

**IMPACT OF CERTAIN NEWER INSECTICIDES ON
SOIL FAUNAL DIVERSITY IN FRENCH BEAN
(*Phaseolus vulgaris* L.)**

A Thesis
Submitted to the
Assam Agricultural University

In partial fulfilment of the requirements for the degree of
MASTER OF SCIENCE (AGRICULTURE)
IN
ENTOMOLOGY



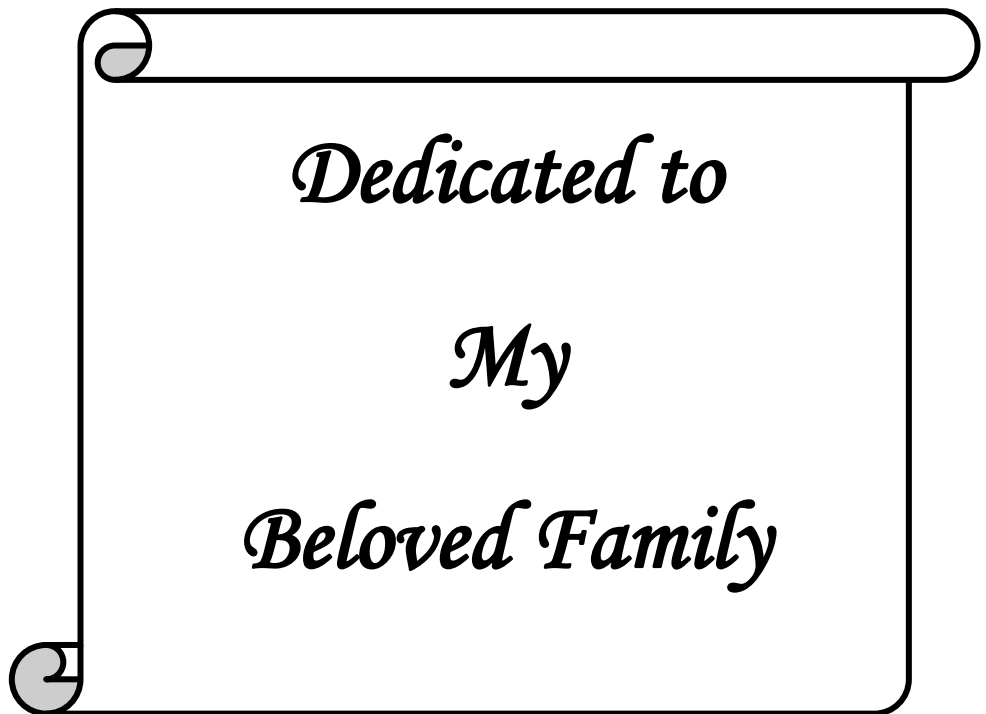
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JORHAT- 785013, ASSAM
DECEMBER, 2021**



Dedicated to

My

Beloved Family

ASSAM AGRICULTURAL UNIVERSITY
Faculty of Agriculture

CERTIFICATE – I

This is to certify that the thesis entitled “**Impact of certain newer insecticides on soil faunal diversity in French bean (*Phaseolus vulgaris* L.)**” submitted to the Faculty of Agriculture, Assam Agricultural University, in partial fulfillment for the degree of **Master of Science (Agriculture) in Entomology** is a record of research work carried out by **Gourismita Nath (Roll No. 2019-AMJ-56)** under my personal supervision and guidance.

All helps received by her have been duly acknowledged.

No part of this thesis has been reproduced elsewhere for any degree.

Dated: Jorhat

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CERTIFICATE – II

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ABSTRACT

Laboratory and field experiments were carried out in the Department of Entomology; Department of Plant Pathology and Horticulture Experimental Farm of Assam Agricultural University, Jorhat during 2020-21 to study the impact of certain newer insecticides on soil faunal diversity in French bean. Six newer insecticides *viz.*, clothianidin 50 WDG, fipronil 0.3 G, thiamethoxam 25 WG, imidacloprid 70 WG, chlorantraniliprole 0.4 GR and fipronil 40%+ imidacloprid 40% WG were selected for conducting the experiment. Sampling for soil macro and microarthropods as well as soil microbial population were done at pre-treatment, 15, 30, 45 60 and 75 days after treatment (DAT). The soil macroarthropods were sampled using pitfall traps whereas the microarthropods were extracted through Tullgren Funnel. Assessment of soil microbial population was done by following the standard pour plate method.

