the accepted range 70-120 at three levels of fortification (0.05, 0.25, 0.50 mg kg^{-1}) with satisfactory rSD (<20%).

On analysis of residues in flowers of culinary melon two hours after spraying of novaluron 10 EC @ 2 mL L^{-1}, the average residue level on flower was observed to be 10.50 mg kg^{-1}. A gradual reduction in amount of residues was observed in subsequent days, i.e., 1, 3, 5, 7 and 10 DAS spraying, the quantified level of pesticide residues being 6.52, 2.47, 1.17, 0.98 and 0.58 mg kg^{-1}. The residue reached below detectable level (BDL) on 15 DAS.

In case of dimethoate 30 EC @ 0.5 mL L^{-1}, the residue level present on the flowers of culinary melon was 9.7 mg kg^{-1} on the day of spraying. On one and three DAS, the residue level of dimethoate 30 EC was 3.20 and 0.28 mg kg^{-1} respectively, the residue dissipated and reached BDL after five days of spraying.

Table 18. Pesticide residues of insecticides in cucumber flowers at different intervals of insecticide application

Treatment	*Pesticide residue (mg kg^{-1}) DAS						
	0	1	3	5	7	10	15
Novaluron 10 EC	10.50	6.52	2.47	1.17	0.98	0.58	BDL
Dimethoate 30 EC	9.70	3.22	0.28	BDL	BDL	BDL	BDL

* Mean of five replications

DAS: Days after spraying

BDL: below detectable limit

LOQ: 0.05 mg kg^{-1}

DISCUSSION

5. DISCUSSION

The present investigation was conducted at the AICRP on honey bees and pollinators, Department of Agricultural Entomology, College of Agriculture, Vellayani to study the field toxicity of new generation insecticides to bee pollinators. The results of the study are discussed below:

Hiving of domesticated honey bees in crop fields is being practised by the farmers for centuries in order to utilize their pollination services. The local and the wide landscape management have been reported to favour natural pollination services, which in turn help to sustain crop diversity and production (Klein *et al.*, 2007).

Pest management is an important aspect of crop production and several management strategies have been practised by the farmers from the beginning itself. Among these, insecticide application gives quicker and better results against crop pests, but their toxicity towards non – target organisms were unknown for a long period of time. As the toxicity of carbamates, organophosphates and chlorinated hydrocarbons in honey bees (Bai and Reddy, 1977) were reported long back, use of these conventional insecticides in crop fields are less appreciated. In order to combat pest problems in agriculture fields, some novel molecules with specific mode of actions (IRAC, 2018) were introduced very recently.

Due to their lower toxicity to other vertebrates (Coats, 1994) and high bio-efficacy in controlling insect pests (Lopez *et al.*, 2005), they got a wide adoption among the farmers particularly vegetable growers. It is essential to know about the safety/ toxicity of these new generation insecticides applied to the entomophilous crops towards their respective pollinators in order to obtain maximum benefit from bee pollination. Though the safety of different insecticides to the pollinators were worked out, there is a need to evaluate the safety of the newer molecules recommended against the pests of vegetable ecosystems to the major pollinators.

The present study sticks on to the safety/ toxicity of certain new generation insecticides *viz.*, thiamethoxam 25 WG, dinotefuran 20 SG, cyantraniliprole 20 SC, novaluron 10 EC, thiamethoxam (17.5 %) + chlorantraniliprole (8.8 %), flubendiamide (19.92 %) + thiacloprid (19.92 %) and one conventional insecticide dimethoate 30 EC to honey bees.

5.1 LABORATORY EVALUATION OF TOXICITY OF THE INSECTICIDES TO HONEY BEES

Two concentrations each of eight insecticides were tested under laboratory conditions in two major hive species of Kerala, *viz., A. cerana indica* and *T. iridipennis* to evaluate their toxicity in them.

Though, the insecticides were observed to have mortality in bees, the treatments cyantraniliprole 20 SC (1.2 mL L^{-1} and 0.6 mL L^{-1}) and novaluron 10 EC (2 mL L^{-1} and 1 mL L^{-1}) recorded least toxicity to *A. cerana indica* and *T. iridipennis* as compared to other insecticides under study. The results of the study are in agreement with the findings of several research workers. O' Neill *et al.* (2014) demonstrated laboratory bioassay of cyantraniliprole 20 SC on pollinators and the results revealed that the formulation poses no potential risks to the pollinators under laboratory condition. Though, the report of EPA (2014) revealed that the label requirements of the formulated product (cyantraniliprole 20 SC) is designed in such a way to mitigate the risks to the pollinators, the active ingredient of cyntraniliprole is reported to have high end toxicity to pollinators under laboratory conditions (May *et al.*, 2015).

Similar results were reported with regard to the safe profile of novaluron to these pollinators from laboratory studies conducted in other parts of the country (Nadaf *et al.*, 2006) and Australia (APVMA, 2013). Novaluron 10 EC, being a growth regulator (IRAC, 2018) does not cause much harm to the adult insects.

Neonicotinoid insecticide, thiamethoxam 25 WG @ 0.3 g $L^{-1}$was found to cause cent per cent mortality in both Indian and stingless bees, within

one HAT. Whereas, the toxicity evaluation of thiamethoxam conducted by Pastagia and Patel (2007) under laboratory condition revealed 85.67 per cent mortality of *A. cerana indica* workers at 24 HAT. The variation may be due to the differences in experimental conditions, dosage of the insecticide and race specific resistances in bee colonies.

Though the field studies on the safety evaluation of insecticides conducted by Boli (2013) revealed that chlorantraniliprole is relatively safer to bee pollinators, in the present study combination insecticide thiamethoxam (17.5 %) + chlorantraniliprole (8.8 %) 300 SC was found to have early mortality in both *T. iridipennis* and *A. cerana indica* when compared to that associated with flubendiamide (19.92 %) + thiacloprid (19.92 %) 480 SC.

This variation may be due to the second component (neonicotenoid-thiamethoxam) of the combination products as their difference in side chain substitution contribute to their differential toxicity in bee pollinators (Iwasa *et al.*, 2004). Cyano-substituted compound, thiacloprid metabolise easily in honey bees as compared to that of nitro-substituted compounds (thiamethoxam) so they are reported to have comparatively lower toxicity in pollinators (Decourtye and Devillers, 2010).

Neonicotinoids (thiamethoxam 25 WG and dinotefuran 20 SG) showed early mortality in *T. iridipennis* than *A. cerana indica* whereas, all other insecticides recorded higher mortality in Indian bees when compared with that of stingless bees (Fig 1). Earlier studies on sensitivity of neonicotinoids to different bee pollinators revealed that *T. iridipennis* is more sensitive than *A. cerana indica* (Arena and Sgolastra, 2014). The sensitivity of honey bees to insecticides is a result of the concerted action of several manifold factors such as body mass, genetic background, physiological characters of the honey bees and the structure of the chemicals (Thompson, 2016). The results of present study support the findings, that the toxicity of insecticides to honey bees is closely associated with the structure of pesticides and their modes of action, but not with the body mass of

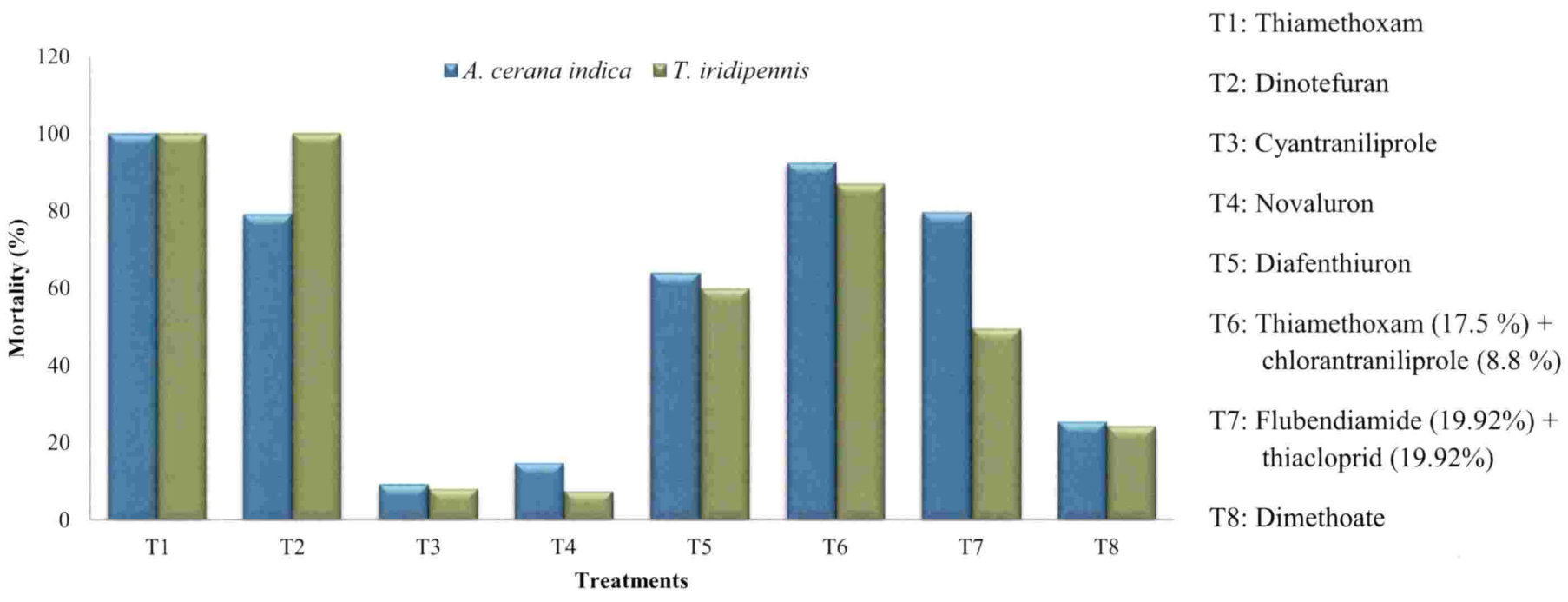


Fig. 1. Mortality of *A. cerana indica* and *T. iridipennis* in laboratory when exposed to field dose of new generation insecticides

bees (Meng *et al.*, 2017). Apart from these, the pesticide toxicity of different pollinators varies from one species to another (Yasuda *et al.*, 2017).

