TOXICITY OF INSECTICIDES TO *Cheilomenes sexmaculata* FABRICIUS (COLEOPTERA: COCCINELLIDAE)

by

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(2018-11-056)

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KERALA, INDIA

2021

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THESIS

Submitted in partial fulfillment of the requirement for the degree of

Master of Science in Agriculture

Faculty of Agriculture

Kerala Agricultural University

DEPARTMENT OF AGRICULTURAL ENTOMOLOGY

COLLEGE OF AGRICULTURE,

VELLANIKKARA, THRISSUR – 680656

KERALA, INDIA

2021

DECLARATION

I hereby declare that the thesis entitled **"Toxicity of insecticides to *Cheilomenes sexmaculata* Fabricius (Coleoptera: Coccinellidae)"** is a bona fide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award of any degree, diploma, fellowship or other similar title, of any other University or Society.

Place: Vellanikkara
Date: 27/04/2021

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CERTIFICATE

Certified that the thesis entitled **"Toxicity of insecticides to *Cheilomenes sexmaculata* Fabricius (Coleoptera: Coccinellidae)"** is a bona fide record of research work done independently by **Mr. Pavithrakumar K. (2018-11-056)** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to him.

Place: Vellanikkara
Date : 27/04/2021

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We, the undersigned members of the advisory committee of **Mr. Pavitharkumar K. (2018-11-056)**, a candidate for the degree of **Master of Science in Agriculture** with major field in **Agricultural Entomology**, agree that this thesis entitled **"Toxicity of insecticides to *Cheilomenes sexmaculata* Fabricius (Coleoptera: Coccinellidae)"** may be submitted by **Mr. Pavitharkumar K.** in partial fulfilment of the requirement for the degree.

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ACKNOWLEDGEMENT

This thesis concludes my pursuit of an M.Sc. in Agricultural Entomology, letting me to fulfill my passion for the field. I convey my sincere gratitude towards all the people who helped, led and encouraged me to complete this journey successfully.

With great respect and pleasure, I would like to express the deepest gratitude to my major advisor ***Dr. Smitha M. S,*** *Assistant Professor, AICRP on Cashew, Cashew Research Station, Madakkathara, for her invaluable support and guidance throughout this venture. I feel fortunate to have had her as my major advisor. I am highly indebted for her patience and co-operation over these years for the completion of this work.*

I also would like to express my gratitude to my advisory committee members am extremely thankful to ***Dr. Mani Chellappan,*** *Professor and Head, Department of Agricultural Entomology,* ***Dr. Madhu Subramanian,*** *professor and Head, AICRP on BCCP, Vellanikkara and Director of Research, KAU and* ***Dr. Anita. S*** *Professor (Agronomy), Instructional Farm, Vellanikkara, KAU, Thrissur for regular support, valuable suggestions and immense help rendered during the study. I would also like to express my special thanks to* ***Dr. Haseena Bhaskar****,* ***Dr. Berin Pathrose*** *and* ***Dr. Prameela*** *for letting me utilize their facilities.*

*Whole hearted thanks to my teachers****, Dr. Deepthy K. B., Dr. Ranjith, M. T., Smitha Revi and Dr. Vidya C. V.*** *for their expert teaching, moral support and co-operation throughout the period of study. I also thank* ***Dr. Dicto Jose*** *for his motivation and care especially during the last semester.*

My special thanks to the Department of Agricultural Entomology and staffs of the department, especially ***Akhila chechi, Akhil, Ancy chechi, Aneesha chechi, Rema chechi, Shahanzitha, Sheela chechi, Soorya chechi and Vipin chettan*** *for their affection and encouragement.*

I also use this opportunity to thank my batchmates, ***Abinsha Ashraf, Beegam Salma M. P., Laya, A. C., Sachin G. Pai*** *and* ***G. Sravanthi*** *whose helping hands, love and affection will never fade from my heart. I take this opportunity to extend my gratitude to my seniors* ***Anna chechi, Anju chechi Anu chechi, Anusree chechis, Athira chechi, Jancy chechi, Nimisha chechis, Priyatha chechi, Sharanu chettan Vineetha chechi and Vishak etten*** *and juniors* ***Abishek, Rahul, Shika, Sreelakshmi and Sreesha*** *for their support in different stages of the study.*

I wish to extend my thanks to my friends ***Akhil KP, Aswathy, Bony, Jyothish Babu, Mounisha, Nidhinraj, Nivethitha, Mahesh, Pooja, Ribin Baby, Sivadharshanapriya, Sharat Prabhakaran, Suhas, Sunny Babu, Thomson, and Vinu*** *for their support and kindness which rendered mental strength to me during the research.*

I also wish to express my special thanks to ***Abin dhivakaran, Arun, Aswin, Gokul Vigneshwaran, Madala chettan, Mathan, Nibin, Nithinetten, Pravin, Rajakumar sir, Saleesh, Sanjay, Saravanakumar sir, Swathi, Syametten and Themuhi*** *for their companionship and invaluable help throughout these years.*

Words would not suffice to express my love, gratitude and indebtedness to my beloved father **Kumarasamy**, *my mother* **Muthulakshmi** *and my brother* ***Ponananth*** *for their love, sacrifices and prayers which helped me to move forward with a smile through all the hardships.*

I apologize to those whom I forgot to mention here. Once again, I would like to thank all those who have helped me in completing this thesis and I wish them all the best in their deeds and endeavours.

Pavithrakumar K.

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Introduction

1. INTRODUCTION

Integrated pest management (IPM) combines cultural, biological and chemical control options to deliver effective and targeted pest management solutions that can be customized to specific climate and habitat. The key postulate for the use of pesticides in an IPM framework is to use those products with verified selectiveness (Garzon *et al.*, 2015). It stresses on the judicious use of a few pesticides and those molecules that are cooperative with the natural enemies. Combined use of chemical pesticides and biocontrol agents can be synergistic, resulting in enhanced pest control (Tengfei *et al*., 2019).

Biocontrol offers the most sustainable and cost effective way of pest management. Under high herbivore pressure, biocontrol agents alone can't provide profitable control as expected and they may need to be backed up with supplemental insecticidal spray. Till the concept of integrated pest management (IPM) was introduced, biological and chemical pest control measures were considered incompatible. The harmonious use of insecticides and biocontrol agents has been formulated and experienced from the past but still stands as a controversial and complex issue.

Insecticidal molecules have been designed to cause effective mortality in target pests, but at the same time adversely harm non target organisms including natural enemies. Apart from the mortality induced, pesticides can also influence the physiology and behavior of natural enemies leading to reduction in growth, survival and reproduction (Muller, 2018; Teder and Knap, 2019). Also, there are reports of repellency and sterility (Croft, 1990). This could result in poor natural regulation and resultant outbreak of insect pests (Shinde and Radadia, 2018). The adverse effects of insecticides can be minimized through logical selection of pesticides that are less disruptive to natural enemies.

Coccinellids, generally known as ladybird beetles, are the most common natural enemies found in agricultural lands contributing to natural regulation of major agricultural pests. The six spotted zigzag ladybird beetle, *Cheilomenes sexmaculata* is an important aphidophagous predator in vegetable ecosystem. Its voracious feeding habit and density responsiveness makes it a very efficient natural enemy of several aphid species in a variety of crops. Apart from aphids, it also feeds on coccids, diaspidids and aleyrodids (Agarwala and Yasuda, 2000).

The key features of *C. sexmaculata* include its wide host range, geographic distribution, broad habitats and intense searching and feeding ability (Venkatesan *et al.*, 2006). With its preferred host *Aphis craccivora*, it is found all round the year in cowpea and lablab (Joshi *et al*., 1997). Aphid control provided by *C. sexmaculata* is analogous to that of insecticides such as malathion and diazinon (Bari and Sardar, 1998).

The accepted potential of *C. sexmaculata* for natural biocontrol either alone or as a component of IPM, is often undermined by indiscriminate use of insecticides. Often investigations on natural enemies only evaluating the short term effects (lethal effects) of insecticides, ignoring the long term sublethal effects on physiology and behaviour. Selection of insecticides safe to predators such as *C. sexmaculata* require knowledge about the effect of these pesticides on life cycle parameters of the predator.

The present research project has been formulated with a goal to identify safer insecticides to *C. sexmaculata* among those presently recommended in cowpea with the objectives

1. To evaluate the toxicity of insecticides to *C. sexmaculata* in the laboratory
2. To assess the impact of insecticides on field efficacy of *C. sexmaculata* in cowpea

Review of Literature

2. REVIEW OF LITERATURE

Arthropod natural enemies are important in crop production owing to their ability to control phytophagous insects. Unscientific selection and application of pesticides adversely affects the efficiency of these natural enemies. Exposure to a particular product may trigger adverse effects not necessarily resulting in the death but with sublethal effects comprising changes in development, longevity and fecundity, as well as behaviours involved in mobility and foraging. Research is being carried out every so often to evaluate insecticides as old ones vanish and new ones enter the market, to identify those with low toxicity to natural enemies.

The coccinellid predator, *Cheilomenes sexmaculata* Fabricius (Coleoptera: Coccinellidae) is an important natural enemy especially in vegetable ecosystems and is an efficient aphidophagous predator in cowpea ecosystem. The literature pertaining to the study on the toxicity of insecticides on *C. sexmaculata* and other coccinellid predators were reviewed and those after 1970s are presented here under.

2.1. Lethal effects of insecticides on coccinellid predators in laboratory

Gupta *et al*. (1971) reported that methyl demeton (0.025%) and dimethoate (0.03%) were moderately toxic and malathion was extremely toxic to adults of *Coccinella septempunctata* Linnaeus. According to Satyanarayana and Murthy (1991), monocrotophos and quinalphos were highly toxic to third and fourth instar larvae of *C. septempunctata* causing 100 and 90 per cent mortality respectively.

Smith and Krischik (2000) opined that insecticidal soap, Botaini Gard, a commercial formulation of *Beauveria bassiana* and Azatin, a neem tree extract with azadirachtin, caused only less than five per cent mortality in four predatory coccinellids, *Hippodamia convergens* Guerin-Meneville, *Coleomegilla maculata* De Geer, *Harmonia axyridis* Pallas and *Cryptolaemus montrouzieri* Mulsant, as compared to the conventional insecticide carbaryl that caused 100 per cent mortality.

Acetamiprid (40 mg a.i./L) caused 100 per cent mortality of eggs, all larval instars and pupae of *H. axyridis* whereas imidacloprid (50 mg a.i./L) induced 100 per cent mortality of only first and second instar larvae. Abamectin at 18.4 mg a.i/L was toxic to all the stages of the predator. Thiamethoxam (50 mg a.i./L), which caused only 50 per cent mortality in first instar larvae was reported as the safest among the four insecticides tested (Youn *et al.*, 2003).

Lucas *et al.* (2004) observed 100 per cent mortality of the third instar larvae of *C. maculata*, a predator of the Colorado potato beetle, *Leptinotarsa decemlineata* Say, upon topical application with imidacloprid (0.0006%). Bozsik (2006) evaluated the lethal effects of insecticides with different modes of action and reported that *C. septempunctata* adults were more susceptible to deltamethrin+heptenphos (127.5mg a.i./L) and lambda-cyhalothrin (12.5mg a.i./L) with mortality more than 80 per cent whereas imidacloprid proved safe with only 30 per cent mortality.

According to Mali *et al.* (2008) even though there was 90 per cent emergence of *Scymnus coccivora* Ayyar adults from imidacloprid (0.045%) treated pupae, only 3.30 per cent of them were found to have survived at 24h after emergence. Jalali *et al.* (2009) reported that the residual exposure of imidacloprid (50mg a.i./L) was highly toxic to the fourth instar larvae of *Adalia bipunctata* Linnaeus while spinosad, even at the highest concentration (2000 mg a.i./L), had no toxic effects.

Ahmad *et al.* (2011) evaluated some commonly used insecticides *viz.*, imidacloprid, acetamiprid, cypermethrin, deltamethrin and profenofos for their residual effects on the adults of the predator *Coccinella undecimpunctata* Linnaeus. According to them, profenofos (8ml/L) was the most toxic insecticide with 87 per cent mortality followed by acetamiprid (1.25ml/L) and cypermethrin (3.3ml/L) with 63 per cent kill whereas imidacloprid (1ml/L) caused the lowest mortality of 53 per cent at 24h after exposure. After 48h, highest mortality (97%) was observed with profenofos and cypermethrin.

Devee *et al.* (2011) calculated the LC_{50} values of deltamethrin, imidacloprid, lambda-cyhalothrin, bifenthrin, and dimethoate for *C. septempunctata* and classified them based on the increasing order of toxicity as bifenthrin < imidacloprid < lambda-cyhalothrin < dimethoate < deltamethrin.

Alexander *et al.* (2013) studied the lethal effect of eight different insecticides to the non-target beneficial insects of papaya mealybug *viz.*, *S. coccivora* and *C. montrouzieri* in the laboratory. Based on LC_{50} values, thiamethoxam showed highest toxicity to adults of *S. coccivora* and grubs of *C. montrouzieri* while imidacloprid was more toxic to adults of *C. montrouzieri*. Based on selective toxicity ratio and sequential testing scheme, all the tested insecticides *viz.*, dimethoate, chlorpyriphos, carbofuran, profenophos, thiamethoxam, buprofezin, imidacloprid and spirotetramat were reported safe to *C. montrouzieri* and *S. coccivora*.

Evaluation of LD_{50} of imidacloprid (60 mg a.i./L), acetamiprid (50 mg a.i./L), bifenthrin (40 mg a.i./L) and deltamethrin (17.5 mg a.i./L) on the larvae of *C. septempunctata* by topical application method revealed the highly toxic nature of deltamethrin and bifenthrin to all the larval instars (Skouras *et al*., 2017).

Mughal *et al.* (2017) studied the toxicity of seven insecticides on the adults of *C. septempunctata* and classified them in the order of toxicity as imidacloprid< leufenoron < emamectin benzoate < acetamprid < spinosad < indoxacarb < chlorpyrifos. According to Barbosa *et al.* (2018) the coccinellid predator, *Tenuis valvaenotata* was killed after application of lambda-cyhalothrin (0.13 g a.i./L), methidathion (2.67 g a.i./L) and thiamethoxam (0.33 g a.i./L) along with nymphs of mealy bug, *Ferrisia dasylirii* Cockerell.

2.2. Lethal effects of insecticides on *Cheilomenes sexmaculata* in laboratory

Gupta and Kushwaha (1970) studied the toxicity of insecticides to *C. sexmaculata* and classified them in the decreasing order of toxicity as phosphamidon < parathion < lindane < thiometon.

Lingappa *et al.* (1978) observed endosulfan as highly toxic to *C. sexmaculata,* as it caused 100 per cent mortality of adults at 24h after exposure. However, according to Marker and Jadhav (1981), endosulfan (0.05%) was less toxic while carbaryl (0.1%) and quinalphos (0.05 %) were highly toxic causing more than 95 per cent mortality in adults and grubs of *C. sexmaculata.*

Rao *et al.* (1989) reported that endosulfan (500 ppm) and fenvalerate (100 ppm) were safer while phosphomidon and dimethoate, at 500 ppm each, were highly toxic to both grubs and adults of *C. sexmaculata.*

According to Thomas and Phakde (1991), chlorpyriphos was more toxic to early instar grubs and adult beetles of *C. sexmaculata* than quinalphos and oxydemeton methyl. Patil *et al.* (1992) in their bioassay, found that methyl demeton and endosulfan were relatively less toxic to adults and grubs of *C. sexmaculata.*

Swaran *et al.* (1995) also reported the safety of methyl demeton and endosulfan. According to them, synthetic pyretheroids were the most toxic group of insecticides to *C. sexmaculata*. According to Patil and Lingappa (1999), acephate was highly toxic to grubs and adults of *C. sexmaculata* and was followed by imidacloprid and carbosulfan while oxydemeton methyl was safe.

The safety of botanicals such as neem oil to *C. sexmaculata* have been documented by several authors. According to Markandeya and Divakar (1999), margosan, a neem formulation was comparably safe to *C. sexmaculata* larvae. Shankarganesh and Khan (2006) opined that neem seed kernel extract (NSKE), custard apple seed extract and pongamia seed extract at 5 per cent concentration were safe to *C. sexmaculata.*

Basappa (2007) recorded that carbosulfan (0.14%) was highly toxic to *C. sexmaculata* adults with 84.4 per cent mortality at 24h after treatment, and was trailed by acetamiprid (0.002%), profenofos (0.05%), monocrotophos (0.05%) and thiamethoxam (0.02%), while imidacloprid (0.01%) was less toxic at 24h after treatment with only 42.22 per cent mortality. However, 72h after treatment, all the insecticides caused 100 per cent mortality.Tank *et al.* (2007) documented the toxicity of ten insecticides to different stages of *C. sexmaculata*. According to them, dichlorovos (0.025%), cypermethrin (0.015%) and fenvalerate (0.05%) were highly toxic to egg, third instar grubs and adults whereas acetamiprid and endosulfan were least toxic.