Experimental results revealed the hymenopterans as the most dominant group (54.74%) among the different soil macroarthropods observed prior to the application of insecticides followed by Coleoptera (13.68%) and Araneae (11.57%). Among the soil microarthropods, the abundance of Collembola and Oribatida were recorded to be 64.72 and 35.28 per cent, respectively in the pre-treated plots. The number of soil macroarthropods was ranged between 89.00 to 95.33/plot prior to the application of insecticides which showed statistical parity with each other. At 15 DAT, the abundance of soil macroarthropods in all the treated plots were reduced significantly (47.33-52.67 numbers) compared to the untreated control (89.33 numbers). However, maximum number of soil macroarthropods/ plot was recorded in chlorantraniliprole 0.4 GR treated plots (52.67) followed by clothianidin 50 WDG (50.33) and thiamethoxam 25 WG (49.67) treated plots. Perusal of data in respect of 30, 45, 60 and 75 days after treatment also showed a significant decrease in the numbers of soil macroarthropods as compared to the control, however, a gradual increase in the total number of soil macroarthropods was observed in each treated plots from 30 DAT onwards. Soil microarthropods obtained in different plots prior to the application of insecticides was ranged between 458.33 to 555.56 numbers/sq. m. All the insecticidal treatments did not exhibit any significant ($p=0.05$) impact on soil microarthropod population during the experimental period.

Assessment of soil microbial population revealed a significant decrease in the total bacterial (58.67-62.67) and fungal (48.00-51.67) population ($\text{cfu} \times 10^4/\text{g}$ of soil) in all the treated plots at 15 DAT as compared to the control (98.67 bacterial and 91.67 fungal $\text{cfu} \times 10^4/\text{g}$ of soil, respectively). However, maximum number of both the bacterial and fungal colonies ($\text{cfu} \times 10^4/\text{g}$ of soil) were recorded in chlorantraniliprole 0.4 GR treated plots (62.67 and 51.67, respectively) followed by clothianidin 50 WDG (62.00 and 51.00, respectively) and thiamethoxam 25 WG (61.33 and 50.33, respectively) treated plots. A similar trend of results were also observed while the total population of bacterial and fungal colonies were assessed at 30, 45, 60 and 75 DAT. However, a gradual increase in the total bacterial and fungal population was observed in each treated plots from 30 DAT onwards showing the ability to overcome the toxic effect of insecticides by the microbes in the subsequent period of the experiment.

CONTENTS

CHAPTER NO.	TITLE	PAGE NO.
I	INTRODUCTION	1 – 4
II	REVIEW OF LITERATURE	5 – 14
III	MATERIALS AND METHODS	15 – 24
IV	EXPERIMENTAL FINDINGS	25 – 44
V	DISCUSSION	45 – 50
VI	SUMMARY AND CONCLUSION	51 – 54
	BIBLIOGRAPHY	55 – 61
	APPENDIX	i – ii

LIST OF TABLES

Table No.	Title	Page No.
3.1	Details of treatments used in the experiment	18
4.1	Abundance of different soil arthropods recorded in the pretreated plots	26
4.2	Effect of certain newer insecticides on soil macroarthropod population (numbers/plot) at different days intervals	31
4.3	Effect of certain newer insecticides on soil microarthropod population (numbers/sq. m.) at different days intervals	35
4.4	Effect of certain newer insecticides on total bacterial population (cfu $\times 10^4$ /g of soil) at different days intervals	38
4.5	Effect of certain newer insecticides on total fungal population (cfu $\times 10^4$ /g of soil) at different days intervals	42

LIST OF FIGURES

Figure No.	Title	Page No.
3.1	Layout of the field experiment	16
4.1	Abundance of different soil macroarthropods recorded in the pretreated plots	27
4.2	Abundance of different soil macroarthropods recorded in the pretreated plots	27
4.3	Effect of certain newer insecticides on soil macroarthropod population (numbers/plot) at different days intervals	32
4.4	Effect of certain newer insecticides on soil microarthropod population (numbers/sq. m.) at different days intervals	36
4.5	Effect of certain newer insecticides on total bacterial population (cfu $\times 10^4$ /g of soil) at different days intervals	39
4.6	Effect of certain newer insecticides on total fungal population (cfu $\times 10^4$ /g of soil) at different days intervals	43

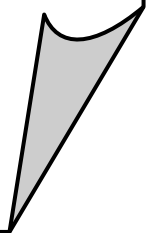
LIST OF PLATES

Plate No.	Title	Page No.
3.1	Layout of the experiment plot	17
3.2	Installation of pitfall traps	20
3.3	Collection of soil samples for extraction of soil microarthropods	20
3.4	Extraction of soil microarthropods from collected soil samples	21
3.5	Assessment of soil microbial population	23
4.1	Coleopteran insects collected through pitfall traps	28
4.2	Other soil macroarthropods observed during the experimental period	29
4.3	Soil microarthropods collected during the experimental period	30