5.2 FIELD EVALUATION OF TOXICITY OF THE INSECTICIDES TO BEE POLLINATORS

Cucurbitaceous vegetables are mostly monoecious and highly cross pollinated. The flowers of these plants possess certain adaptive morphological characters which fit them for insect pollination. The most important features are heavy, large and adhesive pollen grains and stigma, a large amount of high grade nectar and a large, showy corolla (Fronk and Slater, 1956).

Since, bee pollination increases fruit set, seed yield and fruit weight (Cervancia and Bergonia, 1991) farmers can rely on bee pollination to mitigate the yield gap in cucurbitaceous vegetables (Motzky *et al.*, 2015) than on pest and nutrient management of the crop. In the present study, field evaluation of the insecticides to major pollinators was conducted in *C. melo*, commonly known as culinary melon.

5.2.1 Insect Fauna on Culinary Melon

The insect fauna on culinary melon were recorded which constituted pests, natural enemies and pollinators/ flower visitors (Fig. 2). Majority of the insect visitors were from order Hymenoptera (63.54%) followed by Coleoptera (27.26%) and Lepidoptera (9.01%) (Fig. 3). These finding are in close proximity with the experimental results of Cervancia and Bergonia (1991) in Philippines in which they reported that the major pollinators of cucumber belong to order Hymenoptera and all other pollinators were 'chance pollinators'. Similarly, Kumar and Singh (2005) reported that twelve insect species belonging to 11 families under four orders were found visiting the blossoms of *C. melo* among which Hymenopterans were predominant.

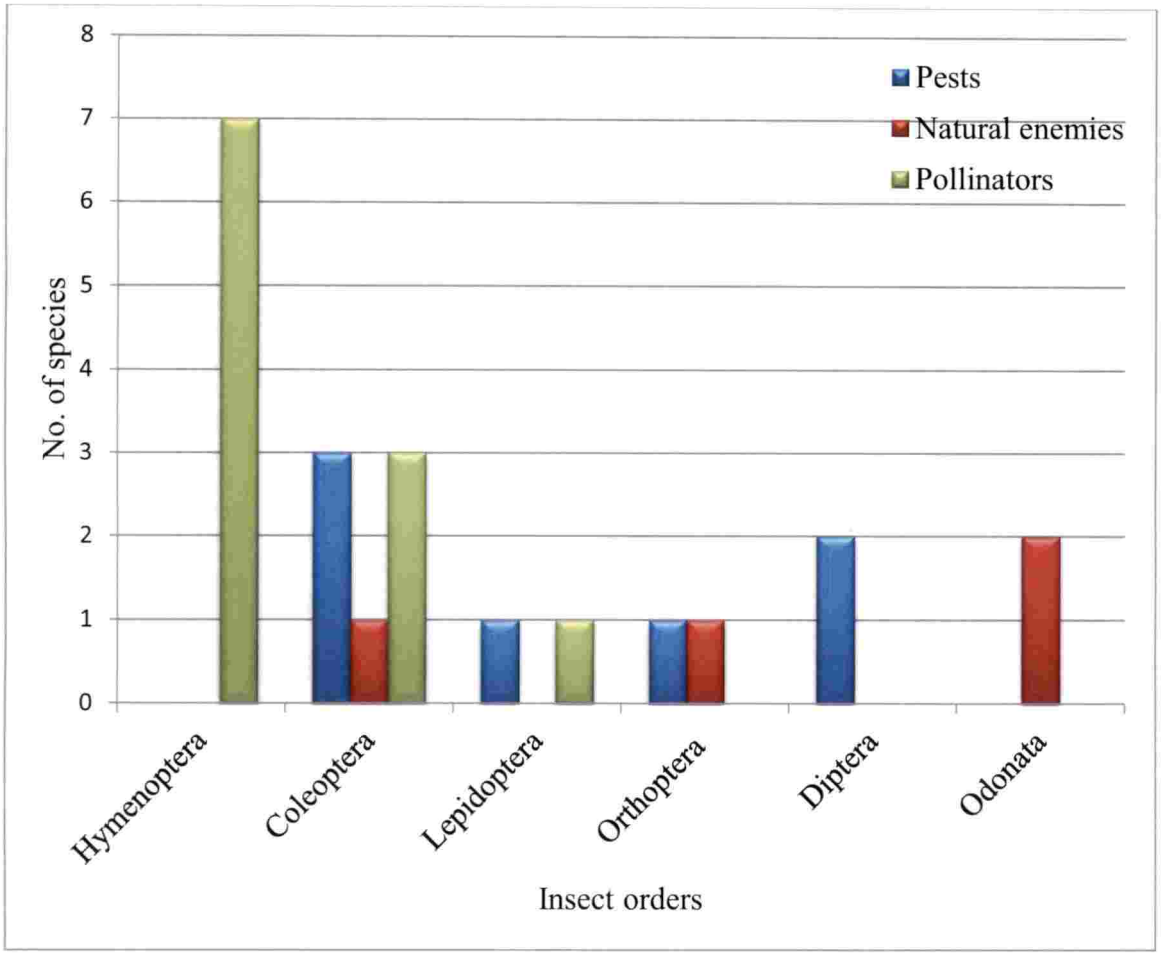


Fig. 2. Insect fauna associated with *Cucumis melo*

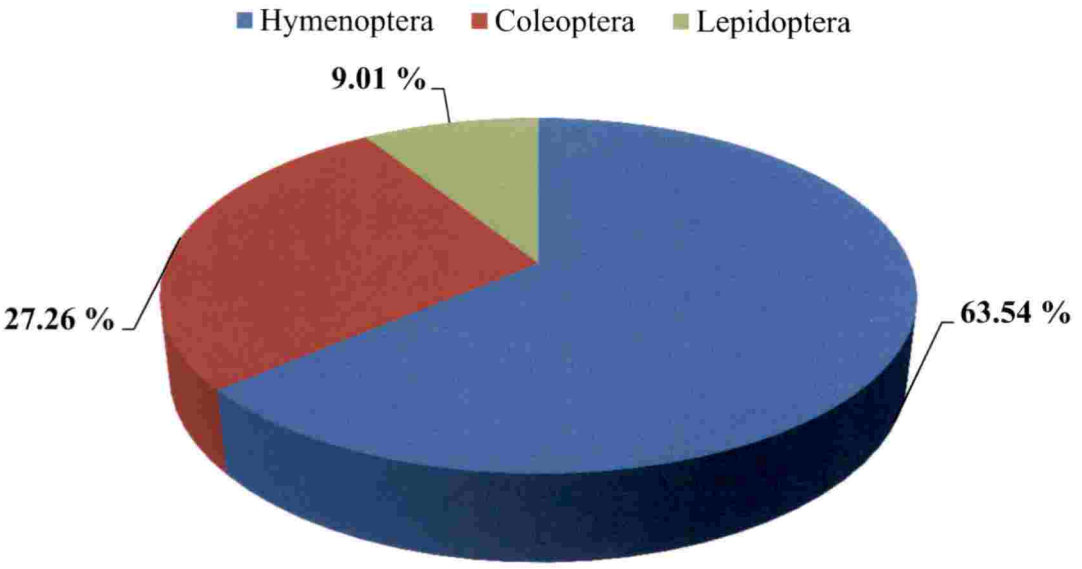


Fig. 3 Pollinators of *Cucumis melo*

The analysis of pollinator population associated with culinary melon revealed that the most abundant pollinator in the crop is *T. iridipennis* followed by *A. cerana indica* with a relative abundance of 5.16 and 3.94 bees m^{-2} 5 $min.^{-1}$ respectively under pesticide free condition (Fig. 4). This result is in agreement with the research findings of Boli (2013) in Kerala.

In the present study, maximum number of pollinators on the crop (*C. melo*) was observed in the period 1000 h to1200 h (Fig. 5) and it was found to be decreasing from 1400 h onwards. For a particular entomophilous crop like cucumber, the pollinator abundance will be at their peak when the floral resources are abundant with pollen and/or nectar. Boli (2013) evaluated the peak time of activity of the major pollinators of culinary melon in terms of time spent by the bees on flowers and their relative abundance in field. It was found that the peak period of activity of both the pollinators was in the period 0900h to 1000h. In the present investigation, peak time of activity was evaluated in terms of their abundance in field and it was found that the abundance of stingless bees were more in the field during 0800 h to 1000 h and that of Indian bees was between 1000 h to 1200 h. The deviation in case of the former may be due to the difference in climatic and weather parameters during the observation period.

Abundance of major pollinators is mostly associated with anthesis, as a depleted floral source is less preferred by the pollinators (Collison and Martin, 1979). They also reported that the floral nectaries of cucurbitaceous vegetables will be opening between 4.00 and 5.00 hours after anthesis while the anthesis in male and female flowers of the crop was observed 5.09 am to 5.20 am (Kiill *et al.*, 2016).