According to Lalithambika (2012), carbaryl (0.1%) and quinalphos (0.05%) were highly toxic to adults of *C. sexmaculata* with 86.67 and 84.99 per cent mortality respectively. Acephate (0.075%) and imidacloprid (0.005%) were less toxic with 31.66 and 51.65 per cent mortality to adults and 16.67 and 35 per cent mortality to grubs.

Neetan and Aggarwal (2012) categorized buprofezin (0.05%) as the safest insecticide, followed by endosulfan, to all life stages of the predator. They also found that pupae of *C. sexmaculata* showed more resistance to insecticides than eggs.

According to Pandi *et al.* (2013) acetamiprid (0.02%) was highly toxic to third instar grubs of *C. sexmaculata*, followed by thiamethoxam (0.02%), imidacloprid (0.05%), buprofezin (0.7%) and neembaan.

Megha *et al*. (2015) observed that dimethoate (0.17%), nimbicidine (0.5%) and malathion (0.2%) were extremely toxic to adults of *C. sexmaculata* with 100 per cent mortality. According to Nazari *et al*. (2016), imidacloprid (0.014%), when exposed through feeding treated prey, caused 100 per cent mortality of *C. sexmaculata* adults, whereas pyriproxyfen had no lethal effect, even at twice the recommended field rate.

According to Khan *et al.* (2015), among the various insecticides tested at recommended field doses, cypermethrin (617.5ml/ha) showed high level of toxicity with 66.67, 83.33, 76.67 & 86 per cent mortality in eggs, larvae, pupae, and adults respectively. Emamectin benzoate (494ml/ha) was safe with significantly less toxicity followed by spinosad to all the stages of *C. sexmaculata*. They observed that neem oil (4940ml/ha) was highly toxic with 80 and 86.67 per cent mortality respectively in egg and pupae, the immobile stages.

Ujjan *et al.* (2017) observed that profenofos (0.5%) and emamectin+bifenthrin (0.5%) were highly toxic to eggs and adults of *C. sexmaculata* with100 per cent mortality but were comparatively less toxic (<55% mortality) to third and fourth instar larvae. Shinde and Radadia (2018) grouped profenofos (0.075%) and indoxacarb (0.01%) as extremely harmful to the grub stage and novaluron (0.0075%) and profenofos (0.075%) as extremely harmful to the adult stage. According to them, imidacloprid (0.005%) and thiamethoxam (0.005%) were comparatively safer to both stages. The extreme toxicity of indoxacarb (0.008%) to eggs (55.19% mortality), grubs (92.96%) and adults (83.33%) were reported by Sanghani *et al.* (2018). According to their study, buprofezin (0.05%) caused least mortality (<25%) to all stages of the predator.

2.3 Effect of insecticides on development and reproduction of coccinellid predators

Galvan *et al.* (2005) studied the effects of spinosad at 10 (0.011 kg a.i./ha), 25 (0.0275 kg a.i./ha), and 50 (0.055 kg a.i./ha) per cent field rate (FR) as well as indoxacarb at 10 (0.0062 kg a.i./ha) per cent FR on first instar grubs of *H. axyridis* and the effect on adult females by exposing them to spinosad at 50 and 100 per cent FR and indoxacarb at 50 per cent FR. The developmental period of the grub was significantly prolonged after exposure to spinosad at 25 and 50 per cent FR with 18.29 ± 0.36 days and 18.86 ± 0.12 days, respectively, followed by indoxacarb (17.96 ± 0.14 days) at 10 per cent FR but no significant difference with spinosad at 10 per cent FR (17.83 days) compared to control (17.14 days). Highest reduction in fecundity (639.27 ± 9.03 eggs) was with indoxacarb at 50 per cent FR followed by spinosad (849.20 ± 38.64 eggs and 889.16 ± 63.12 eggs) at 100 and 50 per cent FR respectively, compared to control with 864.69 ± 16.1 eggs. The study showed that indoxacarb and spinosad adversely affected the population dynamics of *H. axyridis* with prolonged developmental time and reduction in reproduction rate.

The sublethal effects of pyriproxifen and spirotetramat on larvae and adults of *C. montrouzieri* were evaluated by Planes *et al.* (2012) through topical application and ingestion of treated prey, *Planococcus citri*. When adults of *C. montrouzieri* were exposed to spirotetramat, fecundity (82.6 ± 10.6 eggs) and egg hatching (62.3 ± 8.2%) were not significantly reduced compared to control (82.6±10.6 eggs; 62.3±8.2% fertility). The fecundity (95.5±18.1 eggs) and fertility (79.6±4.8) were not affected significantly either when fed with spirotetramat treated prey, in contrast, increase in average fecundity was observed with pyriproxifen (183.5±18.3 eggs), though none of the eggs were fertile. The mortality of larvae (2.0±1.2%) and pupae (2.0±1.2%) were not affected when first instar larvae were topically applied with spirotetramat, as compared to increased larval (6.0±1.8 %) and pupal mortality (89.9±2.3%) with pyriproxifen. Similar scenario was reported in larval and pupal mortality after ingestion of spirotetramat (2.0±1.2; 17.0±4.8%) and pyriproxifen (78.0±0.4; 100.0±0.0%) treated prey.

According to Ibrahim and Kueh (2013), female *C. sexmaculata* could produce only an average of 2.55 female offsprings after sublethal exposure to imidacloprid while the untreated female was able to produce 17.59 female offsprings.

Garzon *et al.,* (2015) examined toxic effects of deltamethrin (12.45 mg a.i./L), sulfoxaflor (63.6 mg a.i./L), flonicamid (60 mg a.i./L), flubendiamide (60 mg a.i./L), metaflumizonen (240 mg a.i./L) and spirotetramat (75 mg a.i./L) on fourth instar larvae and adults of *A. bipunctata*. All the larvae exposed to deltamethrin were dead and hence, no pupae were formed. The pupation was reduced significantly (7.14%) and no adults emerged in sulfoxaflor treated larvae while 100 per cent adult emergence was observed in flonicamid and flubendiamide treated larvae. Significant reduction in pupation (92.86 and 97.62%) was observed with metaflumizone and spirotetramat exposed larvae. Similar trend was observed for adult bioassay with 100 per morality with deltamethrin while reproduction parameters were not affected in other insecticide treatments.

Xiao *et al.* (2016) recorded the impact of sublethal exposure of imidacloprid on F_0 and F_1 generations of *C. septempunctata.* In parental generation, a decline in both fecundity (52.81 and 56.09%) and adult longevity (23.97 and 28.68%) was observed at LC_5 and 10 per cent of LC_5 concentrations, respectively. The development time of immatures of subsequent generation was prolonged by 1.44 days and 0.66 days, and reproduction rate was lessened by 44.03 and 51.69 per cent during oviposition period of 10 and 13 days respectively.

Nawaz *et al.* (2017) studied effects of chlorantraniliprole on *H. axyridis* after exposed to LC_{10} (2.42 mg a.i./L) and LC_{30} (12.06 mg a.i./L) concentrations. The duration of second and fourth instar larvae was increased at LC_{10} (2.64 and 6.16 days) and LC_{30} (2.85 and 6.22 days), compared to control (2.33 and 5.97 days) while no significant difference was observed in duration of third instar larva. Pupal development also was prolonged after exposure to LC_{10} (6.04 days) and LC_{30} (5.98 days). Fecundity and net reproductive rate of adults were markedly reduced at LC_{10} (499.6 eggs; 174.83 offsprings/individual) and at LC_{30} (397 eggs; 99.2 offsprings/individual) compared to control (707.23 eggs; 304.01 offsprings/individual). According to them, chlorantraniliprole at sublethal concentrations impaired the growth and reproductive ability of *H. axyridis*.

Cabrera *et al.* (2018) compared toxic effects of imidacloprid and chlorantraniliprole on reproductive performance of two generalist coccinellid predators found in apple orchards, *C. maculata* and *H. axyridis*. Chlorantraniliprole was safe with no effect on reproduction in either of the predators. The fertility, fecundity and egg hatchability of *H. axyridis* were reduced by 78.7, 29 and 43 per cent, after exposure to imidacloprid, though there was no significant difference in *C. maculata*.

The impact of sublethal doses of buprofezin (0.125 g a.i./L), pymetrozine (0.2 g a.i./L) and pirimicarb (0.375 g a.i./L) on immature and adult stages of *C. undecimpunctata* were assessed by Cabral *et al.* (2008). Significant reduction in survival of predators to adult stage (<33%) were observed after the larvae was exposed to buprofezin, in contrast to other insecticides and control (>45% survival) while average fecundity, adult longevity and fertility were not affected significantly by insecticide treatments. According to them, pymetrozine and pirimicarb were safe to natural enemies and selective to whiteflies and aphids.

The sublethal exposure of various life stages of *Hippodamia variegata* Goeze to imidacloprid at two different concentrations of LC_{10} (3.92 mg a.i./L) and LC_{30} (8.69 mg a.i./L) were evaluated by Skouras *et al.* (2019). The duration of fourth instar larvae was shortened by 3.87 days and 3.32 days (3.50 days in control) while that of pupa was reduced by 3.61 and 3.75 days compared to control (3.50 and 4.03 days). The average fecundity was 387.9, 319.8 and 761.6 eggs at LC_{10}, LC_{30} and control respectively. The female longevity and mean generation time varied significantly at LC_{10} (48.44 and 30.82 days) and LC_{30} (42.36 and 33.81days) compared to control (55.88 and 32.75 days). The study emphasized the negative impact on the predator after extensive use of imidacloprid.

In a recent study, Rasheed *et al.* (2020) observed developmental and reproductive parameters of *H. axyridis* treated with chlorpyrifos at sublethal concentrations of LC_{10} (4.62 mg a.i./L) and LC_{30} (9.59 mg a.i./L). The duration of third instar larvae (2.13 days) was reduced and a significant prolongation of fourth instar larvae (6.44 days) and pupal duration (6.70 days) was recorded at LC_{10} in contrast to control with respective values of 2.68, 5.31 and 5.74 days. However, exposure to LC_{30} increased the developmental time of third and fourth instar larvae and pupae (2.95, 7.44 and 6.14 days) in comparison with control (2.68, 5.31 and 5.74 days respectively). The fecundity of the predator was 379.03, 229.06 and 694.84 eggs at L_{10}, LC_{30} and control respectively. The pre-oviposition period was increased significantly at LC_{10} (11.76 days) and LC_{30} (12.61 days) concentrations compared to control (9.76 days) whereas, female longevity was shortened at either of the concentrations (55.41 days; 47.26 days) compared to control (65.43 days). According to them, chlorpyrifos even at sublethal concentration adversely affected population buildup of predator.

2.4 Effect of insecticides on coccinellid predators under field conditions

According to Joshi and Sharma (1973) carbaryl was extremely toxic while endosulfan was least toxic to the adults of *C. sexmaculata.* The order of toxicity was recorded as carbaryl > dimethoate > dichlorvos >monocrotophos> endrin > malathion >endosulfan.

Effect of a few insecticides on *Coccinella transversalis* population in field was studied by Choudhary and Ghosh (1982). The study showed that endosulfan and methyl demeton were comparatively safer to the predator. According to Upadhyay and Vyas (1986), malathion was extremely toxic to coccinellid predators (*C. sexmaculata* and *C. septempunctata*) while thiometon at 0.03 and 0.05 per cent was least toxic when applied in groundnut crops against sucking pests.

Sharma *et al.* (1991) studied the toxicity of five insecticides applied against aphid, *A. craccivora* on coccinellid predators in three crops namely lentil, *Lathyrus* and chickpea and recorded endosulfan (0.07%) and cypermethrin (0.04%) as highly toxic in chickpea and lentil ecosystems respectively, whereas dimethoate (0.04%) was the most toxic insecticide in all the three ecosystems.

According to Ahmad and Sardar (1994), the synergistic action of malathion (0.001%) spray and predator *C. sexmaculata* was an assured method for control of *A. craccivora* in phaseolus bean as malathion had no effect on the predatory potential. Among various insecticides tested, Dhingra *et al.* (1995) reported that lindane, endosulfan and methyl demeton had high safety value while cypermethrin, decamethrin, phosphamidon, fenvalerate and malathion were nearly 10 times more toxic to the adults of *C. sexmaculata.*

Rathod and Bapodra (2002) observed that dimethoate (0.03%) and endosulfan (0.07%) were safer, followed by phosalone (0.07%), methyl-o-demeton (0.025%) and monocrotophos (0.04%). Phosphamidon (0.03%) and cypermethrin (0.04%) were comparatively more toxic to predatory coccinellids of cotton aphids.

Azadiractin (0.5%), malathion (0.05%), *Pongamia pinnata* pod extract (10%) and endosulfan (0.07%) sprays, against aphids in fenugreek ecosystem were found safer to *C. septempunctata* (Meena *et al.*, 2002).

Sunitha *et al.* (2004) observed that imidacloprid and dichlorvos were comparatively more toxic to *C. sexmaculata* than novaluron and spinosad when applied on okra against sucking pests.

Cypermethrin and malathion showed higher mortality than dimethoate and methyl demeton to *C. septempunctata* on rapeseed when applied against mustard aphids (Bandral, 2006). Ghosh *et al.* (2007) assessed the effect of six insecticides to the predator *C. sexmaculata* in brinjal ecosystem. Population count after insecticide application revealed that DDVP suppressed the predator population by 60.52 per cent followed by malathion (56.97%) and abamectin (46.16%).

Sakthivel and Qadri (2010) studied the effect of a few commonly applied insecticides and botanicals on the predatory coccinellid population in mulberry field. According to them, predatory beetles were reduced upto 88 per cent in dichlorvos, phosalone, dimethoate and metasystox treated plots while in those plots applied with pungam and neem oil, the population reduction was only about 35 per cent.

Mollah *et al*. (2013), conducted a study wherein they assessed the effect of a few insecticides on population buildup and mortality of the predacious coccinellids in bean ecosystem. They recorded neem oil and emamectin benzoate as less toxic compared to fenvalerate, cypermethrin and deltamethrin.

According to Rajesh *et al.* (2013), use of new generation insecticides such as imidacloprid and thiamethoxam were not at all a threat to predatory coccinellids in mustard ecosystem. Choudhary *et al.* (2016), opined that the natural enemy population in mustard plot was not affected after treating with flonicamid, thiamethoxam, dimethoate and imidacloprid for the management of aphid infestation. According to Kaushik *et al*. (2016), use of imidacloprid and thiamethoxam, either as seed treatment or foliar application is a phenomenal aphid management strategy and were safer to natural enemy populations in cow pea ecosystem.

Materials and Methods

3. **MATERIALS AND METHODS**

The study on the "Toxicity of insecticides to *Cheilomenes sexmaculata* Fabricius (Coleoptera: Coccinellidae)" was carried out at the Department of Agricultural Entomology, College of Horticulture, Vellanikkara, Thrissur, Kerala during 2019 to 2020. The facilities at Pesticide Residue Tracer Laboratory were utilized for this purpose. Lethal toxicity as well as effect of insecticides on reproduction of *C. sexmaculata* were studied. The insecticides were also evaluated under field. The materials used and methods followed in the laboratory and field experiments are presented in this chapter.

3.1 Mass culturing of host insect, *Aphis craccivora*

The stock culture of cowpea aphid, *Aphis craccivora* Koch was maintained on cowpea plants at the farm area of Pesticide Residue Tracer Laboratory. Cowpea plants were raised within cages of 2mx2mx1.5m size which accommodated approximately 100 plants and the cage was made of nylon mesh (120micron size) to avoid any natural enemies and to facilitate population build-up. Seeds were sown very closely to have a thick growth of cowpea seedlings enough to multiply aphids. The plants were infested at first trifoliate stage (Jaba *et al.*, 2010) with aphids collected from nearby cowpea fields. Three such caged plots were maintained to satisfy the requirement for conducting the experiments. The plants were irrigated and fertilized with urea at regular intervals to promote vegetative growth. Senesced plants were replaced with fresh sowings to ensure continuous establishment of culture. Aphids were collected from these plants for rearing the predator.

3.2. Mass culturing of *Cheilomenes sexmaculata*

Nucleus culture of the predator was developed from healthy grubs and pupae of *C. sexmaculata* collected from cowpea fields. The adults emerged were paired and were released for egg laying on potted cowpea plants with aphids, maintained in rearing cages at the rate of five pairs per cage of size 30cmX30cmX30cm. The eggs laid on cowpea leaves and glass wall were collected and kept in plastic containers for hatching. The freshly hatched grubs were released on caged cowpea plants infested with aphids and thus the stock culture was maintained in the laboratory.