LIST OF ABBREVIATIONS

%	: per cent
/	: per
μ	: micron
AM	: ante meridiem
°C	: Degree centigrade
CD	: Critical difference
cm	: Centimeter
DAT	: Days after treatment
EC	: Emulsifiable concentrate
<i>et al.</i>	: <i>Et alli</i> (and others)
etc.	: Et cetra
Fig.	: Figure
g	: Gram
G	: Granule
Gr	: Granule
ha	: Hectare (10,000 square meters)
i.e.	: that is
Kg	: Kilogram
L	: Liter
mg	: Milligram
ml	: Milliliter
pH	: $-\log_{10}[\text{H}^+]$
SC	: Suspension concentrate
SD	: Standard Deviation
S.Ed(±)	: Standard Error of Difference
sq. m.	: Square meter
WDG	: Water Dispersable Granule
WG	: Wettable Granules
<i>viz.</i>	: <i>Vide hiet</i> (Namely)

Introduction... 



CHAPTER I

INTRODUCTION

Soil is a living entity that serves as the primary nutrient base and the unique habitat for multitude of organisms which live together as communities. It supports the rich and diverse plant and animal life as well as the most dynamic sites of biological interactions in nature. Every member of this soil fauna and flora has its individual contribution to the soil community. Each occupies its specific trophic position in the criss-crossing food chains and webs and help in the flow of matter and energy in the ecosystems. Their community work ultimately leads to the humification and maximization of the soil quality. Soil fauna, also recognized as the “Ecosystem Engineers” plays a major role in the decomposition of organic matter, nutrient cycling as well as soil functioning and development (Lavelle *et al.*, 2006). They also act as a potential bioindicator in the soil ecosystem due to their great diversity as well as prompt responses towards the environmental variations. The soil fauna consists of bacteria, fungi, virus, tardigrades, rotifers, mites, proturans, diplurans, collembolans, pauropods, symphylans, millipedes, pseudoscorpions, spiders, isopods, centipedes and many other insect species. Singh and Lal (2001) classified the soil fauna into three different categories *viz.*, micro, meso and macrofauna on the basis of their body size. The microfauna consists only the protozoans, whose body size varies within the range of 20 μ to 200 μ and the mesofauna comprises organisms with body size in between 200 μ to 1cm. Mesofauna includes organisms like collembolans, nematodes, rotifers, tardigrades, spiders, mites, chelonethi (pseudoscorpions), opiliones (harvestmen), enchytraeidae (pot worms), insect larva, smaller millipedes, isopods etc., among which Acari (mites) and Collembola are the most important ones. Soil macrofauna, on the other hand comprises of organisms whose body size is greater than 1cm and it includes lumbricidae, Mollusca, arachnids, hexapods and many other soil dwelling vertebrates.

However, the scenario of soil biota in an agricultural field is quite different as compared to natural ecosystems like forest soil. Many agricultural activities starting from cleaning of land, ploughing, harrowing etc. till the harvesting of the crop exerts an important influence on the soil biota, their activities as well as their diversity

(Menta, 2012). The agricultural practices that use high amounts of external inputs such as inorganic fertilizers, pesticides and other amendments may result in continuous environmental degradation, more particularly of soil which ultimately leads to the depletion of the soil fauna. A plenty of applied chemicals and their degraded products are accumulated into the top 10-15 cm layer of soil (Blasco and Pico, 2009). As the top soil is considered to be the region of greatest activity of soil fauna, the accumulated chemicals or their degraded products may cause disturbances leading to both qualitative and quantitative changes in soil diversity. There are many existing reports that most of the deposited pesticides changed the physico-chemical properties of the soil which ultimately influenced the biological properties including metabolic activity of soil microbial and macrobial communities and the overall soil fauna (Filimon *et al.*, 2015).

India is the 7th largest country of the world where agriculture and its allied sectors provide livelihood for more than 70 per cent of the country's population. Use of high yielding crop varieties together with fertilizers and pesticides have helped the Indian farmers in achieving a substantial increase in the agricultural productivity in the past few decades. However, pest infestation to various agricultural commodities both in the field as well as storage conditions, which is estimated to be approximately 45 per cent of the total annual food production have threatened the livelihood of agriculture dependent people of the country (Dhaliwal *et al.*, 2015). To combat the risk associated with the pest infestation, farmers always try to adopt advance technologies including the use of newer pesticides which significant impacts farmer's economy by improving both quality and quantity of food (El-Naggar and Zidan, 2013). In India, the use of synthetic pesticides started in 1948-49 with the use of Dichloro Diphenyl Trichloroethane (DDT) for malaria control and Benzene Hexa Chloride (BHC) for controlling locust. The production of pesticides started in India in 1952 with the establishment of a BHC manufacturing plant at Rishra near Kolkata (Abhilash and Singh, 2009). Currently, India is the largest producer of pesticides in Asia and ranks 12th globally in the utilization of pesticides (Bhardwaj and Sharma, 2013). Moreover, India also ranks 4th in the global suppliers of agrochemicals, after USA, Japan and China. As per the Standing Committee on Chemicals and Fertilizers of India (2013), the total value of annual production of pesticides in the country was about Rs. 8000 crores, out of which pesticides worth Rs. 6000 crores were consumed in the country and the rest were exported to the nearby countries (Devi *et al.*, 2017). According to non-profit Pesticide