The maximum foraging distance of *T. iridipennis* and *A. cerana indica* are within 300 m (Nieuwstadt and Iraheta, 1996) and 500 m (Beekman and Ratnieks, 2000) respectively. In order to get sufficient number of pollinators in the field, one hive each of Indian bee and stingless bee were placed 5 m away from the field. As the distance from the hives to the farther point in the field were within

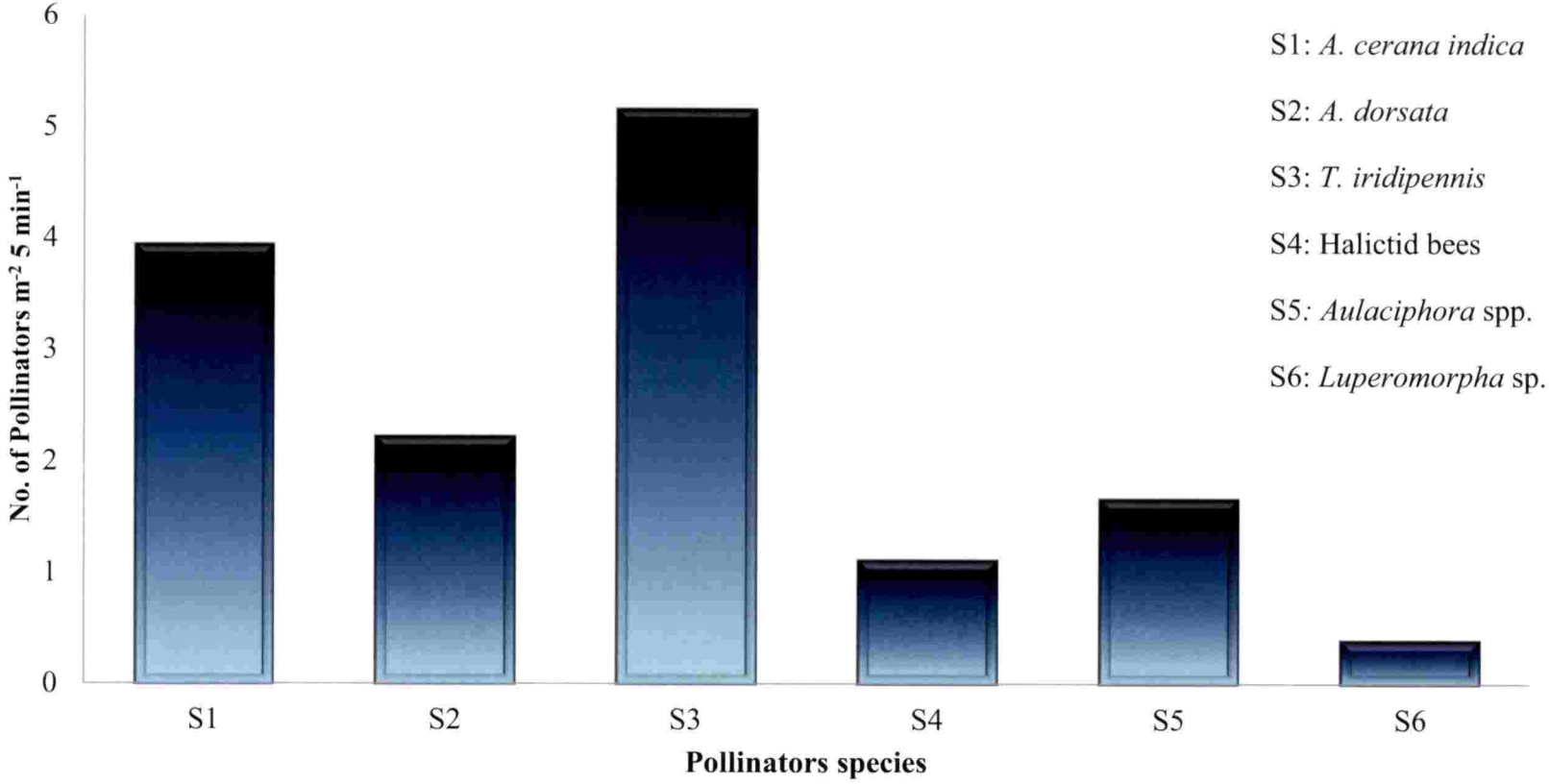


Fig. 4 Abundance of pollinators in *Cucumis melo*

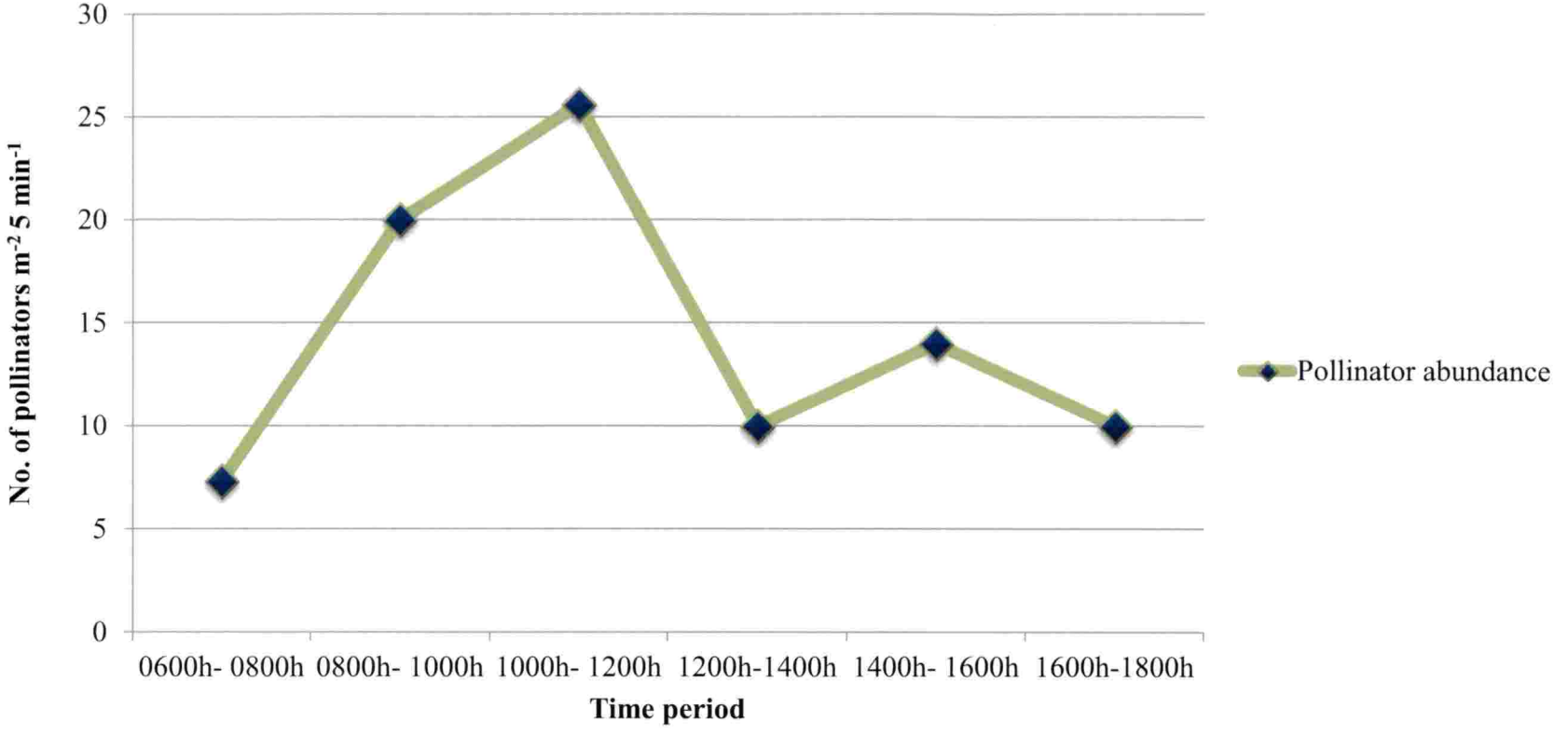


Fig. 5 Pollinator activity in *Cucumis melo* at different hours of the day

the flight range of the two pollinators, their density in the crop field increased significantly by 26.07 per cent and 50.05per cent respectively at their peak periods of activity (Table 6).

5.2.2 Effects of insecticides on foraging behaviour of bee pollinators

5.2.2.1 *Relative abundance*

Significant reduction in relative abundance of *A. cerana indica* and *T. iridipennis* was observed in all the treatments, except in control on the day of spraying the least toxic insecticides. While comparing the relative abundance of the pollinators associated with insecticidal check (dimethoate 30 EC), cyantraniliprole 20 SC @ 1.2 mL L^{-1} and novaluron 10 EC @ 2 mL L^{-1} possess least effect. Though cyantraniliprole 20 SC is having excellent xylem mobility, the studies conducted in rape seed and melon in Germany and France showed that the application of the insecticide through drip irrigation didn't affect the relative abundance of the pollinators in the field (Dinter and Samel, 2014). Similarly, semi field studies conducted at Bangalore also revealed that novaluron is relatively safer to *A. mellifera* @ 1 mL L^{-1} (Ratnakar, 2015).

5.2.2.2 *Foraging rate*

Foraging rate of *A. cerana indica* and *T. iridipennis* were not much affected by the application of cyantraniliprole 20 SC @ 1.2 mL L^{-1} in the field where it was found to be safe to the Indian bees under field condition. O' Neill *et al.* (2014) reported that cyantraniliprole didn't affect the flight activity of pollinators when exposed to its field concentration.

Similarly, May *et al.* (2015) classified novaluron as a low risk compound to bee pollinators. In present study also, the effect of novaluron 10 EC @ 2 mL L^{-1} was lower as compared to that of the insecticidal check, dimethoate 30 EC @ 0.5 mL L^{-1}.

In USA, foraging visits of the bees in the colony placed in the citrus orchard was found to be reduced upto 2 weeks after dimethoate application in the field (Waller *et al.*, 1984). While studies conducted by Decourtye and Devillers (2010) revealed that dimethoate have no effect on foraging behaviour of *A. mellifera* in terms of their proboscis extension from a foraging source contaminated with dimethoate residues.

This variation may be due to the genetic differences associated with different species (*A. mellifera, A. cerana indica* and *T. iridipennis*), as the differential toxicity of insecticides towards different insects are a function of their genetic makeup (Claudianos *et al.*, 2006) which in turn determine the number of genes encoding enzymes that are involved in insecticide detoxification mechanism in them (Johnson, 2010).

5.2.2.3 *Time spent by the bees on flowers*

The observations on time spent by the bees before application of insecticides in the field revealed that both the Indian and stingless bee spent less time on female flowers than that on male flowers. Srikanth (2015) quantified nectar content and sugar present on flowers of bottle gourd and found that male flowers recorded higher value for these two parameters than female flowers. This may be the reason behind the maximum foraging time on male flowers.