3.3 Selection of insecticides

Commercial formulation of five insecticides including a botanical (Table. 1) that are recommended in cowpea, as per Package of Practices Recommendations (POP Crops KAU, 2016) against aphids and pod borers were selected for evaluation of toxicity to the predator, *C. sexmaculata* in the laboratory

3.4 Laboratory bioassay on survival

The selected insecticides were tested for their lethal effects on different life stages of *C. sexmaculata* as per the procedure described by Neetan and Aggarwal (2012).

3.4.1 Toxicity to eggs of *C. sexmaculata*

Eggs of 0-24h stage were collected from cowpea plants maintained in rearing cages. Cowpea leaf with a minimum of 10 intact eggs was dipped for 10 seconds in insecticide solutions prepared at field recommended doses. An untreated control was maintained by dipping the leaves with eggs in distilled water. The treated leaves were then placed on tissue paper to drain off excess insecticide solution and then placed in Petri dishes. Each treatment was replicated four times. In case any replication containing more than 10 egg, excess were picked out. The eggs were observed for hatching and survival of first instar grubs at 24h after hatching.

3.4.2 Toxicity to grubs of *C. sexmaculata*

0 to 24h old third instar grubs of *C. sexmaculata* were selected for conducting this experiment. Eggs from the rearing cages were individually placed in 40ml rearing containers. The hatched grubs were reared till third instar. Using wet camel hair brush, ten grubs were released into Petri dish containing cowpea leaf with aphids. The grubs were let to acclimatize for two hours. Insecticide solutions prepared at recommended doses were sprayed in the Petri dish using hand atomizer, with spray droplets enough to wet the grubs, aphids and cowpea leaf. An untreated control was maintained using a spray of distilled water. Each treatment was replicated four times with 10 grubs/ replication. The grubs were fed with fresh aphids (*ad libitum*) regularly and mortality of grubs was observed at 12, 24, 48 and 72h after spraying.

Plate 1.a: Field cages for aphid culture maintenance

Plate 1.b: Cowpea plants inside field cage

Plate 1.c: Aphids on caged plants

Plate 1. Maintenance of aphids under caged cow pea field

Plate 2.a: Egg of *C. sexmaculata*

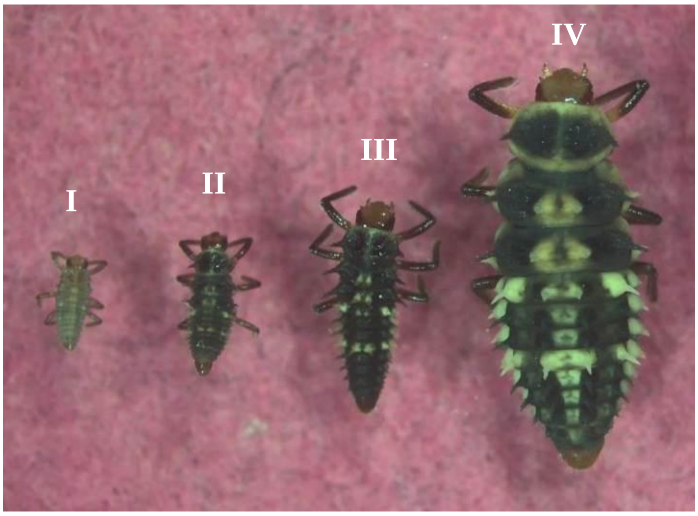

Plate 2.b: I, II, III & IV th instar grubs of *C. sexmaculata*

Plate 2.c: Pupa of *C. sexmaculata*

Plate 2.d: Adult of *C. sexmaculata*

Plate 2. Life stages of *Cheilomenes sexmaculata*

Plate 3.a: Potted cowpea plants in containers

Plate 3.b: Rearing cages for multiplication of *C. sexmaculata*

Plate 3. Maintenance of *C. sexmaculata* culture

Table 1. Details of selected insecticides and concentrations used in conducting the experiment

S.No	Common Name	Trade Name	Formulation	Recommended dose (g ai/ha)	Concentrations used		Manufacturer
					* field dose	**1/10th of field dose	
1.	Dimethoate	Tafgor	30EC	600	0.06%	0.006%	M/s Rallis India Limited
2.	Flubendiamide	Takumi	20WDG	25	0.004%	0.0004%	M/s Rallis India Limited
3.	Spinosad	Taffin	45SC	75	0.015%	0.0015%	M/s Rallis India Limited
4.	Thiamethoxam	Actara	25WG	25	0.005%	0.0005%	M/s Syngenta Crop Protection Private Limited
5.	Neem oil				3%	0.3%	M/s Sterling Farm Research and Services Private. Limited

*To study of effect on survival of *C. sexmaculata*

** To study of effect on reproduction of *C. sexmaculata*

3.4.3 Toxicity to pupa of *C. sexmaculata*

Eggs collected from the rearing cages were individually reared in 40 ml rearing containers with *ad libitum* supply of aphids till pupation. 0 to 24h old pupae were carefully removed using wet camel hair brush and dipped in insecticidal solution for 10 seconds. The dipped pupae were placed on tissue paper to drain excess insecticide solution and then transferred to Petri dishes @10 pupae/Petri dish. An untreated control was maintained by dipping pupae in distilled water. Each treatment was replicated four times with 10 pupae/replication. Treated pupae were observed for adult emergence and survival of adults at 24h after emergence.

3.4.4 Toxicity to adults of *C. sexmaculata*

Ten numbers of one day old adult beetles were released into Petri dishes with cowpea leaf inoculated with aphids and left undisturbed for 2h. The adult beetles were exposed to insecticidal treatments using a hand atomizer. Each treatment was replicated four times. An untreated control was maintained by spraying the adult beetles with distilled water. Mortality of adults was recorded at 12, 24, 48 and 72h after spraying.

3.5 Effect of insecticides on reproduction of *C. sexmaculata*

0 to 24h old first instar grubs were exposed to insecticides at one tenth of the recommended field dose by residue film method (Hassan *et al.*, 1985). For conducting this experiment, 1ml of prepared insecticide solution was pipetted out into a 9cm Petri dish. The dish was then rotated to have a uniform thin film of insecticide solution on it. After drying, ten grubs were released into each Petri dish. Each treatment was replicated four times maintaining 30grubs/replication. Test insects were allowed to crawl in the insecticide treated Petri dishes for 1h. After treatment period, the grubs were transferred into new Petri dishes and were provided with aphids as feed. Observations were made on grub mortality and the survived grubs were transferred to rearing containers for recording observations on developmental and reproductive biology such as developmental duration of life stages, sex ratio, fecundity, hatching per cent and adult longevity.

Plate 4.a: Eggs on cowpea leaf

Plate 4.b: Experimental arena

Plate 4. Bioassay on eggs

Plate 5.a: Grubs left to acclimatize

Plate 5.b: Spraying insecticide solution

Plate 5. Bioassay on grubs

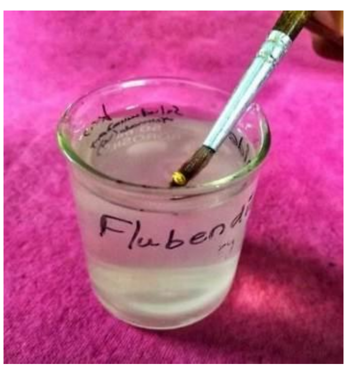

Plate 6.a: Pupa dipped in insecticidal solution

Plate .6b: Dried pupae after dipping

Plate 6. Bioassay on pupae

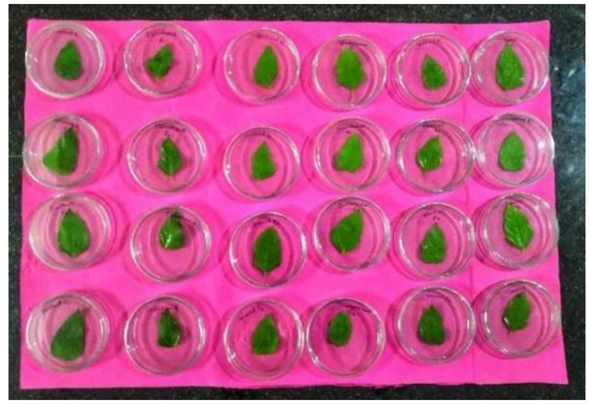

Plate 7.a: Adults left to acclimatize

Plate 7.b: Experimental arena

Plate 7. Bioassay on adults

Plate 8. Studies on effect of insecticides on reproduction

3.5.1 Developmental period of immature stages

3.5.1.1 Larval period

The grubs that survived after insecticide treatment were reared individually with ad libitum aphids and by changing the feed daily. Each grub was observed carefully twice a day to record the developmental period. The duration of each larval instar was observed and the total larval period was calculated.

3.5.1.2 Pupal period

After pupation, each pupa was observed for adult emergence. Pupal period was estimated form the day the pupa was formed till the day of adult emergence.

3.5.2 Sex ratio

After adult emergence, the beetles were differentiated into male and female (Lalithambika, 2012) by size and external genetalia. Sex ratio was calculated based on the number of males and females emerged from the pupa.

3.5.3 Fecundity and hatching per cent

To study the sublethal effect of insecticides on the fecundity and egg hatching, the paired adults were maintained separately in Petri dishes. Each pair was transferred to new Petri dish daily after egg laying. Number of eggs laid by each female was counted daily and the total number of eggs obtained from each pair during the life time was recorded. The eggs were observed daily for hatching and the per cent hatching was worked out.

$$Percent\ hatchability = \frac{Total\ number\ of\ eggs\ hatched}{Total\ number\ of\ eggs\ laid} \times 100$$

3.5.4 Adult longevity

The female and male adults were observed until death of the last individual to record longevity and the average adult life span was calculated.

3.6 Evaluation of toxicity in the field

Field evaluation on toxicity of insecticides on *C. sexmaculata* was carried out at research farm of Department of Plant Pathology, College of Agriculture, Vellanikkara. Following land preparation, cowpea plants of variety, *Bhagyalakshmi* was raised under rainshelter following all management measures as per KAU POP, 2016 except for plant protection, during November - December. The experiment was laid out in Randomized Block Design with five insecticides in plots of size 2.5mX2.7m (Plate 9). An untreated control was also maintained with water spray. Each treatment was replicated four times. The selected insecticides were applied at their recommended doses only once after natural infestation of aphids as well as predators. Aphid and predator population was observed one day prior to spraying as well as 1, 3, 5, 7 and 15 days after spraying. The population was recorded on ten sample plants selected at random in each plot. Aphid population was observed by counting number of aphids/10cm twig and predator population by counting number of *C. sexmaculata*/plant.

3.7 Statistical analysis and interpretation of data

The data was tabulated and transformations were made wherever necessary. Statistical analyses were done using WASP 2.0 and results were interpreted.

Plate 9.a: Experimental field

Plate 9.b: Natural aphid infestation in field

Plate 9.c: Natural occurrence of *C. sexmaculata* in field

Plate 9.c: Spraying of insecticides in field

Plate 9. Field experiment

Result

4.**RESULTS**

The results of the study on “Toxicity of insecticides to *Cheilomenes sexmaculata* Fabricius (Coleoptera: Coccinellidae)” conducted at the Department of Agricultural Entomology, College of Agriculture, Vellanikkara are presented here in this chapter.

4.1 **Effect of insecticides on survival of *Cheilomenes sexmaculata***

Different life stages of the predator, *Cheilomenes sexmaculata* were exposed to selected insecticides at concentration recommended for field application, in the laboratory and the results pertaining to survival and mortality are presented here.

4.1.1 **Effect of insecticides on eggs of *Cheilomenes sexmaculata***

4.1.1.1 Egg hatchability

The hatchability of eggs exposed to insecticides varied from 0 to 85 per cent. None of the eggs treated with dimethoate and neem oil hatched. Flubendiamide and thiamethoxam recorded 77.5 per cent egg hatching and were on par with that of spinosad (Table 2). The eggs treated with spinosad showed 85 per cent hatching which was on par with control in which 92.5 per cent eggs hatched.

4.1.1.2 Survival of grubs at 24h after hatching

The grubs emerged from eggs treated with thiamethoxam had a significantly lower survival of 41.93 per cent. The survival of grubs emerged from spinosad and flubendiamide treated eggs were 93.54 and 94.11per cent respectively at 24h after hatching, which was on par with survival in control with 97.29 per cent.

Table 2. Effect of insecticides on one day old eggs of *Cheilomenes sexmaculata*

Treatment	Egg hatchability (%)	Survival of emerged grubs at 24h after hatching	
		Numbers survived	Survival (%)
Dimethoate	0[c] (0.91)	-	-
Flubendiamide	77.50[b] (62.15)	7.25	93.54[a] (79.39)
Thiamethoxam	77.50[b] (62.15)	3.25	41.93 [b] (40.42)
Neem oil	0.00[c] (0.91)	-	-
Spinosad	85.00[ab] (70.22)	8	94.11[a] (79.13)
Control (Water dip)	92.50[a] (75.95)	9	97.29[a] (84.45)
CD (0.05)	11.84	-	12.29

Values in the parentheses are arc sin transformed

Figures followed by same alphabets did not differ significantly at P=0.05

4.1.2 Effect of insecticides on grubs of *Cheilomenes sexmaculata*

When the insecticides were tested for toxicity on 0-24h old third instar grubs, dimethoate caused significantly highest mortality of 62.50 per cent at 12h after treatment, followed by thiamethoxam with 27.50 per cent, which was on par with neem oil causing 10 per cent mortality. Flubendiamide recorded significantly lower mortality of 7.50 per cent compared to dimethoate, thiamethoxam and neem oil. Spinosad with 5 per cent mortality was statistically on par with control where no mortality was observed at 12h.

At 24h, the mortality in dimethoate treatment was 90 per cent and was significantly highest, followed by thiamethoxam with 67.50 per cent. Neem oil with 10 per cent mortality was followed by flubendiamide and spinosad, each with 7.50 per cent mortality and these treatments were on par with control which recorded 2.50 per cent mortality.

At 48h, dimethoate caused complete mortality of the treated grubs followed by thiamethoxam with 92.50 per cent mortality and these two were on par with each other. A significantly lower mortality of 15 per cent was recorded in neem oil followed by flubendiamide and spinosad respectively with 10 and 7.50 per cent mortality which were on par with neem oil. However, flubendiamide and spinosad were also on par with control which recorded 5 per cent mortality.

Table 3. Effect of insecticides on four days old grubs of *Cheilomenes sexmaculata*

Treatment	**Cumulative mortality (%) of third instar grubs at different hours after treatment**			
	12h	**24h**	**48h**	**72h**
Dimethoate	62.50[a] (52.49)	90.00[a] (73.96)	100.00[a] (89.09)	-
Flubendiamide	7.50[c] (14.05)	7.50[c] (14.05)	10.00[bc] (16.09)	10.00[c] (16.09)
Thiamethoxam	27.50[b] (31.39)	67.50[b] (55.50)	92.50[a] (78.30)	100.00[a] (89.10)
Neem oil	10.00[c] (16.09)	10.00[c] (16.09)	15.00[b] (22.50)	32.50[b] (34.50)
Spinosad	5.00[cd] (9.67)	7.50[c] (14.05)	7.50[bc] (14.05)	7.50[c] (14.05)
Control (Water spray)	0.00[d] (0.91)	2.50[c] (5.29)	5.00[c] (7.32)	5.00[c] (7.32)
CD (0.05)	12.04	13.88	14.19	12.39

Values in the parentheses are arc sin transformed
Figures followed by same alphabets did not differ significantly at P=0.05

At 72h, complete mortality was observed in thiamethoxam. The mortality recorded in neem oil at 32.50 per cent was significantly lower than that in thiamethoxam. Flubendiamide with 10 per cent and spinosad with 7.50 per cent mortality were significantly lower compared to neem oil and were on par with control which recorded a mortality of 5 per cent. A gradual increase in mortality of grubs was observed as the time interval proceeded from 12h to 72h after treatment with dimethoate, thiamethoxam and neem oil,

4.1.3 Effect of insecticides on pupae of *Cheilomenes sexmaculata*

4.1.3.1 Adult emergence

Adult emergence from pupae treated with insecticides varied from 0 to 92.50 per cent. No adults emerged from pupae exposed to neem oil and dimethoate. Even though the adult development was visible within pupae exposed to dimethoate, the adults did not emerge from the pupal case. Thiamethoxam recorded 17.50 per cent adult emergence which was significantly higher than dimethoate and neem oil. Among the insecticides, spinosad recorded maximum adult emergence of 92.50 per cent followed by flubendiamide with 90 per cent emergence and these two were on par with control where 95 per cent adults emerged.