Action Network (PAN), pesticide consumption in India grew by 13.07 per cent in between 2014-15 and 2017-18. Of late, the average pesticide consumption in India is around 0.381 kg/ha and mostly applied on the crops like cotton (50%) followed by paddy (18%) and vegetables (13-14%) (Devi *et al.*, 2017). Per hectare consumption of pesticides in the North Eastern states of India is far below than the national average and the rate of consumption of pesticides in Assam is 0.065 kg/ha (Devi *et al.*, 2017).

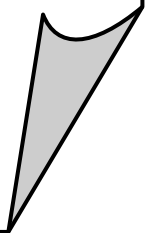
In modern agriculture, the use of pesticides is considered as indispensable and its widespread use has greatly benefited the farming communities of the country. However, the indiscriminate use of pesticides has created several problems in soil and water due to their long persistence nature. Pesticides adversely affect the proliferation of beneficial soil microorganisms and their associated biotransformation in the soil. However, some microbes are capable of utilizing pesticides as an energy source and may benefit from pesticide exposure. Among the pesticides, insecticides and acaricides have direct effects on the soil fauna, whereas the fungicides and herbicides might influence the soil faunal population indirectly by affecting the abundance and diversity of flora and detritus inputs which provides the food resources for most of the soil invertebrates (Pereira *et al.*, 2005). Out of all crop protection chemicals, insecticides, fungicides and herbicides are used predominantly in India, where insecticides dominate the industry with 65 per cent of consumption followed by herbicides (16%), fungicides (15%) and others (4%) (Devi *et al.*, 2017). The warm humid and tropical climate of India provides favourable breeding environment for insects and leads to higher consumption of insecticides for their management. Globally herbicides occupy the major share (44%), followed by fungicides (27%), insecticides (22%) and others (7%). In India, organophosphates were the most frequently used insecticides followed by neonicotinoids and pyrethroids (Yadav and Dutta, 2019). It has already been estimated that over 98 per cent of the sprayed insecticides actually reached a destination other than the target species and most of the insecticidal particles are directly deposited in the soil (Aktar *et al.*, 2009). Most of the conventional insecticides like organochlorines, organophosphates, carbamates and synthetic pyrethroids having broad spectrum of activity have resulted in the development of pest resistance to insecticides, outbreak of secondary pests, objectionable pesticide residues, direct hazard to the users and adverse effect on environment and non-target organisms (Kodandaram *et al.*, 2010). In the recent past, many newer insecticides have been developed having quite different chemical structures over the existing groups and target alternate

physiological and biochemical effects and novel mode of action to combat the problems associated with the conventional insecticides. Of late, several new insecticide groups having green chemistries *viz.*, neonicotinoids, phenylpyrazoles, oxadiazines, diamides, ketoenols, pyridines, flonicamids, mitochondrial electron transport inhibitors, diafenthiurons, tetrazines, thiazolidinones, oxazolines, avermectins, milbemycins, spinosyns, pyrrole insecticides and insect growth regulators have been discovered and also commercialized for uses in modern crop protection system (Deep *et al.*, 2018). These newer insecticides play an important role in managing many arthropod pests with good bio efficacy, high selectivity, low mammalian toxicity and also safer to the environment (Gavkare *et al.*, 2013). Hence, the trend of adopting newer insecticides by the stakeholders is intensifying at a significant rate in the country.

Although the use of newer insecticides showed encouraging results in managing various insect pest problems in recent years, but studies related to their impact on the soil arthropod community as well as microbial diversity under pesticide residue stress is yet in infancy. Most of the eco-toxicological studies carried out in different parts of the globe confined to Collembola and earthworms only. Moreover, already conducted eco-toxicological studies are also limited to conventional insecticides. Hence, there is an urgent need to understand the eco-toxicological effects of the newer insecticides on the soil fauna to maintain the ecological niches. Considering the above facts, the present investigation was undertaken with the following objectives:

1. To study the effect of certain newer insecticides on some soil arthropods in French bean
2. To know the effect of certain newer insecticides on soil microbial population

Review of Literature... ✍️



CHAPTER II

REVIEW OF LITERATURE

Soil fauna consists of extremely diverse group of organisms which plays a pivotal role in the functioning of the ecosystem. Hence, it is important to explore the ecotoxicological effects of insecticides on soil faunal diversity in order to preserve their ecological niches. Some of the important relevant work done in India and abroad in the context of the present study have been reviewed in this chapter under different subheadings.