The time spent by the Indian bees on flowers of culinary melon was least affected by cyantraniliprole 20 SC @ 1.2 mL L^{-1} with average time spent by them on male and female flowers being 3.35 sec. and 2.17 sec. respectively. This can be explained in terms of the nectar contamination by dimethoate (Waller *et al.*, 1979) which may contribute to the repellent effect of dimethoate in bee pollinators in the field (Johansen *et al.*, 1983).

5.2.2.4 *Foraging speed*

Foraging speed of *A. cerana indica* was the highest in treatment with cyantraniliprole 20 SC @ 1.2 mL L^{-1}(5.93 flowers $min.^{-1}$) followed by novaluron 10 EC @ 2 mL L^{-1} (5.60 flowers $min.^{-1}$). In *T. iridipennis* the effect of novaluron 10 EC was negligible (8.82 flowers $min.^{-1}$) as compared to that of cyantraniliprole 20 SC @ 1.2 mLL^{-1}(8.20 flowers $min.^{-1}$), but the effects of the two new generation insecticides were lower as compared to that of insecticidal check.

5.2.3 Effect of Insecticide Application on the Foraging Behaviour of the Colonies Placed Near the Field

To ensure sufficient pollinator population in the field, one hive each of *A. cerana indica* and *T. iridipennis* was placed near the field. In order to study the effect of the insecticides in these hived bees, the number of returning foragers with pollen load on their leg was observed before and after insecticide application.

Significant reduction in number of returning foragers were observed from the day of spraying to five DAS and three DAS in case of stingless bees and Indian bees respectively. The percentage reduction was higher in stingless bees (Fig. 6). Similar studies have been conducted in different crop fields, in citrus orchards application of dimethoate 10 EC resulted in reduced foraging behavior of the hived bees placed in the field upto two weeks after spraying (Waller *et al.*, 1984).

5.3 PERSISTENCE OF INSECTICIDES ON THE FLOWERS OF CULINARY MELON

Analysis of cucumber flowers to quantify pesticide residues in them, it was observed that persistence of novaluron 10 EC was present in detectable levels upto seven DAS (0.98 mg kg^{-1}), whereas detectable levels of dimethoate 30 EC was recorded from the flowers only upto three DAS (0.28 mg kg^{-1}). Though the residue levels in pollen and nectar was quantified in different parts of the world,

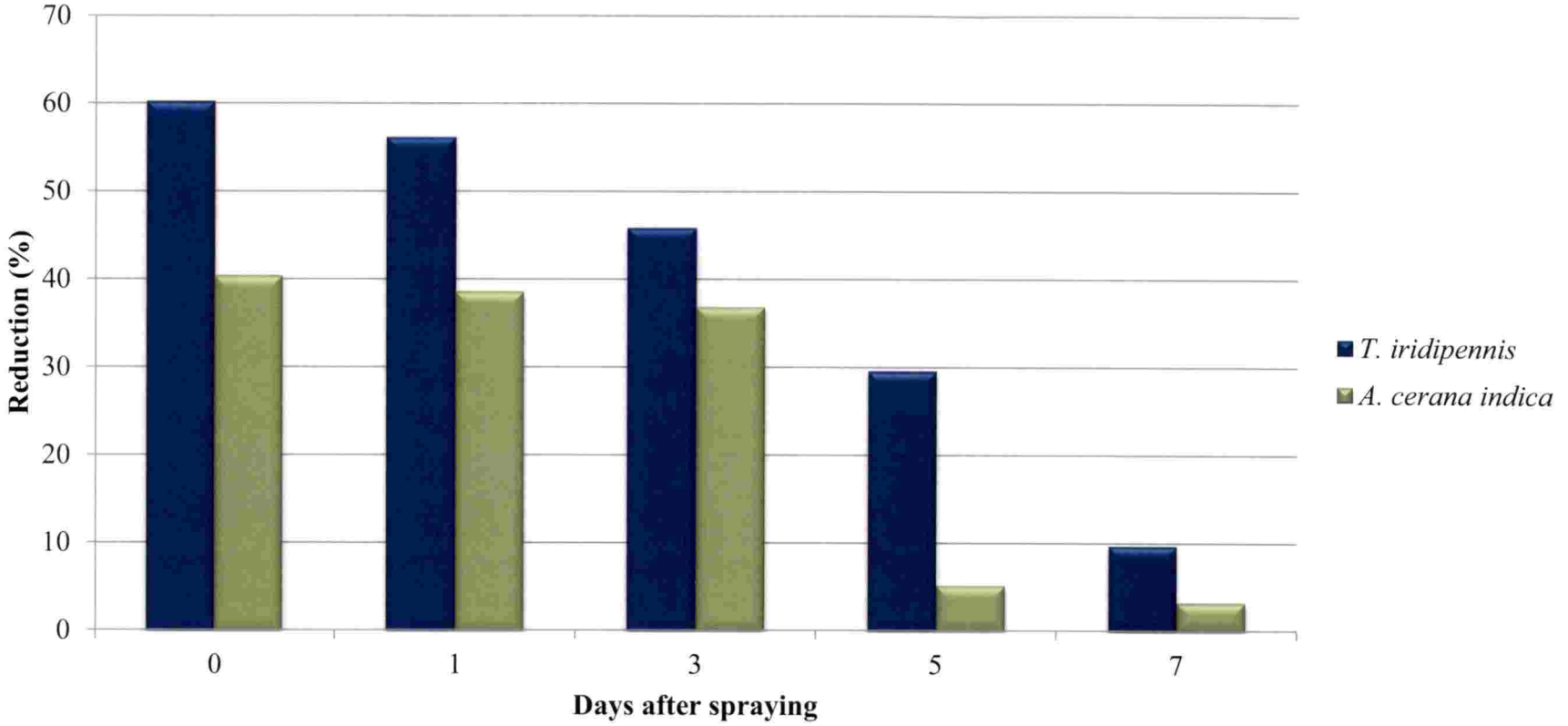


Fig. 6 Percentage reduction in number of foragers in hives placed near the experimental plot

research work on insecticide residues in cucurbitaceous flowers is scanty. However, in 1980, Barker *et al.* reported that the residues of dimethoate 30 EC upto 3 ppm was detected from nectar of alfalfa flowers seven DAS, but the residue level was lower (0.5 ppm) in pollen of flower on one DAS.

In brief, novaluron 10 EC and cyantraniliprole 20 SC pose low risk to the pollinators under both laboratory and field conditions. When talking in light of the residues left on the flowers by the insecticides, residues of dimethoate 30 EC even at below detectable level (<0.05 mg kg^{-1}) could affect the foraging activities of the bee pollinators in field. At the same time 0.98 mg $kg^{-1}$of novaluron 10 EC didn't have any observable effect on their foraging behaviour. Since cyantraniliprole 20 SC (Anthranilic diamide) and Novaluron (Insect Growth Regulator) are effective in controlling insect pests of cucurbitaceous agro ecosystem, they can be incorporated in effective pest management without affecting the pollination provided, application is avoided at their activity periods.

SUMMARY

6. SUMMARY

Experiments carried out in All India Coordinated Research Project (AICRP) on honey bees and Pollinators, Department of Agricultural Entomology, College of Agriculture, Vellayani during 2016– 18 to determine the field toxicity of new generation insecticides to bee pollinators are summarized below;

Contact toxicity of some new generation insecticides commonly recommended in vegetable ecosystem was evaluated under laboratory condition against major pollinators *viz.*, *A. cerana indica* and *T. iridipennis*.

Among the treatments cyantraniliprole 20 SC (1.2 mL L^{-1} and 0.6 mL L^{-1}) and novaluron 10 EC (2 mL L^{-1} and 1mL L^{-1}) were found to be least toxic to *A. cerana indica* as no mortality of the bees was observed at one HAT and three HAT. The treatment which was found to be least toxic to Indian bees at six HAT was cyantraniliprole 20 SC @ 0.6 mL L^{-1} which recorded no mortality followed by novaluron 10 EC @ 1 mL L^{-1} (2.5%), cyantraniliprole 20 SC @ 1.2 mL L^{-1} (5 %). At 12 HAT lower mortality of *A. cerana indica* was observed with cyantraniliprole 20 SC @ 0.6 mL L^{-1} which recorded 15.56 per cent mortality. This was followed by novaluron 10 EC @ 1 mL L^{-1} and cyantraniliprole 20 SC @1.2 mL $L^{-1}$with the mortality percentages being 28.61 per cent and 32.50 per cent respectively.

Safety evaluation in stingless bees, *T. iridipennis* showed that cyantraniliprole 20 SC (@ 1.2 mL $L^{-1}$and 0.6 mL L^{-1}) and novaluron 10 EC (@ 2 mL L^{-1} and 1 mL L^{-1}) were safe among the insecticides tested since, they recorded no mortality in the population under study at one HAT and at three HAT. At six HAT, cyantraniliprole 20 SC @ 0.6 mL L^{-1}, novaluron 10 EC @ 1 mL L^{-1} and novaluron 10 EC @ 2 mL L^{-1} were recorded with no mortality, whereas, the mortality associated with cyantraniliprole 20 SC @ 1.2 mL L^{-1} was 2.5 per cent. Lower mortality of stingless bees at 12 HAT was observed with novaluron 10 EC @1 mL L^{-1}. This was followed by cyantraniliprole 20 SC @

0.6 mL L^{-1}, novaluron 10 EC @ 1 mL L^{-1} and cyantraniliprole 20 SC @ 1.2 mL L^{-1} with their mortality percentages 25.56, 30.55 and 30.56 per cent respectively which didn't differ significantly.

Thiamethoxam 25 WG was found to be highly toxic to Indian and stingless bees at both the concentrations (0.3 g L^{-1} and 0.15 g L^{-1}) as cent per cent mortality was observed within three HAT. Another neonicotinoid, dinotefuran 20 SG was also recorded with higher mortality in *T. iridipennis* at two concentrations (0.3 g $L^{-1}$and 0.15 g L^{-1}) within one HAT. Similarly, among the two combination products, thiamethoxam (17.5 %) + chlorantraniliprole (8.8 %) 300 SC was found to have early mortality in both *T. iridipennis* and *A. cerana indica* when compared to the mortality associated with flubendiamide (19.92 %) + thiacloprid (19.92 %) 480 SC.