Table 4. Effect of insecticides on one day old pupae of *Cheilomenes sexmaculata*

Treatment	Adult emergence (%)	Survival of emerged adults at 24h after emergence	
		Numbers survived	Survival (%)
Dimethoate	0.00[c] (0.91)	-	-
Flubendiamide	90.00[a] (76.26)	8.25	91.67[a] (77.44)
Thiamethoxam	17.50[b] (24.16)	0	0.00[b] (0.91)
Neem oil	0.00[c] (0.91)	-	-
Spinosad	92.50[a] (78.30)	8.75	94.60[a] (80.07)
Control (Water dip)	95.00[a] (82.68)	9.25	97.36[a] (84.71)
CD (0.05)	14.88	-	11.96

Values in the parentheses are arc sin transformed
Figures followed by same alphabets did not differ significantly at P=0.05

4.1.3.2 Survival of adults at 24h after emergence

The adults emerged from pupae exposed to thiamethoxam were found dead within 24h of emergence. The survival of adults at 24h after emergence was 94.60 and 91.67 per cent respectively in spinosad and flubendiamide treatments and were on par with control which recorded a maximum survival of 97.36 per cent (Table 4).

4.1.4 Effect of insecticides on adults of *Cheilomenes sexmaculata*

When 0 -24h old adults were exposed to insecticides, the highest mortality of 87.50 per cent was observed in dimethoate at 12h after treatment. Thiamethoxam recorded 70 per cent mortality which was significantly lower than that of dimethoate. The mortality in neem oil at 20 per cent was significantly lower compared to thiamethoxam. Flubendiamide with 10 per cent mortality trailed neem oil and were on par with each other. The mortality recorded in spinosad at 5 per cent was on par with flubendiamide and also with control, which recorded the minimum mortality of 2.50 per cent.

Table 5. Effect of insecticides on one day old adults of *Cheilomenes sexmaculata*

Treatment	Cumulative mortality (%) of one day old adults at different hours after treatment			
	12	24	48	72
Dimethoate	87.50^{a} (72.25)	92.50^{a} (78.30)	100.00^{a} (89.09)	-
Flubendiamide	10.00^{cd} (16.09)	12.50^{d} (20.47)	12.50^{c} (18.18)	12.50^{c} (18.18)
Thiamethoxam	70.00^{b} (56.95)	80.00^{b} (63.81)	97.50^{a} (84.71)	100.00^{a} (89.09)
Neem oil	20.00^{c} (26.19)	37.50^{c} (37.66)	45.00^{b} (42.05)	50.00^{b} (45.00)
Spinosad	5.00^{d} (9.67)	10.00^{d} (16.09)	12.50^{c} (18.18)	12.50^{c} (18.18)
Control (Water spray)	2.50^{d} (5.29)	5.00^{d} (9.67)	5.00^{c} (9.67)	5.00^{c} (11.70)
CD (0.05)	14.01	13.16	12.12	11.37

Values in the parentheses are arc sin transformed
Figures followed by same alphabets did not differ significantly at P=0.05

At 24h after treatment, dimethoate caused 92.50 per cent mortality, which was significantly higher than all other treatments including control. Mortality in thiamethoxam was 80 per cent which was significantly higher than neem oil with 37.50 per cent mortality. The mortality recorded in flubendiamide and spinosad were 12.50 and 10 per cent respectively, which were significantly lower compared to other insecticide treatments and were on par with control with 5 per cent mortality.

At 48h, complete mortality of adults was recorded in dimethoate treatment followed by thiamethoxam with 97.50 per cent and were on par with each other. Mortality recorded in neem oil at 45 per cent was significantly lower than that of dimethoate and thiamethoxam. Flubendiamide and spinosad caused only 12.50 per cent mortality, which was lowest among the insecticides and was on par with five per cent mortality in control.

At 72h, complete mortality of grubs was observed with thiamethoxam and was significantly higher than all other treatments. Neem oil caused 50 per cent mortality, which was significantly lower compared to thiamethoxam. Mortality recorded in flubendiamide and spinosad at 12.50 per cent was on par with control, which recorded the minimum mortality of 5 per cent.

4.2 Sublethal effects of insecticides on *Cheilomenes sexmaculata*

The insecticides at one tenth of the recommended field rate were evaluated on first instar grubs of *C. sexmaculata* to study their effects on developmental and reproductive biology of the surviving individuals and the results are presented here.

4.2.1 Grub survivability

When 0-24h old first instar grubs of *C. sexmaculata* were exposed to residue films of selected insecticides at one tenth of recommended field rate, the survival of grubs varied from 0 to 84.17 per cent. None of the first instar grubs survived after exposure to dimethoate. Minimum survival at 18.33 per cent was observed in neem oil followed by 20 per cent in thiamethoxam and these two treatments were on par with each other. Flubendiamide and spinosad treatments recorded 82.50 and 84.17 per cent survival respectively which were on par with each other and were significantly higher compared to thiamethoxam and neem oil. Maximum survival was in control with 94.17 per cent and was significantly higher than all other treatments (Table 6).

Table 6. Effect of insecticides on survival of first instar grubs of *Cheilomenes sexmaculata*

Treatment	Survival of first instar grubs (%)
Dimethoate	0.00^{d} (0.52)
Flubendiamide	82.50^{b} (65.50)
Thiamethoxam	20.00^{c} (26.32)
Neem oil	18.30^{c} (24.66)
Spinosad	84.17^{b} (66.63)
Control	94.17^{a} (78.15)
CD(0.05)	7.064

Values in the parentheses are arc sin transformed
Figures followed by same alphabets did not differ significantly at P=0.05

4.2.2 Effect of insecticides on developmental period of *Cheilomenes sexmaculata*

Effect of exposure to insecticides at $^{1}/_{10}$th of the field recommended rate on the developmental biology of the survived individuals was studied. The mean duration of first instar grubs treated with neem oil was 1.57±0.22days and that of thiamethoxam was 1.44±0.21 days which were significantly longer than those treated with either spinosad or flubendiamide with 1.16±0.07 days and the later two were on par with control where a mean duration of 1.12±0.06 days was recorded.

The duration of second instar grubs also was prolonged both in thiamethoxam and neem oil respectively with 1.95±0.35 and1.84±0.29 days and they differed significantly from other treatments (Table 7). The mean duration of second instar grubs recorded in

Table 7. Effect of insecticides on developmental biology of *Cheilomenes sexmaculata*

Treatment	Duration of different developmental periods (Days)					
	Instar 1	Instar 2	Instar 3	Instar 4	Pupal period	Total developmental period
Flubendiamide	1.16±0.07[b]	1.51±0.13[c]	1.95±0.15[a]	2.62±0.15[a]	2.76±0.14[ab]	9.99±0.64[b]
Thiamethoxam	1.44±0.21[a]	1.95±0.35[a]	1.97±0.29[a]	2.78±0.30[a]	2.92±0.33[a]	11.06±1.49[a]
Neem oil	1.57±0.22[a]	1.84±0.29[ab]	1.99±0.34[a]	2.83±0.28[a]	2.89±0.33[a]	11.13±1.46[a]
Spinosad	1.16±0.07[b]	1.56±0.15[bc]	1.93±0.15[a]	2.58±0.10[a]	2.61±0.15[b]	9.84±0.61[b]
Control (Water)	1.12±0.06[b]	1.50±0.12[c]	1.88±0.15[a]	2.52±0.13[a]	2.69±0.14[b]	9.69±0.59[b]
CD(0.05)	0.136	0.321	NS	NS	0.169	0.719

In vertical columns, figures followed by same alphabets did not differ significantly by DMRT P=0.05

flubendiamide and spinosad treatments at 1.51±0.13 and 1.56±0.15 days were on par with control having a mean duration of 1.50±0.12 days. No significant difference was observed between treatments with respect to the mean duration of third and fourth instar grubs. The mean pupal period of survived individuals after exposure of grubs to thiamethoxam and neem oil was 2.92±0.33 and 2.89±0.33 days respectively and was significantly lower than that of other treatments. The pupal period observed with flubendiamide and spinosad treatments were 2.76±0.14 and 2.61±0.15 days respectively and were on par with control in which a mean pupal period of 2.69±0.14 days was recorded.

The total developmental period from first instar to adult was significantly prolonged after exposure to both neem oil and thiamethoxam with mean values of 11.13±1.46 and 11.06±1.49 days respectively as against 9.69±0.59 days in control. At the same time, the total developmental time recorded with flubendiamide and spinosad were 9.99±0.64 and 9.84±0.61days respectively and were on par with control.

4.2.3 Survival of immature stages and sex ratio

After the first moult of grubs that survived the exposure to insecticide, no mortality was observed in further stages. Emergence of adults from survived immatures was minimum in thiamethoxam with 88.89 per cent, followed by neem oil with 96.43per cent, flubendiamide with 97.12 per cent and spinosad with 98 per cent. However, no significant difference was observed among treatments including control, where 99.03 per cent adults emerged. (Table 8).

Sex ratio was highest in thiamethoxam treatment with male:female ratio of 1:1.73 followed by neem oil with 1:1.67, flubendiamide with 1:1.64, control with 1:1.63 and spinosad with 1:1.61. However, there was no significant difference in sex ratio among the treatments.

Table 8. Effect of insecticides on survival of immature stages and sex ratio of *Cheilomenes sexmaculata*

Treatment	Survival of immature stages (%)	Sex ratio (Male: Female)
Flubendiamide	97.12 (85.03)	1:1.64
Thiamethoxam	88.89 (76.31)	1:1.73
Neem oil	96.43 (84.44)	1:1.67
Spinosad	98.00 (85.89)	1:1.61
Control	99.03 (87.17)	1:1.63
CD(0.05)	NS	NS

Values in the parentheses are arc sin transformed
Figures followed by same alphabets did not differ significantly at P=0.05

4.2.4 Effect of insecticides on reproductive biology of *Cheilomenes sexmaculata*

Variations were observed in reproductive parameters of *C. sexmaculata* developed from first instar grubs exposed to insecticides at 1/10th of the field recommended rates. The effects on pre-oviposition period, oviposition period, post-oviposition period, adult longevity, fecundity and fertility are given below.

4.2.4.1 Pre-oviposition period

The pre-oviposition period was maximum in neem oil with 5.38±0.47 days followed by thiamethoxam with 4.87±0.35 days, flubendiamide with 4.38±0.44 days and spinosad with 4.25±0.58 days as against 4.13±0.44 days in control. However, there was no significant difference between the treatments (Table 9a).

4.2.4.2 Oviposition period

Exposure of first instar grubs to thiamethoxam, flubendiamide and neem oil significantly reduced the oviposition period of *C. sexmaculata* to 47.88±3.45, 48.63±4.86 and 50±2.29 days respectively (Table 9a), when compared with 55.88±1.15 days in control.

The oviposition period in spinosad treatment was 53.13±1.33 days and was on par with control as well as with other treatments.

Table 9a. Effect of insecticides on reproductive biology of *Cheilomenes sexmaculata*

Treatment	Reproductive parameters				
	Pre-oviposition period	Oviposition period	Post-oviposition period	Adult longevity	
				Male	Female
Flubendiamide	4.38±0.44	48.63±4.86^{b}	3.88±0.79	43.63±2.39^{bc}	56.38±4.69^{b}
Thiamethoxam	4.87±0.35	47.88±3.45^{b}	4.25±0.53	41.5±1.95^{c}	56.5±3.53^{b}
Neem oil	5.38±0.47	50±2.29^{b}	3.87±0.62	43.25±2.12^{bc}	56.5±2.30^{b}
Spinosad	4.25±0.58	53.13±1.33^{ab}	3.75±0.53	45.75±1.59^{ab}	61±1.24^{ab}
Control (Water)	4.13±0.44	55.88±1.15^{a}	4.38±0.44	47.25±1.41^{a}	64.38±1.15^{a}
CD(0.05)	NS	5.53	NS	3.52	5.29

Values are mean ±SE;
Figures in vertical columns with same alphabets did not differ significantly by DMRT P=0.05

Table 9b. Effect of insecticides on reproductive biology of *Cheilomenes sexmaculata*

Treatment	Reproductive parameters		
	Fecundity/ female	Offspring/female	Hatching (%)
Flubendiamide	1581.63±80.13^{c}	1092.25±67.02^{c}	69.15^{b} (55.96)
Thiamethoxam	1637.13±92.10^{c}	1162.38±58.92^{c}	71.10^{b} (57.48)
Neem oil	1727.13±51.75^{bc}	1242.62±46.79^{c}	71.97^{b} (58.03)
Spinosad	1917±89.43^{b}	1604±72.89^{b}	83.67^{a} (66.16)
Control (Water)	2151.88±27.31^{a}	1868±26.50^{a}	86.80^{a} (68.69)
CD(0.05)	218.68	172.22	5.20

Values are mean ±SE; Values in the parentheses are arc sin transformed
Figures in vertical columns with same alphabets did not differ significantly by DMRT P=0.05

4.2.4.3 Post-oviposition period

With respect to post-oviposition period, no significant difference was observed among treatments (Table 9a). It was minimum in spinosad with 3.75±0.53 days followed by neem oil with 3.87±0.62 days, flubendiamide with 3.88±0.79 days, thiamethoxam with 4.25±0.53 days and control with 4.38±0.44days.

4.2.4.4 Adult longevity

Exposure to insecticides greatly influenced the life span of adults. The life period of males was minimum in thiamethoxam with 41.5±1.95 days followed by neem oil with 43.25±2.12 days and flubendiamide with 43.63±2.39 days and these three were on par with each other. Male longevity recorded in spinosad at 45.75±1.59 days was significantly higher than that of thiamethoxam but was on par with neem oil and flubendiamide and also with control, which recorded the maximum longevity of 47.25±1.41 days (Table 9a).

Female longevity in flubendiamide, thiamethoxam and neem oil treatments was 56.38±4.69, 56.50±3.53 and 59.50±2.30 days respectively which were significantly shorter compared to control with 64.38±1.15 days. The female longevity in spinosad treatment was 61.00±1.24 days, which was on par with control as well as with other insecticides.

4.2.4.4 Fecundity, offsprings/female and hatching per cent

Exposure of 0-24h old first instar grubs to insecticides at $^{1}/_{10}{}^{th}$ of field recommended rate significantly reduced the fecundity of *C. sexmaculata.* The fecundity was minimum in flubendiamide with 1581.63±80.13 eggs followed by thiamethoxam with 1637.13±92.10 eggs (Table 9b). Both these treatments were on par with neem oil recording 1727.13±51.75 eggs but were significantly lower than spinosad with 1917.00±89.43 eggs. In many instances, the laid eggs in a cluster were malformed and turned into an undifferentiated mass in neem oil treatment. Such egg masses were not counted. The fecundity in control with 2151.88±27.31 eggs was significantly higher compared to all other treatments.

The fertility (offsprings/female) of adult females developed from grubs exposed to insecticides was reduced significantly. The number was minimum in flubendiamide with

1092.25±67.02 offsprings/female, followed by thiamethoxam with 1162.38±58.92 offsprings/female and neem oil with 1242.62±46.79 offsprings/female and were on par with each other. However, the number observed with spinosad at 1604.00±72.89offsprings/female (Table 9b) was significantly higher to other insecticides. The number in control with 1868±26.50 offsprings/female was significantly higher than that of all other treatments.

The hatchability of eggs was minimum in flubendiamide treatment with 69.15 per cent followed by 71.10 and 71.97 per cent respectively in thiamethoxam and neem oil and these three were on par with each other. In spinosad, 83.67 per cent eggs hatched, which was significantly higher than all other insecticide treatments (Table 9b) but was on par with 86.80 per cent recorded in control.

4.3 Evaluation of insecticides under field conditions

The insecticides were evaluated for their effect on aphids and toxicity to *C. sexmaculata* in cowpea variety *Bhagyalakshmi* raised in rain shelter. The insecticides were applied after natural infestation with aphids. The population of aphids as well as *C. sexmaculata* were recorded and presented.

4.3.1 Aphids, *Aphis craccivora*

The aphid population was recorded one day before spray and the pre-count ranged from 48.30 to 31.43 aphids/10cm twig with no significant difference among treatments including control (Table 10).

At 1 DAS, no aphid population was recorded in plots treated with thiamethoxam. In plots treated with dimethoate, only 1.45 aphids/10 cm twig were recorded. The mean aphid population in dimethoate and thiamethoxam treatments were on par and were significantly lower than other insecticide treatments. The mean population in spinosad, neem oil and flubendiamide treatments were 19.55, 26.00 and 26.05 aphids/10cm twig, respectively and were on par with each other. In control, the mean aphid population was 41.08numbers/10 cm twig, which was significantly higher than all other treatments.