2.1 Effect of pesticides on soil arthropod diversity

Joy and Chakravorty (1991) investigated the effect of direct and residual toxicities of some insecticides on ecologically important soil microarthropods under field and laboratory conditions in Santiniketan, West Bengal. They reported that the density of total microarthropods and major groups, namely Acarina and Collembola, suffered a statistically significant and persistent decline in the aldrin 30 EC (0.25 %) and endosulfan 35 EC (0.33 %) treated soil of wheat fields. However, dimethoate 30 EC (0.125 %) and phosphamidon 85 EC (0.03 %) applied in mustard fields produced only a temporary decline in the total microarthropod population. Laboratory studies showed that all the above insecticides along with monocrotophos 36 EC (0.2 %), methyl parathion 50 EC (0.05%), chlordane 20 EC (0.125 %) and carbaryl 50 WP (0.625 %) had a direct knockdown effect on *Cyphoderus* sp. but another collembolan species, *Xenylla* sp., seemed to be a bit resistant. Only endosulfan was toxic to a common soil Acarina species, *Lancetoppia* sp.

Frampton (1999) studied the non-target effects of chlorpyrifos, cypermethrin and pirimicarb on Collembola in winter wheat at Mereworth, South-East England. Suction sampling was used for extraction of epigeic arthropods where he reported the reduction of eight genera of Collembola significantly in the chlorpyrifos treated plots. No negative effects of cypermethrin and pirimicarb on Collembola population was detected in his study. Increases in the abundance of Collembola after application of cypermethrin and pirimicarb was also observed due to the knockdown effects of synthetic pyrethroids on potential predators of Collembola.

In an experiment conducted at the University of Kentucky, Lexington, by Kunkel *et al.* (1999), it was observed that the imidacloprid and bendiocarb reduced the capture of predatory coleopteran larvae and hister beetles, however, abundance of ants, carabids, spiders and staphylinids was largely unaffected. In addition, they also reported that a significant impact of bendiocarb on earthworms, mesostigmatid mites and Collembola. Further, they stated that the imidacloprid suppressed earthworms for a short period of time, however, halofenozide caused no reduction in the abundance of any group of beneficial invertebrates.

Marquini *et al.* (2003) worked with an aim of assessing the temporal and spatial effect of imidacloprid on the arthropod community associated with the canopy of *Phaseolus vulgaris* at Coimbra County, Brazil. Imidacloprid 700 WG (147 g *a.i./ha*) was applied to 20 days old plants and arthropods were sampled from the plant canopy at pre application and 3, 8, 14, 22, 29, 37 and 44 days after insecticide application. From their study, they reported that the imidacloprid prevented the population increase of springtails, but it was selective in favor of the most common bean pest predators as well as parasitoids.

Michereff-Filho *et al.* (2004) conducted a study to assess the side-effects of chlorpyrifos on springtails, oribatid mites and ants of cornfields in Brazil. They found that the activity of the ant *Solenopsis saevissima* was lower after two weeks of spraying but recovered afterwards, while the frequency of *Ectatomma brunneum* was significantly reduced only at the third week after spraying and did not show any recovery up to the termination of the study. However, chlorpyrifos did not decrease the overall abundance of Collembola and oribatid mites during the study period.

Joy *et al.* (2005) conducted a field experiment on mustard, wheat and okra where two insecticides *viz.*, heptachlor 20EC @ 3.25 kg *a.i./ha* and endosulfan 35EC @ 0.875 kg *a.i./ha* were applied at the seedling stages. At fortnightly intervals soil microarthropod population was estimated. They revealed an adverse effect of the insecticides, prominently on the density and relative abundance of Collembola and Acari. Further laboratory evaluation of the abovementioned insecticides against *Cyphoderus javanus* (Collembola) and *Archeogozetes longisetosus* (Acari) confirmed the chronic toxicity of the insecticides on adults leading to very low fecundity.

Al-Haifi *et al.* (2006) analyzed the effect of dimethoate and its metabolite omethoate residues on the soil microarthropods population in the Zendan

valley, Yemen. They found that dimethoate and omethoate residues were beyond the Total Threshold Limit Concentration (TTLC) and significantly reduced the population of the non-target soil arthropods *viz.*, mites, collembolans, grubs, cutworms, thrips, symphyla and seed corn maggots. Among these, the reduction in the soil microarthropods (mites and collembolans) population varied from 59 per cent to 69 per cent as compared to the control plot.