The insect fauna on culinary melon were recorded which constituted pests, natural enemies and pollinators/ flower visitors. The pests encountered in the field included three species from order Coleoptera, two species from order Diptera and one species each from orders Lepidoptera, Hemiptera and Orthoptera. Natural enemies observed in the field were predators from orders Coleoptera (ground beetle) and Odonata (dragon flies and damsel flies). Majority of the pollinators recorded belong to order Hymenoptera (63.54 %) followed by Coleoptera (27.26 %) and Lepidoptera (9.01 %).

Abundance of major pollinators under pesticide free condition was recorded at different hours of the day from 6.00 am to 6.00 pm. Most abundant pollinator on culinary melon was *T. iridipennis* followed by *A. cerana indica* with their peak time of activity between 0800 h to 1000 h and 1000 h to 1200 h respectively. Maximum number of pollinators on the crop was observed in the period between 1000 h to1200 h. The population of bees in the field increased after installation of hives in the field. Population of Indian bees and stingless bees were increased by 26.07 per cent and 50.05 per cent respectively at their peak periods of activity.

The insecticides which recorded lower mortality to honey bees in the laboratory (cyntraniliprole 20 SC and novaluron 10 EC) were selected for their field evaluation. Relative abundance of *A. cerana indica* was least affected by the application of cyantraniliprole 20 SC and novaluron 10 EC when compared to that of insecticidal check (dimethoate 30 EC) in field. The reduction in relative abundance of Indian bees were significant up to one DAS in case of both the new generation insecticides, whereas it was significant up to five DAS in case of insecticidal check.

Relative abundance of *T. iridipennis* was also affected on the day of spraying in case of all the three insecticides. Compared to dimethoate 30 EC, the reduction was relatively lower in case of novaluron 10 EC @ 2 mL L^{-1}. There was a significant reduction in relative abundance of stingless bees in the field upto seven DAS in case of dimethoate 30 EC, whereas cyantraniliprole and novaluron affected the relative abundance upto three DAS.

Foraging rate of *A. cerana indica* was not adversely affected by the application of cyantraniliprole 20 SC @ 1.2 mL L^{-1}in the field and was found to be the safer treatment in Indian bees under field condition also. Lower foraging rate was observed with novaluron 10 EC @ 2 mL L^{-1} (1.00 bees $min.^{-1}$) but, was higher than that recorded in dimethoate 30 EC @ 0.5 mL L^{-1} (0.20 bees $min.^{-1}$) treatment at one DAS. Novaluron 10 EC was found to be safer than dimethoate 30 EC in the field. The reduction in foraging rate of Indian bees was significant for a shorter period of time in case of cyantraniliprole 20 SC and novaluron 10 EC (0 and 1 DAS) compared to that in dimethoate 30 EC (3 DAS).

In case of *T. iridipennis*, 1 DAS, the highest foraging rate was observed in novaluron 10 EC (7.60 bees $min.^{-1}$) and was found on par with control. Cyantraniliprole 20 SC and dimethoate 30 EC were found to have significant reduction in foraging rate of stingless bees upto three DAS.

The time spent by the Indian bees on flowers of culinary melon was least affected by cyantraniliprole 20 SC with average time spent by them on male and female flowers being 3.35 sec. and 2.17 sec. respectively. The foraging time was affected by novaluron 10 EC on both male and female flowers up to one DAS only. In case of dimethoate 30 EC, the reduction in time spent by Indian bees was lower on both male and female flowers.

Among the treatments, relatively longer foraging time of stingless bees was associated with novaluron 10 EC and one DAS, the average time spent by the bees on male and female flowers were 29.78 sec. and 20.78 sec. respectively.

Foraging speed of *A. cerana indica* was higher in treatment, cyantraniliprole 20 SC (5.00 flowers min^{-1}) followed by novaluron 10 EC (4.60 flowers min^{-1}) but were comparatively safer than insecticidal check (2.00 flowers $min.^{-1}$). In *T. iridipennis* the effect of novaluron 10 EC was (5.00 flowers $min.^{-1}$) and cyantraniliprole 20 SC (4.40 flowers $min.^{-1}$) on foraging speed was comparatively lower than that in dimethoate 30 EC (1.80 flowers $min.^{-1}$).

Average number of foragers with pollen load getting into the hives that were placed near the field in order to ensure the presence of major pollinators in the field was recorded at their peak time of activity. In the case of stingless bee hive, significant reduction in number of foragers with pollen load were observed upto five DAS. In case of Indian bees, significant reduction in number of foragers lasted for three DAS. The percentage reduction in number of foragers was more in stingless bees than that in Indian bees.

Analysis of cucumber flowers to quantify pesticide residues in them, it was observed that persistence of novaluron 10 EC was longer in flowers as it was present in detectable levels upto seven DAS (0.98 mg kg^{-1}). Whereas, detectable levels of dimethoate were recorded from the flowers only upto three DAS (0.28 mg kg^{-1}) still, its effect was more on foraging behaviour of the bees.

REFERENCES

7. REFERENCES

Abbott, W. S. 1925. A method of computing effectiveness of insecticides. *J. Econ. Entomol.*18: 265-267.

APVMA [Australian Pesticides and Veterinary Medicines Authority]. 2013. Public release summary on the evaluation of the new active constituent cyantraniliprole in the product dupont exirel insecticide. Australian Pesticides and Veterinary Medicines Authority. October, 2013. ISSN: 1443-1335.

Aravind, J. and Samiayyan, K. 2014. Safety evaluation of diafenthiuron 50 WP (NS) to non-target organisms. *Trends Biosci.*7 (13): 1434-1439.

Aravind, J., Samiayyan, K. and Kuttalam, S. 2018. Assessing the impact of diafenthiuron 50 WP on the pest, pollinator and natural enemy diversity in cardamom ecosystem. *J. Entomol. Zool. Stud.* 6 (3): 398-402.

Arena, M. and Sgolastra, F. 2014. A meta-analysis comparing the sensitivity of bees to pesticides. *Ecotoxicol.* 23: 324–334.

Atkins, E. L., Greywood, E. A. and Macdonald, R. L. 1973. Toxicity of pesticides and other chemicals to honey bees – laboratory studies. University of California Agriculture Extension Service M-26. USA

Aupinel, P., Fortini, D., Michaud, B., Marolleau, F.,Tasei, J. N. and Odoux, J. F. C. 2007. Toxicity of dimethoate and fenoxycarb to honey bee brood (*Apis mellifera*), using a new in vitro standardized feeding method. *Pest Manag. Sci.* 63: 1090–1094.

Bai, A. R. K. and Reddy, C. R. 1977. Laboratory toxicity of some insecticides to *Apis cerana indica*. *J. Apic. Res.* 16 (3): 161-162.

Barker, R. J., Lehner, Y. and Kunzmann, M. R. 1980. Pesticides and honey bees: Nectar and pollen contamination in alfalfa treated with dimethoate, *Arch. Environ. Contamin. Toxicol.* 9: 125-127.

Beekman, L. N. and Ratnieks, F. L.W. 2000. Long-range foraging by the honey bee, *Apis mellifera. Funct. Ecol.* 14: 490–496.

Beevi, S. N., Paul, A., Mohan, P., Mathew, T. B. and Devanesan, S. 2004. Toxicity of botanical and synthetic pesticides on stingless bee (*Trigona iridipennis* Smith.) (Meliponinae: Hymenoptera). *Indian Bee J.* 66 (2): 7-10.

Bennet, R., Shailaja, K. K., Devanesan, S. and Saraswathi, P. 2003. Behaviour of stingless bee (*T. iridipennis* Smith.). *International workshop on conservation and management of bees for sustainable development and honey festival. (Apiexpo-2003)*, 13-18 October, 2003, Banglore, P. 24. Abstract No. 5.6.3.

Blackmer, J. L. and Byrne, D. N. 1999. The effect of *Bemisia tabaci* on amino acid balance in *Cucumis melo*. *Entomologia Experimentaliset Applicata* 93: 315–319.

Blacquiere, T., Smagghe, G., Cornelis, A. M., Gestel, V. and Mommaerts, V. 2012. Neonicotinoids in bees: a review on concentrations, side-effects and risk assessment. *Ecotoxicol.* 21: 973–992.

Boli, R. 2013. Safety of new generation insecticides to bee pollinators. M.Sc. (Ag) thesis, Kerala Agricultural University, Thrissur, 85p.

Cervancia, C. R. and Bergonia, E. A. 1991. Insect pollination of cucumber (*Cucumis sativus* L.) in the Philippines. In: Heemert, K.,Ruijter, A. and Holm, S. N. (eds.), *Acta Hortculturae 288*. Proceedings of the Sixth International Symposium on Pollination, 27 August 1990, Tilburg, Netherlands. pp. 278-282.

Chakraborti, S. and Sarkar, C. K. 2011. Management of *Leucinodes orbonalis* Guenee on egg plants during the rainy season in India. *J. Plant Protect. Res.* 51 (4): 325-328.

Chandel, R. S., Thakur, R. K., Bhardwaj, N. R. and Pathania, N. 2004. Onion seed crop pollination: A missing dimension in mountain horticulture. *Acta Hortculturae.* 631: 80-86.

Choudhari, A. U., Choudhari, S. D., Desai, D. S. and Kanvilkar, M. H. 2006. Honey bee foraging activity on Suringi (*Ochrocarpus longifolius*) in Konkan region of Maharashtra. *Indian bee J.* 68 (1-4): 107-109.

Claudianos, C., Ranson, H., Johnson, R. M., Biswas, S., Schuler, M. A., Berenbaum, M. R., Feyereisen, R., and Oakeshott, J. G. 2006. A deficit of detoxification enzymes: pesticide sensitivity and environmental response in the honey bee. *Insect Mol. Biol.*15 (5): 615–636.

Coats, J. R. 1994. Risks from natural versus synthetic insecticides. *Ann. Rev. Entomol.* 39: 489-515.

Collison, C. H. and Martin, E. C. 1979. Behaviour of honey bees foraging on male and female flowers of *Cucumis sativus*. *J. Apicultural Res.* 18 (3): 184-190.