Table. 10 Effect of different insecticides on *Aphis craccivora* in cowpea field

Treatment	Mean no. of aphids/10cm twig						Overall mean no. of aphids/10cm twig after insecticide application
	1DBS	1DAS	3DAS	5DAS	7DAS	15DAS	
Dimethoate	48.30 (6.91)	1.45[c] (1.28)	0.00[c] (0.71)	0.00[c] (0.71)	0.00[c] (0.71)	3.20[c] (1.81)	0.93
Flubendiamide	39.40 (6.22)	26.05[b] (5.09)	24.10[b] (4.91)	22.45[b] (4.72)	21.50[b] (4.65)	17.90[b] (4.27)	22.40
Thiamethoxam	35.55 (5.77)	0.00[c] (0.71)	0.00[c] (0.71)	0.00[c] (0.71)	0.00[c] (0.71)	1.80[c] (1.37)	0.36
Neem oil	44.75 (6.66)	26.00[b] (5.27)	22.35[b] (4.87)	21.70[b] (4.68)	21.30[b] (4.65)	17.35[b] (4.33)	21.74
Spinosad	49.75 (7.02)	19.55[b] (4.48)	17.38[b] (4.21)	20.10[b] (4.53)	20.45[b] (4.57)	17.00[b] (4.17)	18.90
Control	31.43 (5.57)	41.08[a] (6.42)	39.00[a] (6.23)	39.33[a] (6.27)	37.13[a] (6.10)	33.88[a] (5.83)	38.08
CD (0.05)	NS	0.87	0.74	0.79	0.66	0.99	-

Values in the parentheses are square root transformed

Figures in vertical columns with same alphabets did not differ significantly by DMRT P=0.05

At 3 DAS, no aphid population was recorded in plots treated with thiamethoxam and dimethoate, the lowest among treatments. The mean population in spinosad, neem oil and flubendiamide treatments were 17.38, 22.35 and 24.10aphids/10cm, twig respectively and were on par with each other but were significantly lower than control which recorded.39.00 aphids/10 cm twig.

At 5 DAS, similar trend was recorded with no aphid population in plots treated with thiamethoxam and dimethoate. The mean population in spinosad, neem oil and flubendiamide treatments were 20.10, 21.70 and 22.45aphids/10 cm twig, respectively which were on par with each other. A significantly higher aphid population was recorded in control with 39.33 aphids/10 cm twig.

Aphid population was not observed in plots treated with thiamethoxam and dimethoate at 7 DAS also. The mean aphid population in spinosad, neem oil and flubendiamide treatments were 20.45, 21.30 and 21.5 numbers/10 cm twig respectively which were on par with each other whereas in control, significantly higher population with 37.13 aphids/10 cm twig was observed.

At 15 DAS, mean aphid population in plots treated with thiamethoxam and dimethoate were 1.8 and 3.2 aphids/10 cm twig, which were on par with each other and significantly lower than other treatments. Spinosad recorded 17.00 aphids/10cm twig, followed by neem oil and flubendiamide respectively with 17.35 and 17.90 aphids/10cm twig and these three treatments were on par with each other. The mean aphid population in control was 33.88 numbers/10 cm twig which was significantly higher compared to other treatments.

4.3.2. *Cheilomenes sexmaculata*

The population of *C. sexmaculata* recorded one day before spray ranged from 1.1 to 1.3 per plant and no significant difference was observed among treatments (Table 11).

Table. 11 Effect of different insecticides on *Cheilomenes sexmaculata* in cowpea field

Treatment	Mean no. of *C. sexmaculata* /plant						Overall mean no. of *C. sexmaculata* /plant after insecticide application
	1DBS	1DAS	3DAS	5DAS	7DAS	15DAS	
Dimethoate	1.10 (1.04)	0.00 [c] (0.71)	0.00 [c] (0.71)	0.00 [b] (0.71)	0.00 [b] (0.71)	0.00 [b] (0.71)	0.00
Flubendiamide	1.25 (1.12)	1.05 [b] (1.24)	1.05 [b] (1.24)	1.35 [a] (1.36)	1.80 [a] (1.513)	1.90 [a] (1.55)	1.43
Thiamethoxam	1.15 (1.02)	0.00 [c] (0.71)	0.00 [c] (0.71)	0.00 [b] (0.71)	0.00 [b] (0.71)	0.00 [b] (0.71)	0.00
Neem oil	1.30 (1.14)	1.00[b] (1.22)	1.25[ab] (1.32)	1.35[a] (1.36)	1.85[a] (1.53)	2.00 [a] (1.58)	1.49
Spinosad	1.25 (1.11)	1.05 [b] (1.24)	1.20[ab] (1.30)	1.45[a] (1.38)	1.80 [a] (1.51)	1.90[a] (1.55)	1.48
Control	1.30 (1.13)	1.45 [a] (1.39)	1.55 [a] (1.43)	1.70 [a] (1.47)	1.75 [a] (1.50)	2.15 [a] (1.63)	1.72
CD (0.05)	NS	0.12	0.15	0.18	0.16	0.10	-

Values in the parentheses are square root transformed

Figures in vertical columns with same alphabets did not differ significantly by DMRT P=0.05

At 1 DAS, the number of *C. sexmaculata* decreased significantly in all the treatments compared to control. Population of *C. sexmaculata* was not recorded in plots treated with dimethoate and thiamethoxam. The mean number of *C. sexmaculata* in plots treated with neem oil was significantly higher than that of dimethoate and thiamethoxam with 1.00number/plant. Plots treated with spinosad and flubendiamide recorded 1.05 *C. sexmaculata* /plant which were on par with neem oil. The mean number in control was 1.45 which was significantly higher than all other treatments.

At 3 DAS, no *C. sexmaculata* was recorded in plots treated with dimethoate and thiamethoxam. The mean number of 1.05, 1.20 and 1.25 *C. sexmaculata* per plant recorded respectively in flubendiamide, spinosad and neem oil were on par each other but were significantly higher than that of dimethoate and thiamethoxam. However, the population in spinosad and neem oil were also on par with 1.55 *C. sexmaculata* /plant recorded in control.

At 5 DAS, similar trend was observed in plots treated with dimethoate and thiamethoxam where no *C. sexmaculata* population was recorded. A significantly higher population of 1.35 *C. sexmaculata* /plant was observed with flubendiamide and also with neem oil followed by 1.45 numbers/plant in spinosad and these three treatments were on par with 1.7 numbers/plant recorded in control.

C. sexmaculata was not recorded in plots treated with dimethoate and thiamethoxam at 7 DAS also. The mean number of 1.80 *C. sexmaculata*/plant in flubendiamide as well as spinosad and 1.85 numbers/plant recorded in neem oil were on par with one another and also with control which recorded 1.75 *C. sexmaculata*/plant.

Even at 15 DAS, no *C. sexmaculata* was recorded in plots treated with dimethoate and thiamethoxam. The mean number of *C. sexmaculata* in flubendiamide and spinosad was 1.90 and that in neem oil was 2.00 which were on par with each other. These three treatments were also on par with control, which recorded 2.15 *C. sexmaculata*/plant.

Discussion

5. **DISCUSSION**

Lethal toxicity of five insecticides on different life stages of *Cheilomenes sexmaculata*, their sublethal effects on development & reproduction as well as their effect on field efficacy of the predator were studied at College of Agriculture, Vellanikkara. The findings of the study are discussed in this chapter.

5.1 Effect of insecticides on survival of *Cheilomenes sexmaculata*

The insecticides were evaluated at field recommended dose for their toxicity to different life stages of *C. sexmaculata* following dip or spray method.

5.1.1 Effect of insecticides on eggs of *Cheilomenes sexmaculata*

Neem oil and dimethoate were highly toxic to egg stage of *C. sexmaculata*. None of the eggs exposed to neem oil and dimethoate hatched. Eggs treated with thiamethoxam though recorded 77.50 per cent hatching, only 41.93 per cent of the hatched grubs survived after 24h (Fig.1). Flubendiamide and spinosad were relatively harmless to eggs, recording 77.50 and 85 per cent hatching with relatively higher survival of 93.65 and 93.93 per cent respectively that were comparable to control.

The highly toxic nature of neem oil (1%) with 80 per cent mortality of eggs recorded by Khan *et al.* (2015) closely matches with the present findings. The mortality of eggs after exposure to neem oil was attributed to the adherence of oil particles to the chorion that impedes with respiration (da Silva, 2004). However, an earlier record of neem extract as harmless to eggs of *C. septempunctata* by Kaethner (1991) is a contradiction to present findings. This variation might be due to the difference in the species tested. The ovicidal effect of dimethoate (0.03%) with 84.1 per cent mortality of eggs observed by Shanmugapriya and Muralidharan (2017) is agreeable with the present findings as complete mortality of eggs was observed with dimethoate (0.06%). Whereas, Tank *et al.* (2007) noticed only 42 per cent mortality with dimethoate but at a lower concentration of 0.015 per cent, which was only quarter of the concentration used in the current study.

The present observations with thiamethoxam are in close proximity with that of Shanmugapriya and Muralitharan (2017) who noticed 77.1 per cent mortality of eggs after exposure with thiamethoxam (0.005%). However, in contrast to this, Sanghani *et al.* (2018) recorded only 24 per cent mortality of eggs even at a higher concentration of 0.01 per cent. The report on spinosad (0.05%) as slightly toxic with 47 per cent mortality of eggs by Khan *et al*. (2015) is not in line with the present findings of 15 per cent mortality which might be due to the higher concentration compared to 0.015 per cent used in the present study. Similar studies on toxicity with flubendiamide is lacking for a comparison.

5.1.2 Effect of insecticides on grubs of *Cheilomenes sexmaculata*

Dimethoate caused significantly higher mortality of 62.50 per cent and was followed by thiamethoxam with 27.50 per cent in 0-24h old third instar grubs at 12h after treatment. A gradual increase in mortality was observed both in dimethoate and thiamethoxam and reached 100 per cent at 48 and 72h respectively (Fig.2). These insecticides were categorized as harmful to grubs of *C. sexmaculata* as per IOBC protocol (Hassan *et al.*, 1985). Neem oil recorded only 10 per cent mortality at 12h which, however gradually increased to 15 per cent at 48h and 32.50 per cent at 72h and was categorized as harmless based on IOBC rating. Flubendiamide and spinosad were harmless to third instar grubs recording only 7.50 and 5 per cent mortality respectively, at 12h. A further increase to only 10 and 7.50 per cent respectively was observed till 72h which was on par with control.

Similar trend of increasing mortality with progress of time after exposure of third instar grubs with thiamethoxam and dimethoate causing 87 and 83 per cent mortality respectively, at 72h was reported by Shanmugapriya and Muralidharan (2017). The present results with dimethoate broadly agrees with Tank *et al.* (2007) who categorized dimethoate (0.015%) as highly toxic, inducing 66.69 per cent mortality to third instar grubs at 72h. The present findings with neem oil are in line with Khan *et al.* (2015) who observed 20 per cent mortality in third instar grubs at 48h. The report by same authors on spinosad (0.02%) as slightly toxic with 50 per cent mortality to third instar grubs of *C. sexmaculata* is not agreeable with the present result and that might be due to slightly high concentration of spinosad used in their study.

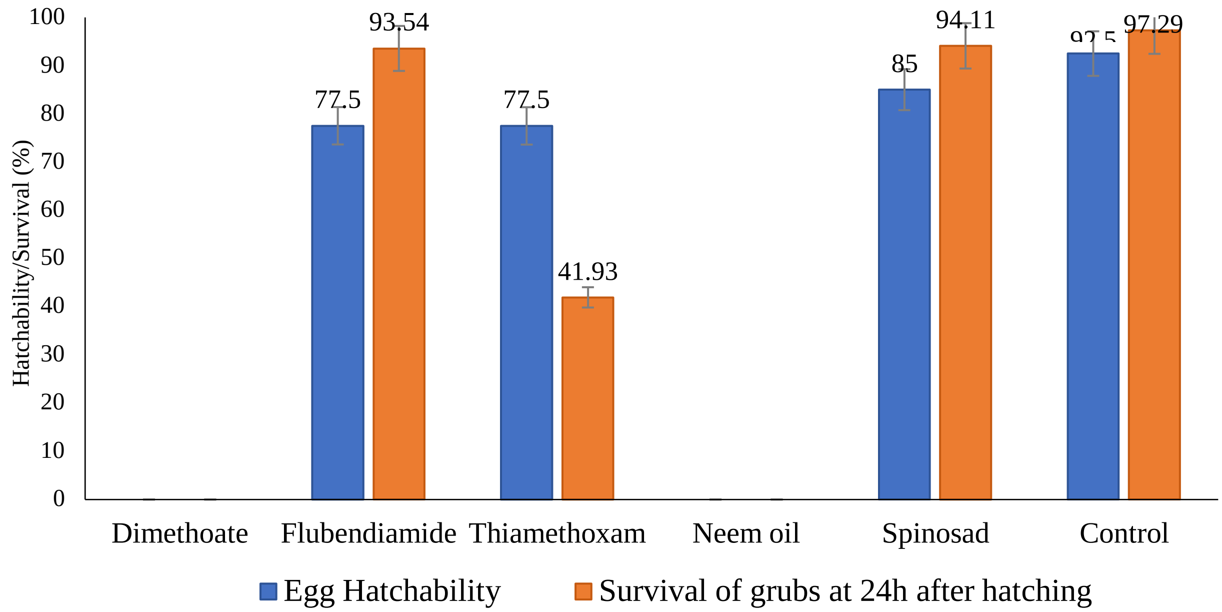

Fig 1: Effect of insecticides on eggs of *Cheilomenes sexmaculata*

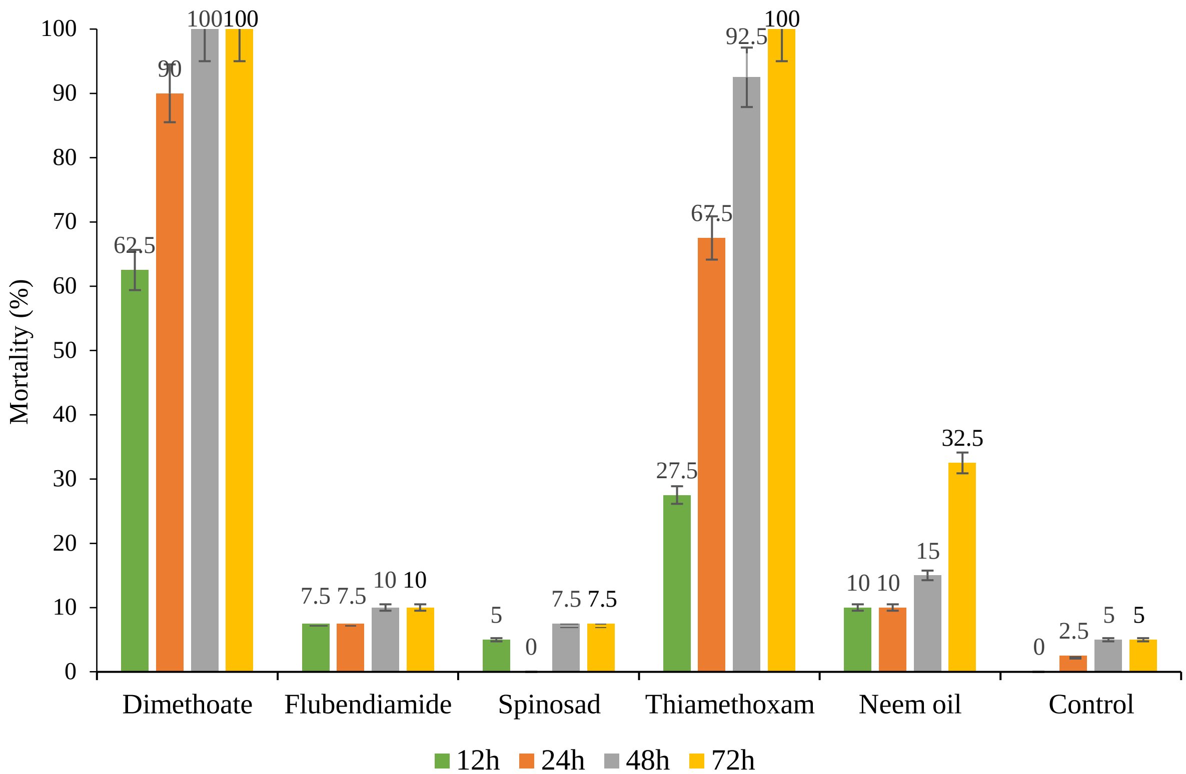

Fig 2: Effect of insecticides on grubs of *Cheilomenes sexmaculata*

5.1.3 Effect of insecticides on pupae of *Cheilomenes sexmaculata*

No adults emerged from pupae treated with neem oil as well as dimethoate and only 17.50 per cent adult emergence was observed from pupae treated with thiamethoxam. Thus, these three insecticides were categorized as harmful to pupae as per IOBC evaluation protocol. Harmful nature of neem oil on pupae of *C. sexmaculata* is in accordance with Khan *et al.* (2015), who documented highest mortality of pupae with only 13.30 per cent adult emergence. Same authors documented the effect of spinosad on pupae and according to them, spinosad is harmless to pupal stage with 60 per cent adult emergence, which broadly confirms the present results where 92.50 per cent adults emerged. A comparison on the results of dimethoate, thiamethoxam and flubendiamide reporting their level of toxicity on pupae could not be made for want of similar previous studies.