Endosulfan and quinalphos persistence and their effect on soil microarthropods after repeated applications was studied by Vig *et al.* (2006) in the cotton fields situated in the University of Delhi, India where Acarina was found to be more sensitive than collembolans to both the insecticides. They further recorded 94.50 and 71.20 per cent decrease in the Acarina population at 3 days after treatment of endosulfan and quinalphos, respectively, but did not record any significant changes in Collembola population.

Frampton and Van den Brink (2007) investigated the non-target effects of some broad-spectrum insecticides *viz.*, chlorpyrifos, cypermethrin as well as the selective insecticide pirimicarb on terrestrial arthropod communities in winter wheat fields at South East England. They reported that the chlorpyrifos showed a negative effect on arthropod abundance and taxonomic richness whereas cypermethrin exhibited an adverse effect on predatory arthropods but a positive effect on Collembola. The ratio of Collembola-predator was significantly higher after cypermethrin treatment, indicating that cypermethrin has a negative effect on collembolan's potential predators. Further they stated that the pirimicarb had a minor effect on aphids and their antagonists, with no effect on the collembolan community.

Impact of deltamethrin on non-target soil arthropods were studied by Badji *et al.* (2007) in maize fields of Brazil. From the study, they have recorded a species of Nitidulidae which was highly suppressed due to the application of deltamethrin along with springtails, Oribatida and Gamasida mites. However, the interruption of deltamethrin on population of soil arthropods from tropical fields varied among species and lower than expected.

While studying the phenylpyrazoles impact on *Folsomia candida* at France, Miguel *et al.* (2008) reported a significant reduction in juvenile production due to fipronil application. They revealed that the young organisms have a weaker

detoxification efficiency along with thinner integument and a smaller mass body, thus more sensitive than adults towards fipronil and phenylpyrazole derivatives.

Adamski *et al.* (2009) extensively studied the effects of diflubenzuron and mancozeb on soil microarthropods in Morasko, Poland. In that experiment, soil microarthropods were exposed to the mancozeb @ 240 mg/sq. m. and diflubenzuron @ 7.2 mg/sq. m. for once and studied for 6 months after application. They examined the population of Collembola, Myriapoda and 4 groups of mites *viz.*, Actinedida, Gamasina, Uropodina and Oribatida in the study and found significant reduction in the treated plots with Myriapoda, being the most highly affected group.

Daniel (2009) investigated selective impact of imidacloprid on certain non-target turf grass arthropods at Cornell University, Geneva. Soil core heat extraction and pitfall traps were used to quantify the soil arthropods and revealed no any significant impact of imidacloprid on pitfall captures. On the contrary, the abundance of Hemiptera, Thysanoptera, Coleoptera and Collembola were suppressed in soil core captures. Among Coleoptera, adults of Carabidae and Staphylinidae were affected but not the grubs whereas impact was expressed only on Entomobridae in case of springtails.

A study was conducted by Giglio *et al.* (2011) to investigate the toxicity of dimethoate on *Pterostichus melas italicus*, a carabid beetle living in olive groves agroecosystem of Calabria, Italy. The toxicity test, total haemocyte counts and morphometric analyses were used for quantification and found a reduction in activity and a mortality of 10 per cent after 72 hours of exposure. They further reported significantly less number of circulating haemocytes count in treated beetles at 48 hours and morphometric analyses revealed long-term sub-lethal effects of dimethoate on the beetle population. They also observed a significant reduction in the body size of females from long-term treated olive grove and a sexual dimorphism alteration in those non-target carabid species.

Larson *et al.* (2011) studied the impact of chlorantraniliprole, clothianidin and clothianidin-bifenthrin combination on beneficial invertebrates and their ecosystem services in turf grass at University of Kentucky, Lexington. They found no adverse effect of chlorantraniliprole on populations of earthworms, Collembola or oribatid mites. In contrast, they recorded significant negative impact of both

clothianidin and the clothianidin-bifenthrin combination on earthworms and Collembola population.

Santos *et al.* (2012) conducted an experiment to investigate the impact of pesticide application to non-target soil organisms with application of pesticides *viz.*, chlorpyrifos, endosulfan and glyphosate in a Mediterranean agricultural field located at Coimbra, Portugal. They studied the avoidance behaviour and reproduction of the Collembola, *Folsomia candida* and the earthworm, *Eisenia andrei*. They observed a significant avoidance of chlorpyrifos by collembolans (64 %) and endosulfan by earthworms (60 %) whereas glyphosate did not have any significant effect either on earthworms or on collembolans in the recommended doses.