Decourtye, A. and Devillers, J. 2010. Ecotoxicity of neonicotinoid insecticides to bees. In: Thany S. H. (ed.) *Insect Nicotinic Acetylcholine Receptors. Advances in Experimental Medicine and Biology*. Springer, New York.

Deodikar, G.B. and Suryanarayana, M. C. 1977. Pollination in the service of increasing farm production in India. In: Nair, P. K. K. (eds.) *Advance in Pollen Spore Research; Today and tomorrow*. Maharashtra Association for the Cultivation of Science, New Delhi, India, pp. 60-82.

Devanesan, S., Nisha, M. M., Bennet, R. and Shailaja, K. K. 2002. Foraging behaviour of stingless bee (*Trigona iridipennis* Smith.). *Insect Environ.* 8 (3): 131-133.

Devi, P. I., Thomas, J. and Raju, R. K. 2017. Pesticide Consumption in India: A Spatiotemporal Analysis. *Agric. Econ. Res.Rev.*30 (1): 163-172.

Dinter, A. and Samel, A. 2014. Cyantraniliprole: Pollinator profile of the novel insecticides under laboratory, semi-field and field conditions. In: Oomen, P. A. and Pistorius, J. (eds.), *Proceedings, 12th International Symposium of the ICP-PR Bee Protection Group*. 15–17 September, 2014. Julius Kuhn-Institute, Ghent, Belgium. pp. 28- 49.

Dinter, A., Brugger, K. E., Frost, N. M. and Woodward, M. D. 2009. Chlorantraniliprole (Rynaxypyr): A novel DuPont™ insecticide with low toxicity and low risk for honey bees (*Apis mellifera*) and bumble bees (*Bombus terrestris*) providing excellent tools for uses in integrated pest management. In: Ooomen, P. A. (ed.), *Hazards of pesticides to bees*. Proceedings of 10th International Symposium of the ICP-PR Bee Protection Group, 15–17 September, Julius Kuhn-Institute, Ghent, Belgium.pp. 84–96.

Dively, G. P. and Kamel, A. 2012. Insecticide residues in pollen and nectar of a cucurbit crop and their potential exposure to pollinators. *J. Agric. Food Chem.*60 (18): 4449–4456.

Dorjay, N., Abrol, D. P. and Shankar, U. 2017. Insect visitors on cucumber and bitter gourd flowers and impact on quantity of crop production by different pollination treatment. *J. Apic. Res.* 32 (2): 77-88.

Dutta, N. K., Alam, S. N., Mahmudunnabi, M., Khatun, M. F. and Kwon, Y. J. 2016. Efficacy of some new genearation insecticides and a botanical

against mustard aphid and their toxicity to Coccinellid predators and foraging honey bees. *Bangladesh J. Agril. Res.* 41 (4): 725-734.

Elbert, A., Erdelen, C., Kuhnhold, J., Nauen, R., Schmidt, H. W. and Hattori, Y. 2000. Thiacloprid, a novel neonicotinoid insecticide for foliar application. In: Heberhadt, E. J. (ed.), *The BCPC Conference: Pests and diseases*. 13-16 November, 2000. Proceedings of an international conference, Brighton Hilton Metropole Hotel, Brighton, UK, pp.21-26.

EPA [Environmental Protection Agency]. 2014. *New Active Ingredient Review: Cyantraniliprole*. United States Environmental Protection Agency, 2 p.

Eswarappa, G. 2001. Pollination potentiality of different species of honey bees in increasing the productivity of chow-chow (*Sechium edule* (Jacq) S.W.). M.Sc. thesis, University of Agricultural Sciences, Bangalore.112 p.

Fairbrother, A., Purdy, J., Anderson, T. and Fell, R. 2014. Risks of neonicotinoid insecticides to honey bees. *Environ. Toxicol. Chem.* 33 (4): 719-731.

Fakuda, M. 1987. Utilization of honey bees for pollination of the melons in plastic green house in Kumamoto prefecture. *Honey bee Sci.* 4: 155-158.

Fischer, J., Muller, T., Spatz, A. K., Greggers, U., Grunewald, B. and Menzel, R. 2014. Neonicotinoids interfere with specific components of navigation in honey bees. PLoS ONE 9(3): e91364. https://doi.org/10.1371/journal.pone.0091364.

Fronk, W. D. and Slater, J. A. 1956. Insect fauna of cucurbit flowers. *J. Kans. Entomol. Soc.* 29 (4): 141-145.

Gallai, N., Salles, J. M., Settele, J., and Vaissiere, B. E. 2013. Economic valuation of the vulnerability of world agriculture confronted with pollinator decline. *Ecol. Econ.* 6 (8): 810– 821.

Ghule, T. M., Uikey, B. L., Barma, P. and Jha, S. 2014. Incidence studies on some important insect pests of cucumber (*Cucumis sativus* L.). *The Ecosan.* 8 (2): 177-180.

Girish, P. P. 1981. Role of bees and pollination of summer squash (*Cucurbita pepo* Linn.) with special reference to *Apis cerana* F. M.Sc. thesis, University of Agricultural Sciences, Bangalore. 97 p.

Gontijo, P. C., Moscardini, V. F., Michaud, J. P. and Carvalho, G. A.2014. Non-target effects of chlorantraniliprole and thiamethoxam on *Chrysoperla carnea* when employed as sunflower seed treatments. *J. Pest Sci.* 87(4): 711–719.

Gough, H. J., McIndoe, E. C. and Lewis, G. B. 1994. The use of dimethoate as a reference compound in laboratory acute toxicity tests on honey bees (*Apis mellifera* L.) 1981–1992. *J. Apic. Res.* 33 (2): 119-125.

Gradish, A. E., Scott-Dupree, C. D. and Cutler, G. C. 2012. Susceptibility of *Megachile rotundata* to insecticides used in wild blueberry production in Atlantic Canada. *J. Pest Sci.* 85: 133–140.

Gradish, A. E., Scott-Dupree, C. D., Frewin, A. J. and Cutler, G. C. 2012. Lethal and sublethal effects of some insecticides recommended for wild blueberry on the pollinator *Bombus impatiens*. *Can. Entomol.*144: 478–486.

Gradish, A. E., Scott-Dupree, C. D., Shipp, L., Harrisa, C. R. and Ferguson, G. 2010. Effect of reduced risk pesticides for use in greenhouse vegetable production on *Bombus impatiens* (Hymenoptera: Apidae). *Pest Manag. Sci.* 66: 142–146.

Grewal, G. S. and Sindhu, A. S. 1978. Insect pollinators of some cucurbits in Punjab. *Indian J. Agric. Sci.* 48: 79-83.

Gulati, R., Sharma, S. K. and Chhillar, B. S. 2015. Foraging behaviour and efficiency of Asian bees in cotton (*Gossypium hirusutum* L.) in India. *Indian honey bee sci.* 26(1): 25-28.

Haldhar, S. M., Bhargava, R., Choudhary, B. R., Pal, G. and Kumar, S. 2013. Allelochemical resistance traits of musk melon (*Cucumis melo)* against the fruit fly (*Bactrocera cucurbitae*) in a hot arid. *Phytoparasitica.* 41: 473–481.

Henry, M., Beguin, M., Requier, F., Rollin, O., Odoux, J. F., Aupinel, P., Aptel, J., Tchamitchian, S. and Decourtye, A. 2012. A common pesticide decreases foraging success and survival in honey bees. *Sci.* 336: 348–350.

Hodgeson, E. W., Pitts-Singer, T. L. and Barbour, J. D. 2011. Effects of the insect growth regulator, novaluron on immature alfalfa leaf cutting bees, *Megachile rotundata. J. Insect Sci.* 11(43): 1-10.

Holi, K. S. 1997. Foraging behaviour and palynology of Indian bee, *Apis cerana* Fab. under Dharwad conditions, M.Sc. (Ag.), thesis, University of Agricultural Sciences, Dharwad. 69p.

Horvat, A. 2001. Calypso: insecticide harmless to bees is opening new horizons in crop protection. In: Gorica, N. (ed.), *Proceedings: 5th Slovenian Conference on Plant protection.* 21-27 August, 2001. The Hotel Paka, Rudarskacesta, Slovenia. pp. 367-370.

IRAC [Insecticide Resistance Action Committee]. 2018. Mode of Action Classification of insecticides. Insecticide Resistance Action Committee. 4 p.

Iwasa, T., Motoyama, N., Ambrose, J. T. and Michael, R. R. 2004. Mechanism for the differential toxicity of neonicotinoid insecticides in the honey bee, *Apis mellifera. Crop Protec.* 23: 371–378.

Jangaiah, V. 2007. Insect community analysis in cucurbitaceous vegetable and impact of insecticides on insect pollinators. M.Sc. (Ag) thesis, Kerala Agricultural University, Thrissur, 112p.

Jaycox, E. R. 1964. Effects on the honey bees of nectar from systemic insecticide treated plants. *J. Econ. Entomol.* 57(1): 31-35.

Jeschke, P., Moriya, K. and Lantzsch, R. 2001. Thiacloprid (Bay YRC 2894) –A new member of the chloronicotinyl insecticide (CNI) family [J]. *Crop protec.* 54(2): 147–160.

Johansen, C. A. 1983. Pesticides and pollinators. *Ann. Rev. Entomol.* 22: 177-192.

Johnson, R. M., Ellis, M. D., Mullin, C. A., and Frazier, M. 2010. Pesticides and honey bee toxicity – USA. *Apidologie*. 41: 312–331.

Jyothi, J. V. 2003. Pollen foraging activity of *Apis cerana* Fab. and *Apis mellifera* under tropical conditions of Karnataka, India. *J. Environ. Ecol.* 21(2): 417-419.

Kalmesh, M. 2012. Floral resources of stingless bee (*Trigona laeviceps*) in Uttarakhand. *Indian bee J.* 9(2): 217-217.

KAU [Kerala Agricultural University]. 2016. Package of practices recommendations for crop plants. Kerala Agricultural University. pp. 182-183.