5.1.4 Effect of insecticides on adults of *Cheilomenes sexmaculata*

Dimethoate caused the highest mortality of 87.50 per cent to adults of *C. sexmaculata* followed by thiamethoxam with 70 per cent mortality at 12h after spraying. The mortality gradually increased and peaked to 100 per cent at 48h and 72h respectively in dimethoate and thiamethoxam. As per IOBC protocol, these two insecticides were harmful to the adults of *C. sexmaculata.* Neem oil, though recorded only 20 per cent mortality at 12h, it gradually increased to 50 per cent at 72h and was rated as slightly harmful to adults. Flubendiamide and spinosad were harmless with only 12.50 per cent mortality of adults at 72h (Fig.4) which was on par with 5 per cent mortality in control.

Complete mortality of adults after exposure to dimethoate and thiamethoxam, as observed by Basappa (2007) confirms the present findings. The harmful nature of dimethoate was also in confirmity with earlier reports of Tank *et al.* (2007) and Megha *et al.* (2014). In contrast, a recent study by Sanghani *et al.* (2018) reported only 33.3 per cent mortality with thiamethoxam (0.01%) but following a different method of exposure by releasing the test insects in sprayed surface rather than spray on test insects followed in the present study.

However, varied results with neem products were recorded in earlier findings as Megha *et al.* (2014) observed 100 per cent mortality with nimbicidine (0.5%) at 12h and Khan *et al.* (2015) recorded 22 per cent mortality with neem oil (1%) at 48h in adults of *C. sexmaculata*. The studies with flubendiamide and spinosad on adults of *C. sexmaculata* are lacking for a comparison. However, the findings with spinosad as harmless to adults in the present study is in agreement with observations of Jalali *et al*. (2009) with adults of *Adalia bipunctata* even at ten times the recommended field concentrations and that of Galvan *et al.* (2005) with adults of *H. axyridis* at field recommended concentration. Studies with flubendiamide were not available for a discussion.

The insecticides evaluated for their lethal toxicity in the present study had a similar effect as inflicted by same or other insecticides with similar mode of action on *C. sexmaculata* and other closely related coccinellid predators as reported by earlier records. It is noted that variations can be observed among reports, especially with neem oil. These variations might be attributed to different formulations or quality of products available in different localities. Slight variation in effect of insecticides might be due to variation in concentration, method as well as stage of the predator used in the experiment.

As per IOBC testing scheme, laboratory bioassay on lethal toxicity is the first step in assessing the safety of insecticides (Hassan *et al.*, 1994). In the present study, with respect to IOBC evaluation categories, dimethoate was harmful causing lethal effects to all the life stages of the predator. Thiamethoxam was harmless only to egg stage and harmful to other stages. Neem oil was harmful only to the non-feeding stages and was slightly harmlful to feeding stages. Flubendiamide and spinosad were categorized as harmless insecticides to *C. sexmaculata*. Flubendiamide has a high activity against Lepidoptera (Lahm *et al*., 2009), and there is no evidence of activity in other insect orders (Gentz *et a*l., 2010). Several studies have reported its safety to parasitoids and predators (Tohnishi *et al*., 2005; Garzon *et al*., 2015).

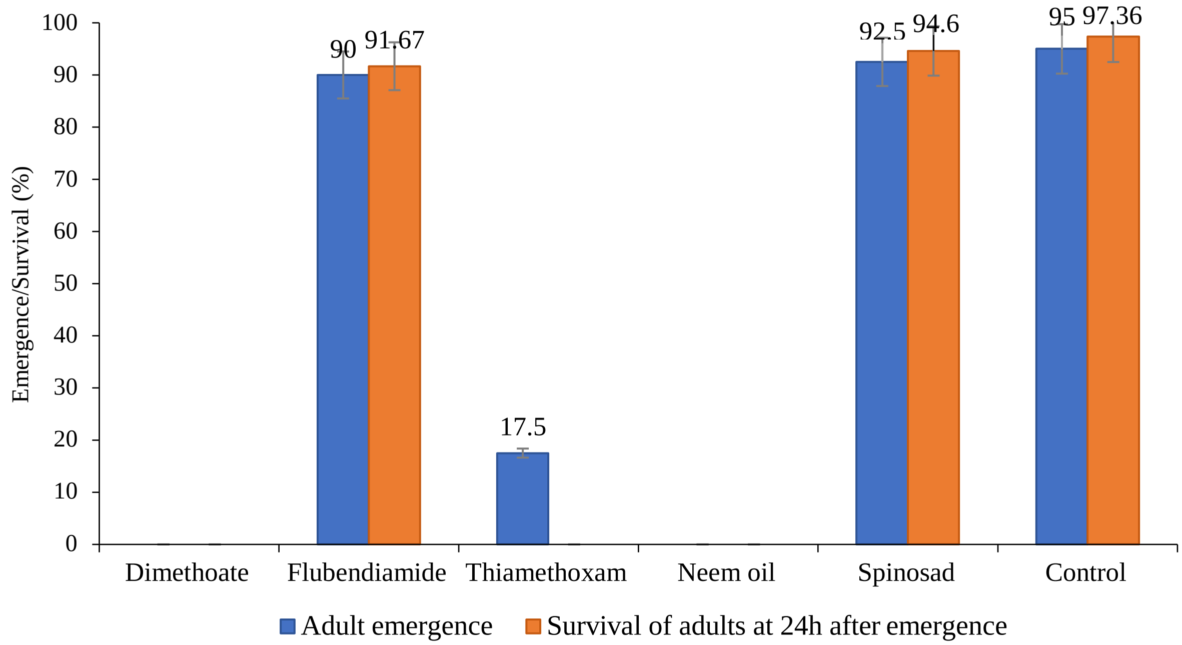

Fig 3: Effect of insecticides on pupae of *Cheilomenes sexmaculata*

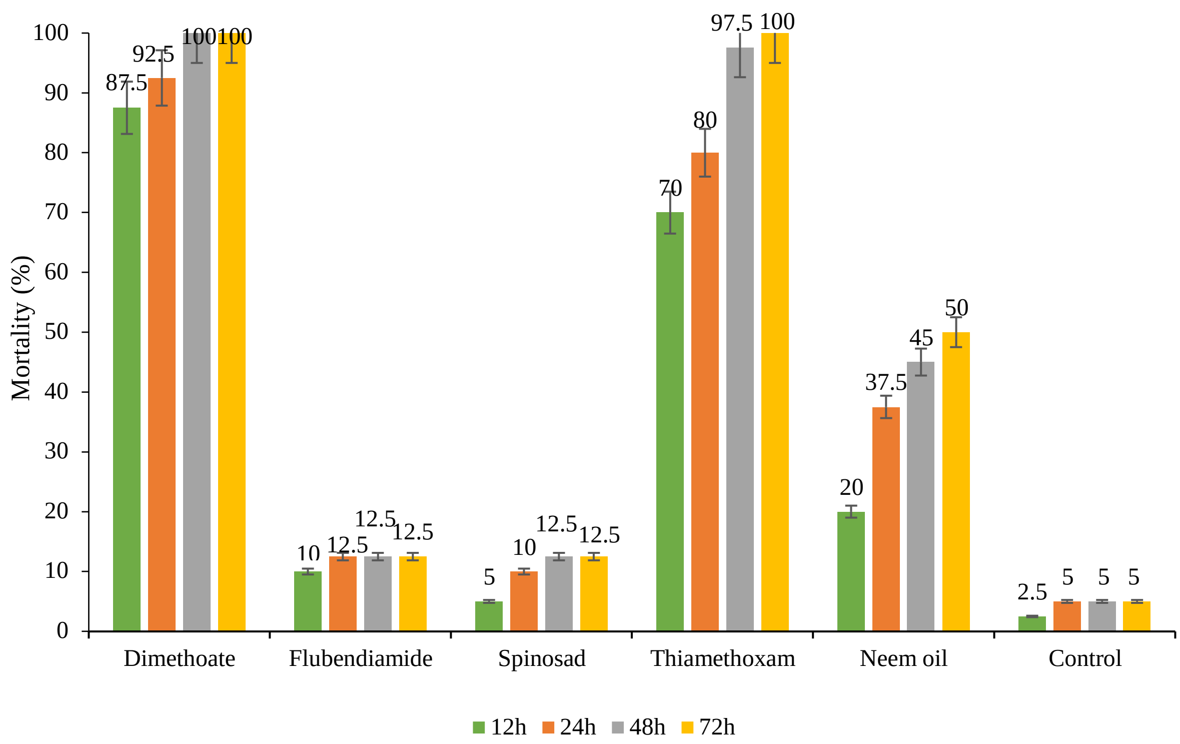

Fig 4: Effect of insecticides on adults of *Cheilomenes sexmaculata*

5.2 Sublethal effects of insecticides on *Cheilomenes sexmaculata*

The effect of insecticides on development and reproduction of *C. sexmaculata* was studied by exposing 0 to 24h old first instar grubs to insecticides at $^{1}/_{10}{}^{th}$ of the field dose.

5.2.1 Effect of insecticides on survival and development of *Cheilomenes sexmaculata*

After exposure to insecticide at $^{1}/_{10}{}^{th}$ of the field dose, none of the grubs survived in dimethoate. The survival of grubs in neem oil and thiamethoxam was 18.30 and 20.00 per cent respectively which were significantly higher than dimethoate. Flubendiamide and spinosad recorded significantly higher survival of 82.50 and 84.17 per cent respectively among the insecticides but were significantly lower to control.

Developmental time of *C. sexmaculata* varied to a certain extent after exposure of first instar grubs to insecticides at sublethal doses. The total developmental time was prolonged by two days after exposure of first instar grubs to neem oil (0.3%). The exposure to thiamethoxam prolonged the pre-adult period of *C. sexmaculata* nearly 2 days (Fig.5). Whereas, Flubendiamide and spinosad had no significant effect on the developmental time of *C. sexmaculata.*

Similar studies on evaluation of toxicity of insecticides at sublethal doses of neem oil on *C. sexmaculata* are lacking for a discussion. However, the developmental period of the spotless ladybird beetle, *Cycloneda sanguinea* Linnaeus was reported to be unaffected after exposure of second instar grubs to neem oil even at 0.5 per cent concentration. This variation might be attributed to the stage, second instar being less vulnerable compared to first instar grubs (da Silva and Martinez, 2004).

The effect of thiamethoxam at sublethal concentration, LC_{10} on coccinellid predators was documented earlier with prolongation in duration of fourth instar grub and reduction in the pupal period of aphidophagous coccinellid, *H. variegata* (Rahmani and Bandani, 2013). Though literature pertaining to the sublethal effect of flubendiamide was not available, a previous record by Nawaz *et al.* (2017) with chlorantraniliprole, an anthralic diamide on significant prolongation of larval and pupal duration of *H. axyridis* after exposure of second instar grubs to the insecticide at LC_{30} was not in line with the present finding. The variation could have been due to the different chemical and test species. The present findings with spinosad are in conformity with the observation of Galvan *et al*. (2005) that spinosad at 10 per cent of field rate had no influence on developmental period of *H. axyridis*. However, according to them, significant extension of development period was observed after exposure to 25 and 50 per cent of the field rates.

The prolongation of developmental period upon insecticide exposure occurs as the immature stages spent more energy to detoxify the chemicals rather than diverting on growth and development (Hanning *et al.*, 2009).

5.2.2 Effect of insecticides on reproduction of *Cheilomenes sexmaculata*

Exposure of 0-24h old first instar grubs to insecticides at $^{1}/_{10}{}^{th}$ of field recommended dose had no influence on pre-oviposition and post-oviposition period. However, flubendiamide, thiamethoxam and neem oil significantly reduced the oviposition period and adult longevity of *C. sexmaculata* (Fig.6) while spinosad had no significant effect. In contrast, significant increase in the pre-oviposition period was reported by Rahmani and Bandani (2013) with thiamethoxam after exposing third instar grubs of *H. variegata* and by Nawaz *et al.* (2017) with chlorantraniliprole, a diamide after exposing second instar grubs of *H. axyridis* at LC_{30}. However, the findings of Nawaz *et al.* (2017) on reduction in adult longevity at LC_{30} is in confirmity with present results.

The present finding on pre-oviposition period after exposure to neem oil is in line with observations of da Silva and Martinez (2004) where neem oil even at 5 per cent concentration had no significant influence on the pre-oviposition period of the predator, *C. sanguinea* when the second instar grubs were exposed. However, their observation that neem oil had no influence on adult longevity is a contradiction to the present finding, where the adult longevity was significantly lower than control.

All the insecticides significantly reduced the fecundity as well as fertility of *C. sexmaculata* when the first instar grubs were exposed to the insecticides at $1/10^{th}$ of field rate. The reduction in egg hatching was also significant except for spinosad.

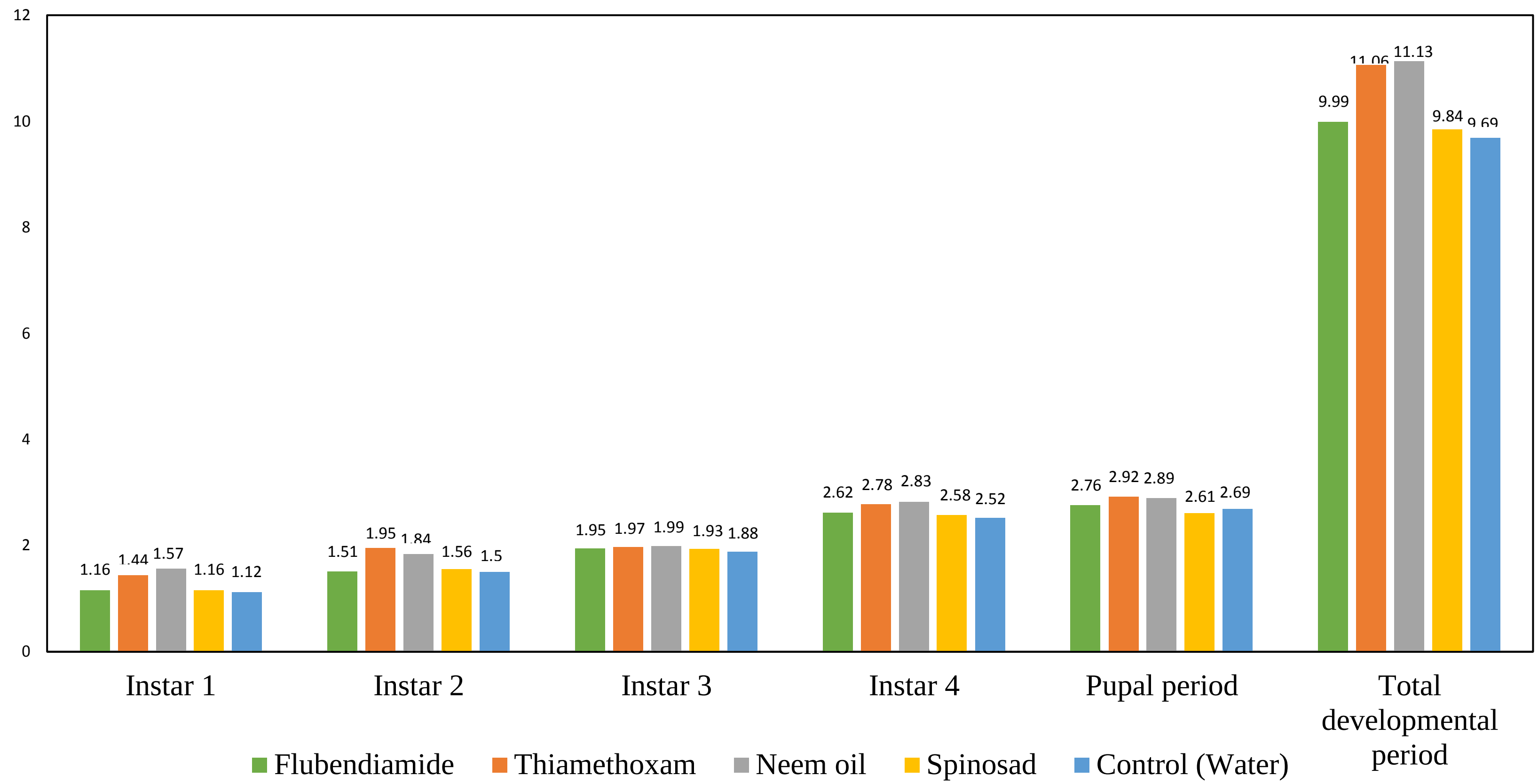

Fig 5: Effect of insecticides at sublethal doses on pre-adult development of *C. sexmaculata*