Anbarashan and Gopalswamy (2013) conducted a comparative analysis between the effects of biopesticides and synthetic pesticides on beneficial arthropods in Puducherry, India. They reported the long-term suppression of beneficial arthropods of order Collembola, Arachnida and Hymenoptera after insecticide application whereas bio-insecticides had a non-significant effect on non-targeted species in organic fields and they started to increase their abundance after 7 days of spraying.

An experiment was carried out by El-Naggar and Zidan (2013) to evaluate the effectiveness of imidacloprid and thiamethoxam against sucking pests in cotton and their side effects on soil fauna at Sakha Agriculture Research Station, Egypt. The result showed that the pesticide application increased the overall collembolan density and decreased the overall Psocoptera, Oribatida, Actinedida and Gamasida density compared to the control plots. They further reported that imidacloprid had more adverse effects on soil fauna than thiamethoxam.

The ecotoxicity of chlorantraniliprole to non-target soil invertebrates was studied by Lavtizar *et al.* (2016) and their observations revealed that chlorantraniliprole at high concentrations (1000 mg/kg_{dw} soil) did not affect the survival and reproduction of *Enchytraeus crypticus* (enchytraeids), *Oppia nitens* (oribatid mites) and *Porcellio scaber* (isopods). On the contrary, the survival and reproduction of *Folsomia candida* (springtails) was severely affected by low concentrations of chlorantraniliprole (0.14 mg/kg_{dw} soil). They further reported that chlorantraniliprole quickly hampers the locomotor abilities of the exposed springtails, preventing them from escaping the chlorantraniliprole contaminated soil. Their study revealed that the soil arthropods

belonging to Insecta class were severely affected by chlorantraniliprole while the other soil invertebrates were comparatively less sensitive.

In order to study the effect of pesticide residues on soil arthropods in okra crop, Shar *et al.* (2016) conducted an experiment at Tando Allahyar, Pakistan and mentioned that the application of insecticides *viz.*, bifenthrin, chlorpyrifos, endosulfan and imidacloprid at recommended doses severely reduced the population of spiders, ants, field cricket, snow bug and silverfish in soil just after 1 day of treatment.

To evaluate the toxicity of malathion on the population density and diversity of soil microarthropods, an experiment was conducted by Sharma and Parwez (2017) at Department of Zoology, Aligarh Muslim University, Aligarh. The extraction of soil microarthropods was done by modified Tullgren funnel where the results showed a negative effect of malathion on soil mesofaunal population.

The effect of thiamethoxam and clothianidin on colonisation of invertebrate population and survival in small ephemeral ponds was observed by Basley and Goulson in 2018. The results showed that thiamethoxam generally produces stronger negative effects to the invertebrates than clothianidin. Populations of chironomids (Diptera) and Ostracoda were negatively affected by both the chemicals while Culicidae appeared to be unaffected by clothianidin.

El-Sherbeni *et al.* (2018) evaluated the efficiency of seven insecticides *viz.*, flonicamid, imidacloprid, thiamethoxam, emamectin-benzoate, chlorpyrifos, methomyl and deltamethrin against *Aphis gossypii* and their side effects on the associated predators, soil fauna and plant defense enzymes in cotton fields at Sakha Agriculture Research Station, Egypt. They found that flonicamid, emamectin-benzoate, imidacloprid and thiamethoxam were the least harmful to the associated predators causing less than 50 per cent mortality while the others were highly toxic. Flonicamid exhibited the highest degree of safety to the soil micro-arthropods followed by emamectin-benzoate, methomyl and deltamethrin. In contrast, chlorpyrifos and imidacloprid were the most toxic to the soil microarthropods.

Arfan *et al.* (2018) analyzed the effect of abamectin application on arthropod diversity in the agroecosystem of red onion crop. From the study, they reported 7 orders of arthropods belonging to 29 families and 42 species with a total of 4,412 individuals in the control plots whereas 8 orders belonging to 22 families and 31 species with a total of 12,078 individuals were recorded in the abamectin treated plots.

They further analyzed the diversity index of both the plots where the diversity was recorded maximum (2.06) in the abamectin treated plots as compared to the control plots (1.28).

Majeed *et al.* (2018) conducted an experiment to explore the impact of pesticides and biopesticides on the diversity and population dynamics of edaphic invertebrate communities in a citrus agroecosystem at Sargodha, Pakistan. A synthetic pyrethroid- bifenthrin, a bio-insecticide- spinosad, a synthetic fungicide-aliette and a bio-fungicide *Trichoderma harzianum* were applied and reported a significant effect of all the pesticides on the population abundance of springtails. The study revealed that insecticides were more suppressive for soil invertebrates than fungicides. Consequently, synthetic conventional pesticides were more disruptive to soil dwelling invertebrates than biopesticides.