Kiill, L. H. P., Feitoza, E. A., Siqueira, K. M. M., Ribeiro, M. F. and Silva, E. M. F. 2016. Evaluation of floral characteristics of melon hybrids (*Cucumis melo* L.) in pollinator attractiveness. *Revista Brasileira de Fruticultura*. 38 (2): 530-531.

Klein, A. M., Vaissiere, B. E., Cane, J. H., Dewenter, I. S., Cunningham, S. A., Kremen, C. and Tscharntke, T. 2007. Importance of pollinators in changing landscapes for world crops. *Proc. R. Soc. B*. 274: 303–313.

Kumar, N. and Singh, 2005. Relative abundance of honey bees on toria bloom (Brassica campestris var. toria) Shashpa. 2(1): 26-30.

Laurino, D., Manino, A., Patetta, A. and Porporato, M. 2013. Toxicity of neonicotinoid insecticides on different honey bee genotypes. *Bull. Insectol.* 66 (1): 119-126.

Laurino, D., Porporato, M., Patetta, A. and Manino, A. 2011. Toxicity of neonicotinoid insecticides to honey bees: laboratory tests. *Bull. Insectol.* 64 (1): 107-113.

Lopez, O., Bolanosa, J. G. F. and Gil, M. V. 2005. New trends in pest control: the search for greener insecticides. *Green Chem.* 7: 431–442.

Mall, P. and Rathore, R. S. 2003. Impacts of some commonly used insecticides with different doses on foraging of *Apis mellifera. International workshop on conservation and management of bees for sustainable development and honey festival (Apiexpo-2003).*13-18 October, Banglore, India. Abstract: 82p.

Malone, L. A., Scott-Dupree, C. D., Todd, J. H. and Ramankutty, P. 2007. No sub-lethal toxicity to bumble bees, *Bombus terrestris*, exposed to Bt-corn pollen, captan and novaluron, *N. Z. J. Crop and Hortic. Sci.* 35(4): 435-439.

Manghanvi, K., Khan, M. S. and Srivastava, P. 2012. Foraging activity of stingless bee (*Trigona laeviceps*). *Res. J. Agric. Sci.* 3(1): 168-171.

Matsumoto, T. 2013. Reduction in homing flights in the honey bee *Apis mellifera* after a sublethal dose of neonicotinoid insecticides. *Bull. Insectol.* 66 (1): 1-9.

May, E., Wilson, J. and Isaacs, R. 2015. Minimizing pesticide risk to bees in fruit crops. *Ext. Bull.* 55: 32-45.

Meng, Y., Luo, S., Liu, J. and Jie, W. 2017. *Apis cerana* is less sensitive to most neonicotinoids, despite of their smaller body mass. *J. Econ. Entomol.*111 (1): 39–42.

Moreira, D. R., Gigliolli, A. P. S., Falco, J. R. P., Julio, A. H. F., Volnistem, E. A., Chagas, F., Toledo, V. A. A. and Takasusuki, M. C. C. R. 2018. Toxicity and effects of the neonicotinoid thiamethoxam on *Scaptotrigona bipunctata lepeletier* (Hymenoptera: Apidae). *Environ. Toxicol.* 00: 1–13 (in press).

Motzky, I., Tscharntke, T., Wanger, T. C. and Klein, A. M. 2015. Pollination mitigates cucumber yield gaps more than pesticide and fertilizer use in tropical smallholder gardens. *J. Appl. Ecol.* 52: 261-269.

Mupade, R. V. and Kulkarni, S. N. 2010. Foraging behaviour of *Apis* spp. and *Trigona iridipennis* on onion. *Indian J. Entomol.* 2010. 72 (4): 307-309.

Nadaf, A. M., Udikeri, S. S. and Sattigi, H. N., 2006, Safety of novaluron to honey bees *Apis cerena indica* Fab. *Pestol.* 30 (4): 12-14.

Neupane, K. R., Dhakal, D. D., Thapa, R. B. and Gautam, D. M. 2006. Foraging preference of giant honey bee, *Apis dorsata* F. to selected horticultural crops. *J. Inst. Agric. Anim. Sci.* 27: 87-92.

Nieuwstadt' M. G. L. and Iraheta, C. E. R. 1996. Relation between size and foraging range in stingless bees (Apidae, Meliponinae). *Apidologie.* 27 (4): 219-228.

Nogueira-Cautao, R. H. and Calmona, R. C. 1993. Insect pollination of cucumber (*Cucumis sativus* var. Aodai Melhorada). *Naturalia.*18: 77-82.

NRAVC [National Registration Authority for Agricultural and Veterinary Chemicals. 2009. Public Release Summary on Evaluation of the new active Flubendiamide in the product/s belt 480 SC insecticide & belt 240 WG insecticide. National Registration Authority for Agricultural and Veterinary Chemicals. pp. 1443-1335.

O'Neill, B. F., Dinter, A. and Samel, A. 2014. Cyantraniliprole (Cyazypyr™) – A testing program for honey bees. [online]. Available: https:// www.researchgate.net/ publication/299256794 Cyantraniliprole Cyazypyr - A testing program for honey bees [08 July 18]

Pastagia, J. J. and Patel, M. B. 2007. Relative contact toxicity of some insecticides to worker bees of *Apis cerana* F. *J. Plant Protec. Environ.* 4 (2): 89.

Pateel, M. C. and Sattagi, H. N. 2010. Effect of different attractants on attracting the bees to cucumber (*Cucumis sativus* L.) crop. *Karnataka J. Agric. Sci.* 20 (4): 761-763.

Perveen, N., Alhariri, M., Ahmad, M. and Suhail, A. 2000. Insecticidal mortality, foraging behavior and pollination role of honey bee *(Apis mellifera* L.) on Sarson (*Brassica campestris* L.) Crop. *Int. J. Agric. Biol.* 2 (4): 332 - 333.

Peter, C. A. and David, B. V. 2008. Population dynamics of the pumpkin caterpillar, *Diaphania indica* (Saunders) (Lepidoptera: Pyralidae). *Trop. Pest Manag.* 37 (1): 75-79.

Pilling, E., Campbell, P., Coulson, M., Ruddle, N. and Tornier, I. 2013. A four-year field program investigating long-term effects of repeated exposure of honey bee colonies to flowering crops treated with thiamethoxam. PLoS ONE 8(10): e77193. doi:10.1371/journal.pone.0077193.

Pitts-Singer, T. L. and Barbour, J. D. 2016. Effects of residual novaluron on reproduction in alfalfa leaf cutting bees, *Megachile rotundata* F. (Megachilidae). *Pest Manag. Sci.* 73: 153–159.

Prakash, K. B. 2002. Pollination potentiality of Indian honey bee *viz.*, *Apis cerana* on the production of cucumber (*Cucumis sativus* (Linn.) Cucurbitaceae). M.Sc. thesis, University of Agricultural Sciences, Bangalore. 88p.

Prasad, R. and Chand, S. 2003. Foraging behaviour of honey bees and other insects on onion (*Allium cepa* L.). *International workshop on conservation and management of bees for sustainable development and honey festival (Apiexpo-2003)*, 13-18 October, 2003, Banglore, 72 p. Abstract No. 5.6.2.

Rabia, K., Dethe, M. D. and Sharma, D. 2005. Relative toxicity of some insecticides to *Apis cerana indica*. *Indian bee J.* 67 (2): 52-56.

Rana, B. S. and Goyal, N. P. 1996. Field toxicity of methyl demeton and dimethoate to foragers of honey bee (*Apis cerana indica* F.) visitors to *Brassica chinensis* L. *Indian bee J.* 53 (1-4): 73-77.

Rao, M. G. and Suryanarayana, M. C. 1988. Studies on pollination of watermelon (*Citrulus lanatus* (Thumb.) Mansf.). *Indian Bee J.* 50 (1): 5-8.

Ratnakar, V., 2015, Safety evaluation of certain insecticides to European honey bee, *Apis mellifera* Linnaeus. M.Sc. thesis, Professor Jayashankar Telangana State Agricultural University, Hyderabad. 112p.

Rosa, A. D., Teixeira, J. S., Vollet-neto, A., Queiroz, E. P., Blochtein, B., Pires, C. S. S. and Imperatriz-Fonseca, V. L.2016. Consumption of the neonicotinoid, thiamethoxam during the larval stage affects the survival and development of the stingless bee, *Scaptotrigona* aff. *Depilis*. *Apidologie* 47: 729–738.

Roy, D. C. and Pande, Y. D. 1991. Seasonal incidence, host preference and feeding rate of red-pumpkin beetle (*Raphidopalpa foveicollis*) in Tripura. *Indian J. Agric. Sci.* 61 (8): 603-607.

Saeed, R., Razaq, M. and Hardy, I. C. W. 2015. The importance of alternative host plants as reservoirs of the cotton leaf hopper, *Amrasca devastans*, and its natural enemies. *J. Pest Manag. Sci.* 88: 517-531.

Sattigi, S. M., Lingappa, S. and Kulkarni, K. A. 1996. Seasonal variation of foraging behaviour of Indian bee (*Apis cerana indica* Fab.). *J. Karnataka Agrl. Sci.* 1: 43-48.

Scott-Dupree, C. D., Conroy, L., and Harris, C. R. 2009. Impact of currently used or potentially useful insecticides for Canola agroecosystems on *Bombus impatiens* (Hymenoptera: Apidae), *Megachile rotundata* (Hymentoptera: Megachilidae), and *Osmia lignaria* (Hymenoptera: Megachilidae). *J. Econ. Entomol.* 102 (1): 177-182.

Sharma, S. K., Singh, J. R. and Kaushik, H. D. 2001. Efficiency of two wild honey bees (Apis spp.) as pollinators of onion (*Allium cepa* L.). *Haryana J. Hortic. Sci.* 30 (2): 134-135.

Shi, T. F., Yu-Fei, W., Liu, F., Qi, L. and Lin-Sheng, Y. 2017. Sublethal effects of the neonicotinoid insecticide thiamethoxam on the transcriptome of the honey bees (Hymenoptera: Apidae). *J. Econ. Entomol.* 20 (10): 1–7.

Singh, J., Mishra, R. C. and Agarwal, O. P. 2006. Foraging rates of different *Apis* species visiting parental lines of *Brassica napus* L. *Zoos' print J.* 21 (4): 2226-2227.