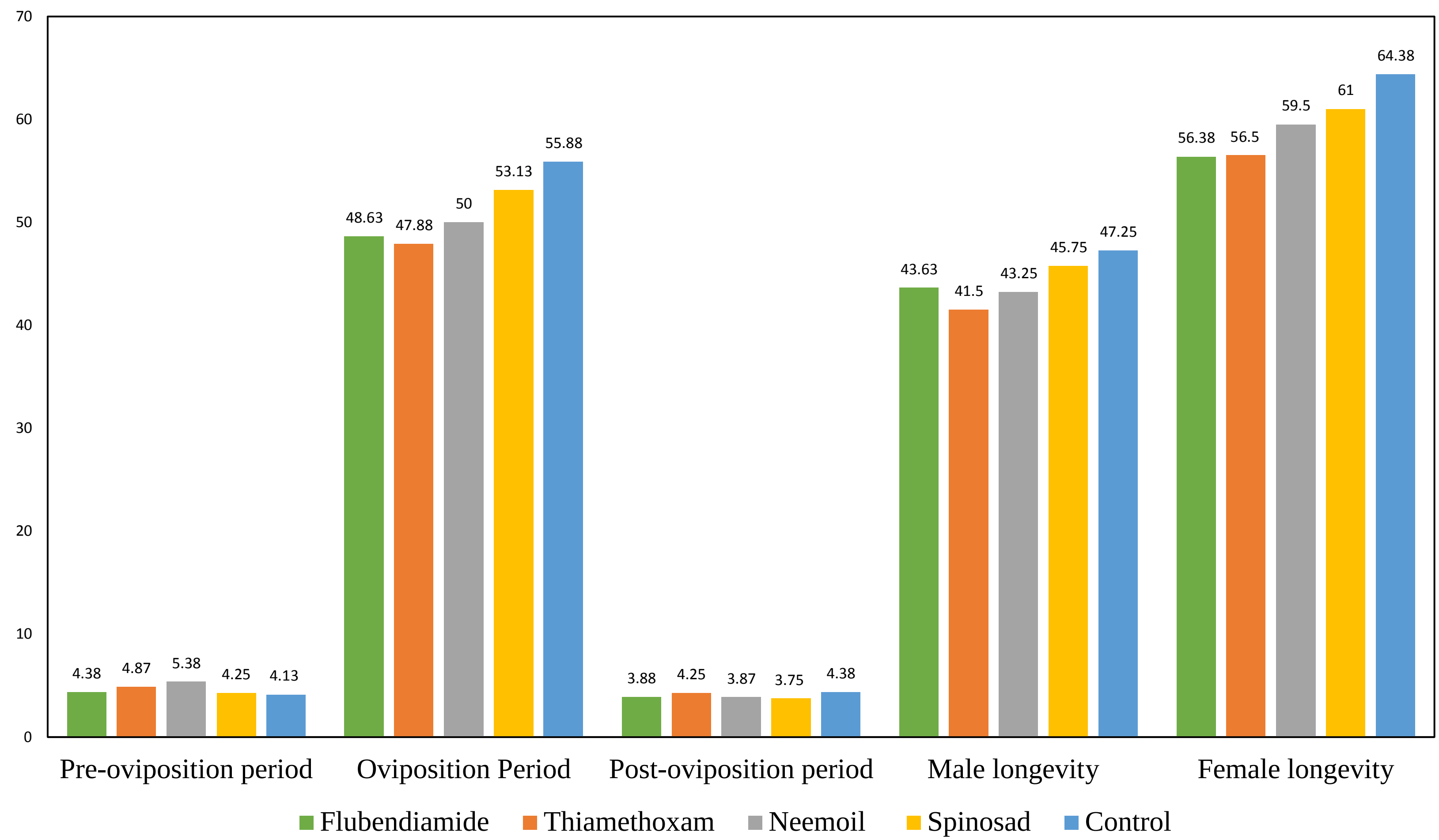

Figure 6: Effect of insecticides at sublethal doses on post adult development of *C. sexmaculata*

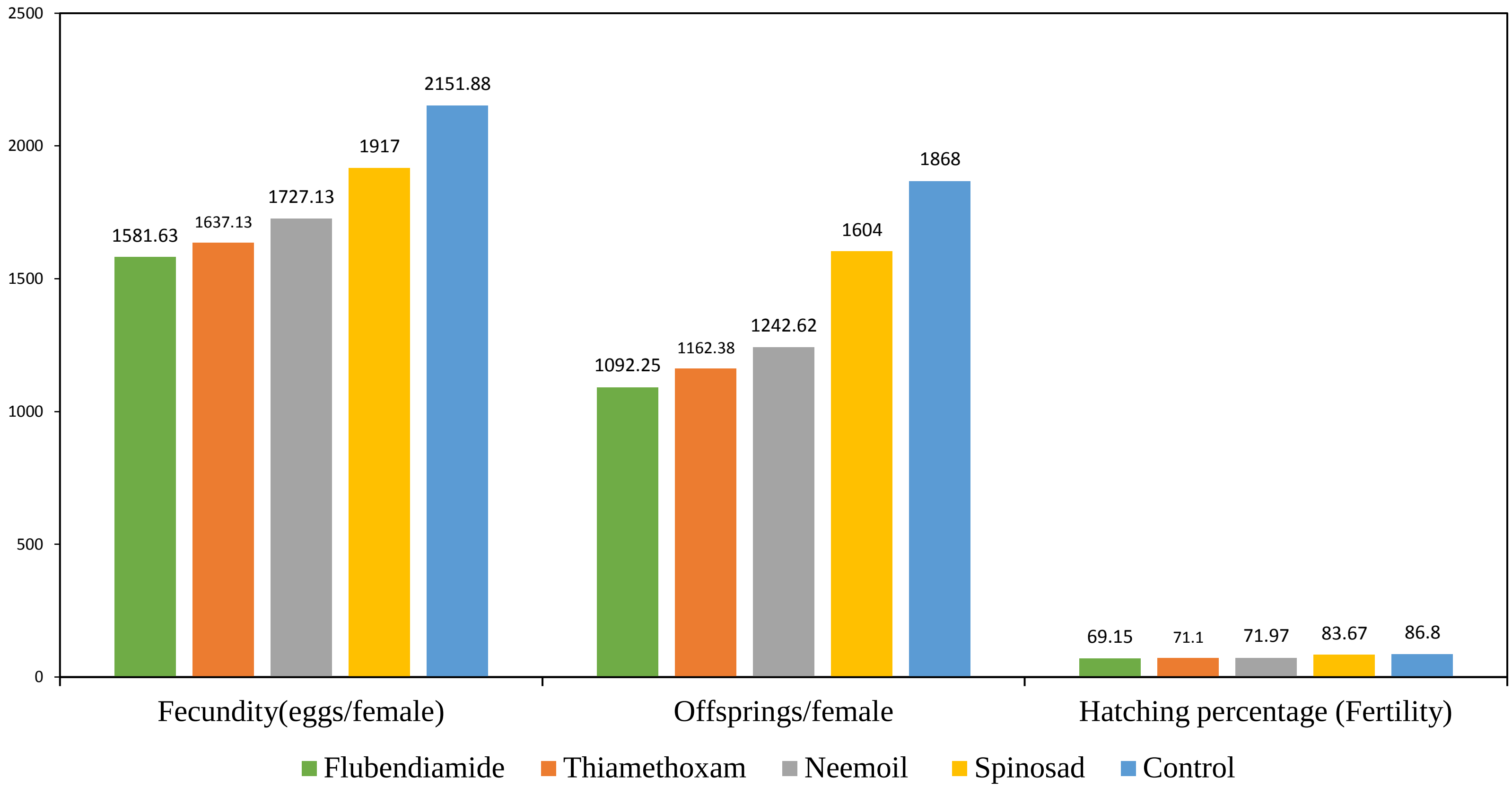

Figure 7: Effect of insecticides at sublethal doses on reproduction of *C. sexmaculata*

Flubendiamide caused maximum reduction in fecundity with 1581.63±80.13 eggs/female and fertility with 1092.25±67.02off springs/female resulting only 69.15 per cent hatching compared to respective values of 2151.88±27.31eggs/female and1868±26.5offsprings/female with 86.8 per cent hatching in control. The fecundity in thaiamethoxam, neem oil and spinosad treatments were 1637.13±92.10, 1727.13±51.75 and 1917.00±89.43 eggs/female with 1162.38±58.92, 1242.62±46.79 and 1604.00±72.89 offsprings/ female respectively.

In contrast to present findings with flubendiamide, there was report that no reduction in fecundity and fertility was documented in coccinellid predator, *A. bipunctata* after exposure of fourth instar grubs to flubendiamide even at field recommended rate (Garzon *et al*., 2015).

The earlier records on decrease or complete loss in fecundity and fertility of *C. septempunctata* with commercial neem formulations, after exposure of larvae to neemix (Banken and Stark, 1998) and after exposure of adults to Neemraj super (Regmi *et al.*, 2019) is broadly agreeable to the present findings with neem oil, where fecundity and fertility were significantly reduced (Fig.7).

Reduction in fecundity and fertility was observed after exposure to thiamethoxam also. The observations of Rahmani and Bandani (2013) on reduction in fecundity and fertility of *H. variegata* after exposure of late instar grubs to sublethal doses of thiamethoxam agrees with the present findings. On contrary, an increase in fecundity with reduction in number of offsprings/female was documented by Fernandez *et al.* (2016) when the last instar grubs of *C. sanguinea* were exposed to thiamethoxam at LC_{20}.Exposure to spinosad had no significant influence on reproductive parameters except for fecundity and offsprings/female. Cole *et al.* (2010) also recorded similar findings with spinosad after exposure of first instar larvae, but of *C. transversalis.* While Galvan *et al.* (2005) observed reduction in fertility with no effect on fecundity, after exposure of adults of *H. axyridis* to both recommended and half of the recommended field rate of spinosad. Similarly, Depalo *et al.* (2017) also observed significant reduction in fertility with no difference in fecundity of *A. bipunctata* after exposure of adults to spinosad at field recommended rate.

When fecundity and fertility were plotted against age of the adults of *C. sexmaculata*, it was observed that the fecundity varied greatly among treatments including control but fertility was always higher in control at all the age intervals (Fig. 8). Even though the fecundity observed in neem oil was higher than all other treatments including control till 20th day of age, the fertility was always lower to control. Except in spinosad, after 30th day of age, the fecundity drastically reduced in all the insecticide treatments, compared to control.

With the exception of spinosad, all the evaluated insecticides at sublethal concentration adversely affected the developmental and reproductive biology of *C. sexmaculata*.

According to Casida and Durkin (2013), neonicotinoids exhibited a variety of lethal and sublethal effects on behavior such as feeding, oviposition and fecundity in arthropods. Sublethal effects of organophosphate insecticide residues resulted in an immediate reduction in the efficiency of coccinellids to locate their prey (Singh *et al.,* 2004). The study clearly indicates on the caution to be taken while inclusion of organophosphates and neonicotinoids in integrated management programmes involving a system with *C. sexmaculata*.

5.3 Evaluation of insecticides under field conditions

5.3.1* Aphids, *Aphis craccivora

Application of thiamethoxam and dimethoate was effective recording less aphid population ranging from 0 to 3.2 numbers/10cm twig in cowpea at different intervals of observations (Fig. 8) as against 33.88 to 41.08 numbers/10cm twig recorded in control. In plots treated with spinosad, neem oil and flubendiamide, though drastic reduction in aphid population was observed at one day after spray with 19.55 to 26.05numbers/10cm twig, the population was persistent till 15 days of spray without much further reduction and were comparatively less effective when compared to thiamethoxam and dimethoate. However, aphid population was significantly lower ranging from 17.00 to 26.05 numbers/10cm twig during post spray observations compared to 33.88 to 41.08 numbers/10cm twig observed in control. The observations of Yadav *et al.* (2015) with maximum reduction in aphid population till 15 days after

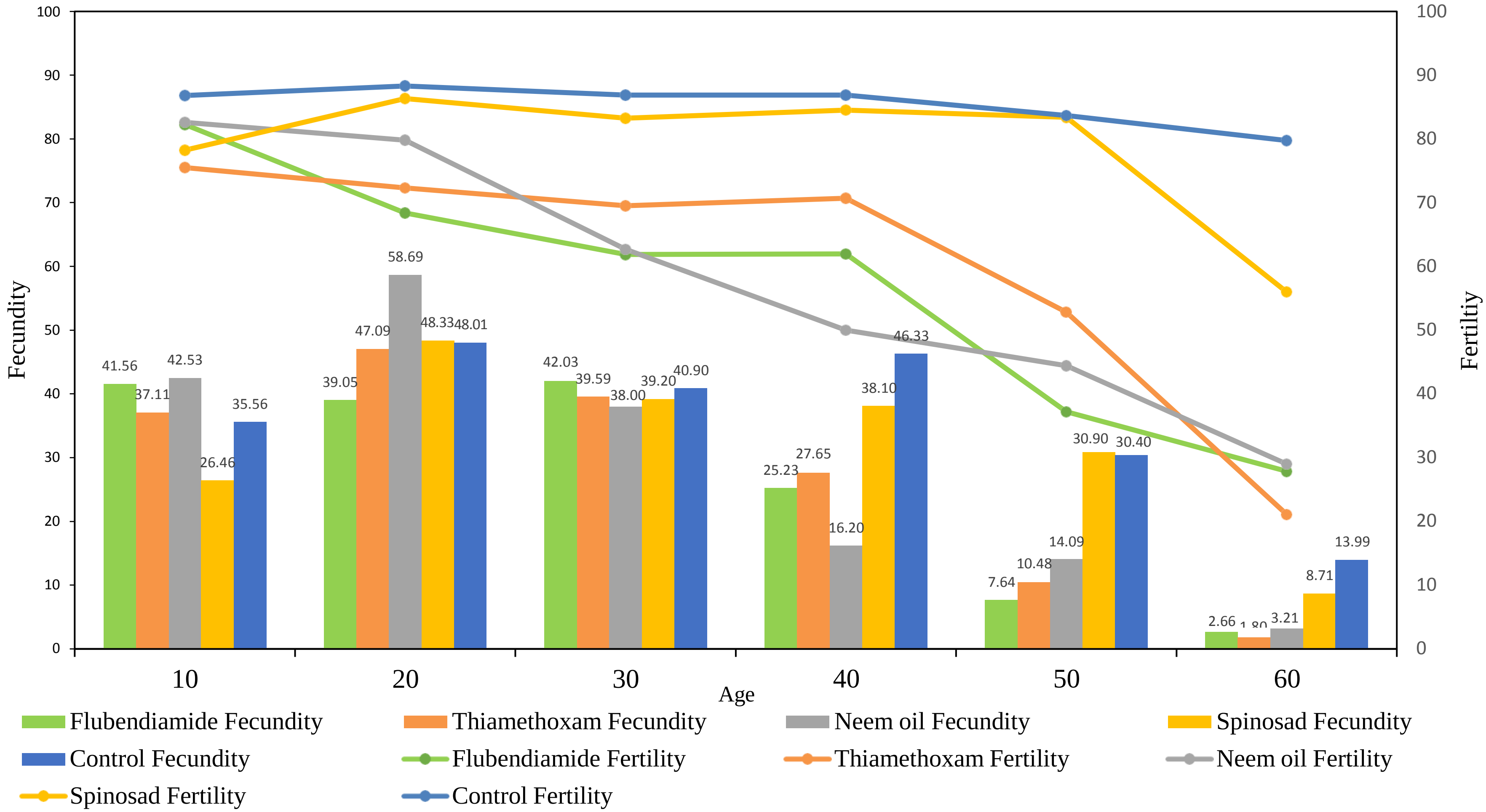

Figure 8: Effect of insecticides on fecundity and fertility of *C. sexmaculata* at different age intervals