Skerl, M. I. S., Bolta, S. P. V., Cesnik, H. B. and Gregorc, A. 2009. Residues of pesticides in honey bee (*Apis mellifera carnica*) bee bread and in pollen loads from treated apple orchards. *Bull. Environ. Contamin. Toxicol.* 83: 374–377.

Soni, J., Thakur, R. K., Chauhan, A. and Sharma, M. 2010. Foraging activity of insect pollinators on pepino (*Solanum muricatum* Aiton). *Pest Manag. Econ. Zool.* 18 (1/2): 266-270.

Srikanth, D. 2015. Insect pollinators diversity with special reference to role of attractants in insect pollination for increasing the productivity of Bottle gourd (*Lagenaria siceraria* L.). M.Sc. thesis, University of Agricultural Sciences, Banglore. 115 p.

Srivastava, U., 1991, Insect pollination in some cucurbits In: Heemert, K., Ruijter, A. and Holm, S. N. (eds.), *Acta Hortculturae 288*. Proceedings of the Sixth International Symposium on Pollination, 27 August 1990, Tilburg, Netherlands. pp. 445-451.

Stanley, D. A., Smith, K. E. and Raine, N. E. 2015. Bumble bee learning and memory is impaired by chronic exposure to a neonicotinoid pesticide. *Sci. Rep.* Series 5:16508. DOI: 10.1038/srep16508.

Stanley, J., Chandrasekaran, S., Preetha, G. and Kuttalam, S. 2010. Toxicity of diafenthiuron to honey bees in laboratory, semi-field and field conditions. *Pest Manag. Sci.* 66: 505–510.

Stanley, J., Preetha, G., Chandrasekaran, S. and Kuttalam, S. 2009. Honey bees of the cardamom ecosystem and the selective toxicity of diafenthiuron to four different bee species in the laboratory, *J. Apicultural Res.* 48 (2): 91-98.

Stanley, J., Sah, K., Jain, S. K., Bhatt, J. C. and Sushil, S. N. 2015. Evaluation of pesticide toxicity at their field recommended doses to honey bees, *Apis cerana* and *A. mellifera* through laboratory, semi-field and field studies. *Chemosphere.* 119: 668–674.

Stoner, K. A. and Eitzer, B. D. 2012. Movement of soil-applied imidacloprid and thiamethoxam into nectar and pollen of squash (*Cucurbita pepo*). PLoS ONE 7(6): e39114. doi:10.1371/journal.pone.0039114.

Thapa, R. B. 2006. Honey bees and other insect pollinators of cultivated plants: A review. *J. Inst. Agric. Anim. Sci.* 27: 1-23.

Thompson, H. 2016. Extrapolation of acute toxicity across bee species. *Integrated Environ. Assess. Manag.* 12: 622–626.

Tome, H. V. V., Barbosa, W. F., Correa, A. S., Gontijo, L. M., Martins, G. F. and Guedes, R. N. C. 2015. Reduced-risk insecticides in neotropical stingless bee species: impact on survival and activity. *Ann. Appl. Biol.* 167: 186–196.

Tosi, S., Burgio, G., Nieh, J. C., 2017. A common neonicotinoid pesticide, thiamethoxam, impairs honey bee flight ability. *Sci. Rep.* 7: 1201. https://doi.org/10.1038/s41598-017- 01361-8.

Viraktamath, S. 1990. Foraging profile of Indian honey bee, *Apis cerana* in Raichur, Karnataka state, India. In: Proc. of social insects and environment.- Veeresh, G. K., Mallik, B. and Viraktamath C. A. (Eds.) Oxfordand IBH publishing Co. New Delhi. 572 p.

Waller, G. D., Barker, R. J. and Martin, J. H. 1979. Effects of dimethoate on honey bee foraging. *Chemosphere.* 7: 461 - 463.

Waller, G. D., Erickson, B. J., Harvey, J. and Martin, J. H. 1984. Effects of dimethoate on honey bees (Hymenoptera: Apidae) when applied to flowering lemons. *J. Econ. Entomol.* 77 (1): 70-74.

Wedberg, J. L. and Erickson, E. H. 1986. Protecting honey bees in Wisconsin from pesticides and other toxic chemicals. *Nature* 7: 22-26.

Yamada, T., Yamada, Y., Yamada, K. and Nakamura, H. 2015. Long-term field experiment on the impacts of the neonicotinoid dinotefuran and the organophosphate fenitrothion on a honey bee colony. *J. Apic. Res.* (In press).

Yasuda, M., Sakamoto, Y., Goka, K., Nagamitsu, T. and Taki, H. 2017. Insecticide susceptibility in Asian honey bees (*Apis cerana* (Hymenoptera:

Apidae) and implications for wild honey bees in Asia. *J. Econ. Entomol.* 110(2): 447–452.

Yu, C. Z., Adamczyk, J., Rinderer, T., Yao, J., Luttrell, R. D. R. and Gore, J. 2015. Spray toxicity and risk potential of 42 commonly used formulations of row crop pesticides to adult honey bees (Hymenoptera: Apidae). *J. Econ. Entomol.* 93: 1–8.

FIELD TOXICITY OF NEW GENERATION INSECTICIDES TO BEE POLLINATORS

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ABSTRACT

The study entitled "Field toxicity of new generation insecticides to bee pollinators" was carried out at AICRP on Honey Bees and Pollinators, Department of Agricultural Entomology, College of Agriculture, Vellayani, during 2016-18 with the objective to evaluate the field toxicity of new generation insecticides to major pollinators namely, *A. cerana indica* and *T. iridipennis* and to assess the insecticide residues in cucumber flowers.

The toxicity of new generation insecticides (at field concentration and half of the field concentration) recommended for the pest management in vegetable crops *viz.*, thiamethoxam 25 WG, dinotefuran 20 SG, cyantraniliprole 20 SC, novaluron 10 EC, diafenthiuron 50 WP, their combinations *viz.*, thiamethoxam (17.50 %) + chlorantraniliprole (8.8 %) 300 SC, flubendiamide (19.92 %) + thiacloprid (19.92 %) 480 SC along with a conventional insecticide (dimethoate 30 EC) to the major pollinators were evaluated in terms of bee mortality in the laboratory. From the laboratory study, two new generation insecticides with least mortality were evaluated under field conditions along with an insecticidal check (dimethoate 30 EC @ 0.5 mL L^{-1}) and an untreated control in order to study their effect on foraging activity of major pollinators of the crop. The persistence of the insecticides was studied by assessing the residues in the flowers.

Laboratory evaluation of the insecticides revealed that upto three HAT, no mortality was recorded in Indian bees, *A. cerana indica* and stingless bees, *T. iridipennis* when treated with cyantraniliprole 20 SC (1.2 mL L^{-1} and 0.6 mL L^{-1}) and novaluron 10 EC (2.0 mL L^{-1} and 1.0 mL L^{-1}). Mortality of Indian bees ranged from 0 to 15 per cent and that of stingless bees from 0 to 2.50 per cent at six HAT, when treated with cyantraniliprole and novaluron at their respective concentrations. At 12 HAT the mortality of Indian bees ranged from 15.56 to 44.72 per cent while that of the stingless bees ranged from 19.44 to 30.56 per cent. Thus, two insecticides, cyantraniliprole 20 SC @ 1.2 mL L^{-1} and

novaluron 10 EC @ 2.0 mL L^{-1} which recorded the lowest mortality in the laboratory evaluation were selected for field evaluation.

Observations on the pollinator diversity in *C. melo* L. flowers, prior to the insecticide treatment, revealed that majority of the pollinators belong to order Hymenoptera (63.54 %) followed by Coleoptera (27.26 %) and Lepidoptera (9.01 %). Among the hymenopterans, *T. iridipennis* (9.33 m^{-2} 5 min^{-1}) and *A. cerana indica* (7.67 m^{-2} 5 min^{-1}) were the dominant pollinators with their peak time of activity being 0800 h to 0900 h and 1000 h to 1100 h respectively. For the sufficient pollinator population, one hive of each bee species was installed near the experimental plot which resulted in the percentage increase of field population of Indian bees and stingless bees by 26.07 and 50.05 per cent.

Field evaluation of insecticide toxicity was assessed in terms of bee foraging behaviour *viz.*, relative abundance, foraging rate (no. of bees visited m^{-2} $min.^{-1}$), foraging speed (no. of flowers visited in a single plot min^{-1}) and the time spent by the bees on male and female flowers separately (in sec.). Significant variation in relative abundance of pollinators among the treatments was observed upto five DAS in the case of Indian bees and seven DAS in stingless bees. Lowest relative abundance was recorded for stingless bees from plots treated with dimethoate 30 EC @ 0.5 mL L^{-1} which ranged from 12.33 to 23.87 per cent. The foraging rate varied significantly among the treatments upto five DAS in both the bees. The stingless bees showed significant variation in the time spent on flowers upto seven DAS, while Indian bees had significant variation only upto three DAS. Though the foraging speed of Indian bees showed significant variation among the treatments upto three DAS, dimethoate 30 EC @ 0.5 mL L^{-1} recorded lowest foraging rate when compared to other treatments. In the case of stingless bees, significant variation in foraging speed among the treatments was observed upto one DAS with least foraging speed from the treated check.

Observations on the number of returning foragers in the hive before and after application of insecticides revealed significant reduction of foragers upto

five DAS in stingless bees and three DAS in Indian bees. Thus, the foraging activities of stingless bees were found to be more affected by the insecticide application than that of Indian bees.

The study could establish that the new generation insecticides, cyantraniliprole 20 SC @ 1.2 mL $L^{-1}$and novaluron 10 EC @ 2.0 mL L^{-1} which recorded the lowest mortality in the laboratory are safe to the pollinators in terms of their foraging behaviour when compared to the dimethoate 30 EC @ 0.5 mL L^{-1}. Considering the safety of new generation insecticides to the dominant pollinators, they can be used for effective pest management in cucurbits, though the residues of novaluron 10 EC was detected upto 10 DAS when compared to dimethoate 30 EC (upto three DAS) on flowers of culinary melon.