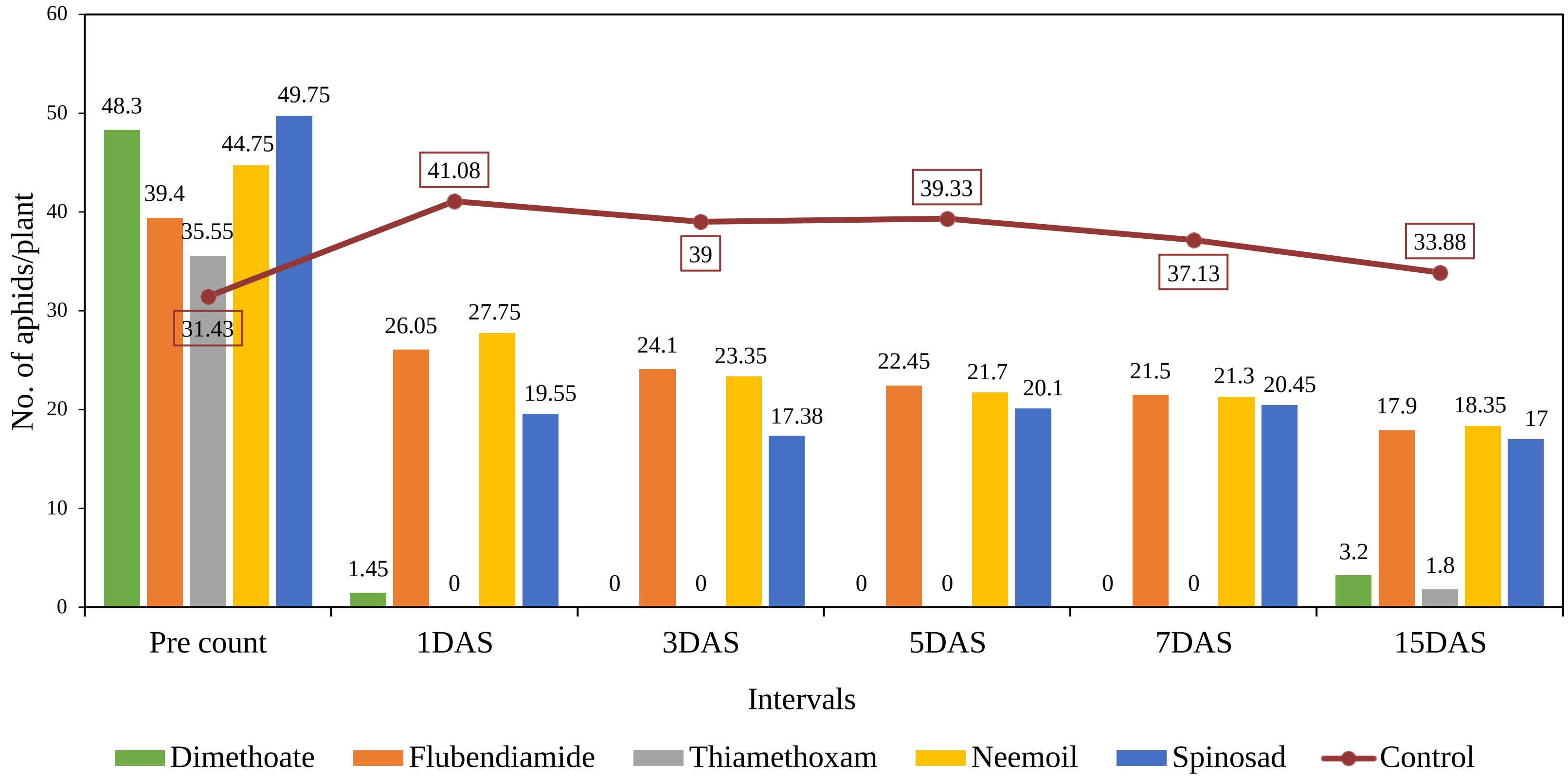

Fig 9: Effect of insecticides *Aphis craccivora* in field

application of dimethoate (0.03%) and thiamethoxam (0.025%) in cluster bean confirm the present results. Similar findings were also documented by Choudhary *et al.* (2017) in cowpea with maximum reduction in aphid population till 10 days after spraying. The effectiveness on aphids could be attributed to the broad-spectrum activity of neonicotinoids to sucking pests (Chao *et al*., 1997) and the more toxic effects of plant metabolites produced by neonicotinoid insecticides than the original insecticide molecules (Gervais *et al*., 2010).

Varied results were recorded on effectiveness of spinosad on aphids. As per Akbar *et al.* (2010), spinosad was least effective against cabbage aphid (*Myzus persicae*), with only 11.26 per cent reduction but 75.07 per cent reduction of *A. craccivora* was reported by Ghosh (2020) in groundnut. The variation in effectiveness might be due to the difference in crop system.

The observations of Ghelani *et al.* (2014) with neem oil (1%) providing 43.8 per cent reduction of *A. gossypi* in cotton is in line with present result. However commercial neem formulations were reported as more effective against *A. craccivora* with 85.67 per cent reduction in cowpea (Soratur *et al.* 2017) and 71.77 per cent reduction in groundnut (Ghosh, 2020). According to Rajwat *et al.* (2017), flubendiamide was not an effective insecticide against aphids in blackgram even with two sprays at 15 days interval which is also in accordance with the present findings.

5.3.1 *Cheilomenes sexmaculata*

Thiamethoxam and dimethoate completely suppressed the population of *C. sexmaculata* till 15 days after spray while a gradual increase in population was observed with 2.15 *C. sexmaculata*/plant at 15th day in control. The population of *C. sexmaculata* in plots treated with flubendiamide neem oil and spinosad were on par with control except for 1st day of spray. The restoration of predator population was observed within three day after spray and was on par with control from 3rd day onwards.

The statement of Sakthivel and Qadri (2010) with least coccinellid population of 8.53 numbers/plant at 10 days after application of dimethoate in mulberry plots and the findings of Ghelani *et al.* (2014) reporting least population of coccinellids with 1.32 numbers/plant at 15th days after application of thiamethoxam (0.01%) in cotton is in agreement with the present findings. The current findings with flubendiamide closely matches with Sudhanan *et al.* (2017) who commented only short-term reduction of predatory coccinellid population following application of flubendiamide in sugarcane fields, as the population restored within a fortnight. The findings of Ghelani *et al.* (2014) on the occurrence of more coccinellid predators in cotton field after spray with neem oil and that of Natikar *et* al. (2017) in soybean field after spray with spinosad are in accordance with the present results.

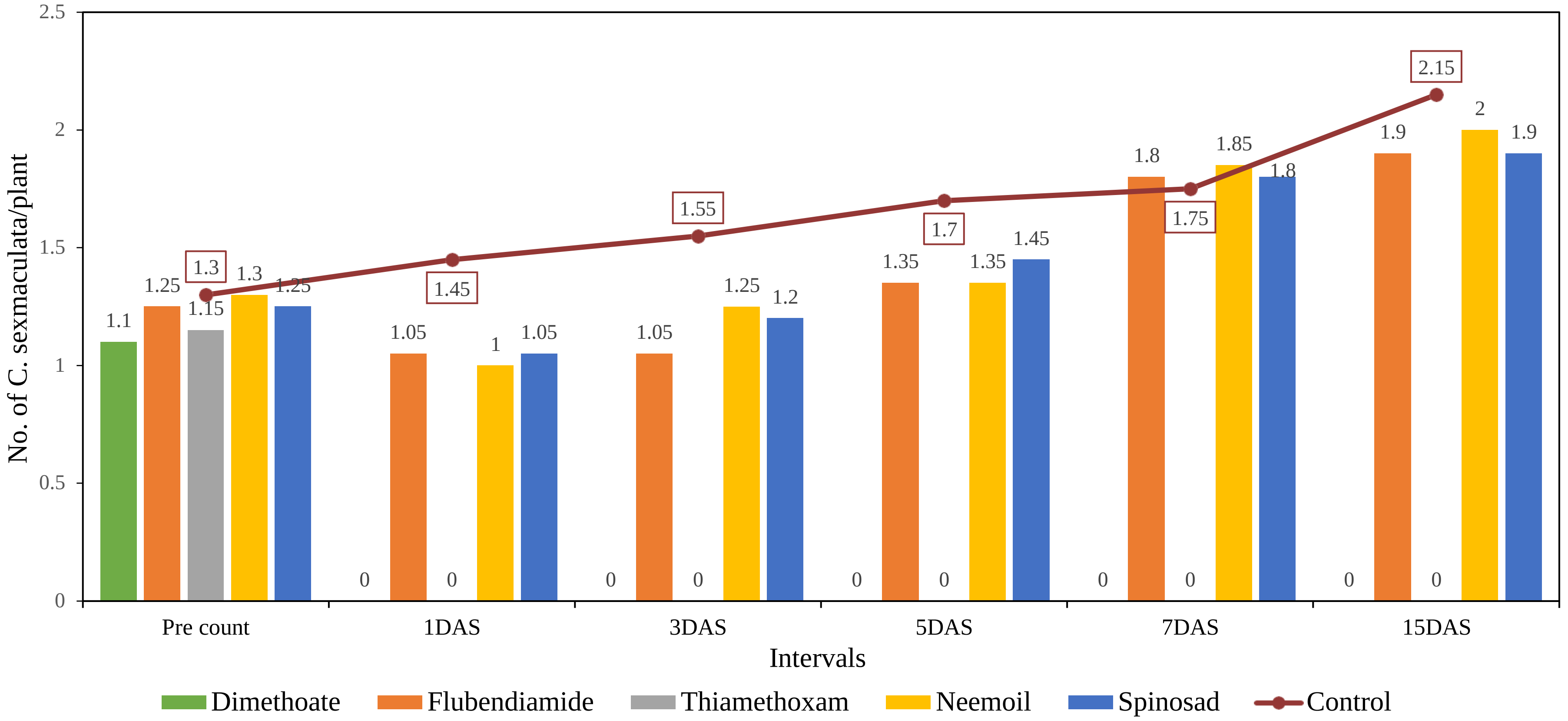

Fig 10: Effect of insecticides *Cheilomenes sexmaculata* in field

Summary

6. SUMMARY

The research study entitled "Toxicity of insecticides to *Cheilomenes sexmaculata* Fabricius (Coleoptera: Coccinellidae)" was carried out at the Department of Agricultural Entomology, College of Agriculture, Vellanikkara, Thrissur, Kerala during 2018- 2020. The objectives were to evaluate the toxicity of insecticides to *C. sexmaculata* in the laboratory and to assess the impact of insecticides on field efficacy of *C. sexmaculata* in cowpea. Direct toxicity was tested by exposing the life stages to insecticides at the field recommended dose and the effect at sublethal level was assessed by exposing 12h old grubs to insecticides at $^{1}/_{10}{}^{th}$ of the field recommended dose.

The salient findings of the present study are summarized below

- Dimethoate at recommended dose was harmful to all the life stages of *C. sexmaculata* causing complete mortality.
- Thiamethoxam was safe to egg stage of the predator but was moderately harmful to pupae with 82.50 per cent mortality and was harmful to grub and adult stages with 100 per cent mortality.
- Neem oil was categorized as harmful to eggs and pupae as it inflicted 100 per cent mortality to those stages, but was slightly harmful to adults and harmless to grubs of *C. sexmaculata* with 50.00 and 32.50 per cent mortality respectively.
- Flubendiamide and spinosad were harmless to all the stages of *C. sexmaculata* with 22.50 and 15.00 per cent mortality in eggs, 10.00 and 7.50 per cent mortality in grubs and pupae and 12.50 per cent mortality in adults of *C. sexmaculata.*
- The insecticides, tested at $^{1}/_{10}{}^{th}$ of the field dose for assessing toxicity at sublethal level, had effect on development and reproduction either by increasing the developmental period of immatures or by impairing the reproductive parameters.
- Exposure to thiamethoxam and neem oil prolonged the developmental period of *C. sexmaculata* nearly by 2 days and reduced the oviposition period by 8 and 5 days respectively. The longevity of the adults was also reduced by 4-8 days, after exposure to these insecticides. The reduction in fecundity was 37.7 and 33.5 per cent respectively and fertility was reduced by 15 per cent.

- Flubendiamide had no influence on the developmental periods of *C. sexmaculata* but reduced the oviposition period by 7 days, male and female longevity by 4 and 8 days respectively, fecundity by 41 per cent, and fertility by 14 per cent.
- Spinosad showed minimal effects on the developmental and reproductive parameters. Though fecundity was less compared to control, the eggs were fertile as normal.
- Dimethoate and thiamethoxam were effective in reducing aphid population with only 3.20 and 1.80 aphids/10cm twig respectively. but *C. sexmaculata* population was not observed in the treated plots till 15th day after spray.
- Flubendiamide, neem oil and spinosad reduced the aphid population to 17.90, 17.35 and 17.00 numbers/10cm twig, However, there was a gradual increase in the predator population after third day of spray from 1.05, 1.25 and 1.20 numbers/plant to 1.90, 2.00 and 1.90 numbers/plant at 15 DAS in flubendiamide, neem oil emulsion and spinosad treatments, respectively.
- The study points out the deleterious effects of the insecticides, dimethoate and thiamethoxam recommended against cowpea aphid, to *C. sexmaculata*. The findings also caution use of the botanical, neem oil when *C. sexmaculata* is active in the field as it can cause significant mortality of the predator. However, spinosad and flubendiamide are harmless to *C. sexmaculata*.

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Abstract

TOXICITY OF INSECTICIDES TO *Cheilomenes sexmaculata* FABRICIUS (COLEOPTERA: COCCINELLIDAE)

by

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(2018-11-056)

ABSTRACT OF THE THESIS

Submitted in partial fulfillment of the requirement for the degree of

Master of Science in Agriculture

Faculty of Agriculture

Kerala Agricultural University

DEPARTMENT OF AGRICULTURAL ENTOMOLOGY

COLLEGE OF AGRICULTURE

VELLANIKKARA, THRISSUR – 680656

KERALA, INDIA

2021

Abstract

Biocontrol represents a sustainable and economically feasible way of pest management. However, under high herbivore pressure, bioagents alone are often unable to provide quick reduction of pest population, calling for insecticide based interventions. Chemical control, though designed to cause rapid mortality of target pests also adversely impact the natural enemies, leading to reduction in their growth, survival and reproduction. Hence it becomes imperative to evaluate the effects of insecticides on efficient natural biocontrol agents, to select safe insecticides and use them harmoniously.

The six spotted zigzag lady beetle, *Cheilomenes sexmaculata* (Coleoptera: Coccinellidae), is an efficient natural enemy of aphid species in various crops due to its voracious feeding habit and density responsiveness. It is very common in cowpea and plays a major regulatory role against the cowpea aphid, *Aphis craccivora*. However, biocontrol by *C. sexmaculata* is often destabilized by the indiscriminate use of insecticides. Selection of insecticides safe to *C. sexmaculata* requires knowledge on direct and indirect effects of insecticides on the growth and development of the predator. Hence five insecticides and a botanical that are recommended in cowpea for pest management were evaluated for their toxicity to *C. sexmaculata* in the laboratory as well as their impact on field efficacy of the predator.

Assessment of direct lethal impact, by exposing the life stages of the predator to field doses of insecticides, revealed the highly toxic nature of dimethoate and thiamethoxam to grub, pupa and adult stages of *C. sexmaculata*, leading to 100.00 per cent mortality. Exposure to dimethoate caused complete mortality of eggs while thiamethoxam was harmless to eggs with only 22.50 per cent mortality. Neem oil emulsion (3%) was found harmful to the non-feeding egg and pupal stages by completely inhibiting hatching and adult eclosion. In grub and adult stage, neem oil caused a mortality of 32.5 and 50 per cent respectively. Flubendiamide and spinosad were relatively harmless to all stages, inducing 22.50 and 15.00 per cent mortality in eggs, less than 10 per cent mortality to grubs and pupae, and 12.5 per cent mortality to adults of *C. sexmaculata.*

Exposure of first instar grubs to sub lethal doses of insecticides also revealed adverse effects on development and reproduction of the predator. While exposure to

dimethoate led to complete mortality of grubs, exposure to thiamethoxam and neem oil prolonged the development period by two days and reduced the oviposition period by eight and five days, respectively. Adult longevity also was reduced by 4-8 days, after exposure to these insecticides. The fecundity was also reduced to 1637.13±92.10 and 1727.13±51.75 eggs/female respectively in thiamethoxam and neem oil as compared to 2151.88±27.31 in control. In both thiamethoxam and neem oil treatments, fertility was reduced by 15.00 per cent of 1868±26.50 offsprings/female recorded in control. Flubendiamide had no influence on the development period but reduced the oviposition period by seven days and male and female longevity by four and eight days respectively. There was a reduction in fecundity and fertility by 27.00 and 14.00 per cent respectively as well. Spinosad had no significant influence on developmental period of immatures but reduced the fecundity to 1917.00±89.43 eggs/female.

Evaluation of insecticides in cowpea field demonstrated the effectiveness of dimethoate and thiamethoxam in managing aphids with drastic reduction in population till 15th day with 3.20 and 1.80 aphids/10cm twig compared to 33.88 aphids in control 15 days after spray. The plots treated with flubendiamide, neem oil emulsion and spinosad recorded 17.90, 17.35 and 17.00 aphids/10cm twig respectively at 15 DAS and were inferior to dimethoate and thiamethoxam in aphid management. However, there was a gradual increase in the predator population after third day of spray from 1.05, 1.25 and 1.20 numbers/plant to 1.90, 2.00 and 1.90 numbers/plant at 15 DAS in flubendiamide, neem oil emulsion and spinosad treatments respectively compared to control (1.55 and 2.15 numbers/plant). This indicates the safety of these insecticides to predator in field. Whereas, no predator population was observed in dimethoate and thiamethoxam treatments till 15 DAS.

The study provides a measure of safety of insecticides in an IPM programme with *C. sexmaculata*, a promising bioagent against the key pest, *A. craccivora* in cowpea. The results point out the deleterious effect of dimethoate and thiamethoxam to *C. sexmaculata*. Botanical, neem oil is harmless to grubs but harmful to other life stages of the predator and also adversely affects growth and reproduction at sublethal doses. Flubendiamide, though harmless at field doses, alters the reproductive parameters at sublethal level. Spinosad, with only minimal reduction in fecundity, is harmless to *C. sexmaculata*.