Ghosal and Hati (2019) conducted an experiment at Sonarpur, West Bengal to know the impact of some newer insecticides on soil arthropod community. They reported that the insecticides *viz.*, rynaxypyr, cartap hydrochloride, fipronil and chlorpyrifos showed non-significant detrimental effect on collembolan population whereas carbofuran and phorate treated plots showed significant reduction of collembolan population registering 27.65 and 13.47 per cent, respectively. They further revealed that imidacloprid, chlorpyrifos and phorate showed a negative impact on earthworm population while non-significant effect was observed in case of other soil microarthropods.

The toxicity of thiamethoxam and clothianidin was explored by Ritchie *et al.* (2019) using three soil dwelling species *viz.*, *Oppia nitens*, *Eisenia andrei* and *Folsomia candida* in Ontario, Canada. They reported that clothianidin was highly toxic to the test species compared to the thiamethoxam. Further they stated that among the three test species *F. candida* was the most sensitive to thiamethoxam and clothianidin while *O. nitens* was the least sensitive.

2.2 Effect of pesticides on soil microbial population

The effect of organophosphorus insecticides isofenphos, phorate and fonofos were investigated on soil microbiota by Digrak and Kazanici (2001) at Kahramanmaras Sutcu Imam University in Turkey. They reported that isofenphos treated soil sample recorded more number of total viable bacteria than that of the control groups during incubation. Moreover, they found no inhibitory effect of isofenphos,

fonofos and phorate on the development of other microorganism group *viz.*, actinomycetes, anaerobic bacteria, aerobic endospores, proteolytic bacteria, cellulolytic microorganisms and yeast-mold.

An experiment was conducted by Pandey and Singh (2003) to study the effect of chlorpyrifos and quinalphos on the total bacterial and fungal population in a groundnut field at Agricultural Research Station, Durgapura, Jaipur and they reported that the total bacterial population was reduced significantly after chlorpyrifos and quinalphos application which recovered after 60 days of seed treatment and 45 days of soil treatment. They also reported that the fungal population was significantly enhanced after chlorpyrifos treatment whereas quinalphos inhibited the fungal population during the initial days of treatment, however, no effect was observed after 60 days.

Ahmed and Ahmed (2006) conducted the laboratory and field studies to determine the effects of chlorpyrifos 40 EC, imidacloprid 200 SL, cypermethrin 10 EC, endosulfan 35 EC, carbofuran 20 EC and bifenthrin 10 EC on total number of soil bacterial population. The results revealed that chlorpyrifos caused significant reduction in the number of soil bacteria whereas effect disappeared at 21 days after application in the field trials. In the laboratory studies, bifenthrin increased the number of bacterial population at 250 ppm and 500 ppm while in the field condition, bacteria population was unharmed.

An experiment was conducted by Sarnaik *et al.* (2006) to observe the effect of pesticides *viz.*, phorate, carbofuran, carbosulfan, thiomethoxam, imidacloprid, chlorpyrifos and monocrotophos on soil microflora in soybean. They found that there was no significant change in the total viable count of rhizobia and phosphate solubilizing bacteria due to the application of pesticides which showed their ability to degrade these pesticides. The viable count of rhizobia and phosphate solubilizing bacteria from rhizospheric soil of soybean ranged between 10^7 - 10^8 cfu/g of soil which was comparable with the count of bacteria from untreated soil.

The impact of fungicides triadimefon and propiconazole on soil bacterial populations was investigated by Yen *et al.* (2009) from a strawberry field located at Nai-Hu, Taipei. They applied both the fungicides in the soil under two concentrations *viz.*, 10 mg/kg and 100 mg/kg. They reported that the propiconazole was more persistent than triadimefon in soils affecting the microbial compositions which were not recovered

upto 75 days. According to them, different soil bacteria exhibited different chemical exploitation patterns depending on their population in the presence of the chemicals.

The effect of imidacloprid on the structural, genetic and physiological diversity of soil bacterial community was studied by Markowicz *et al.* (2013) and they reported that the metabolic activity of microbial communities was drastically reduced due to the application of imidacloprid and its impact was recorded negative on the richness and functional biodiversity of the soil microbial communities.

The impact of three organophosphorus insecticides malathion, diazinon and dimethoate on soil microbial communities were studied by Haleem *et al.* (2013) at University of Technology, Iraq. Their effects were investigated using the standard dilution plate technique at 24, 48 and 72 hours on the population of bacteria, fungi and actinomycetes under laboratory conditions. All the three insecticides recorded significant negative impact on population of microorganisms which basically depends on the concentration of insecticides and time of exposure as well.

Filimon *et al.* (2015) studied the enzymatic and bacteriological analysis to know the effect of certain insecticides on soil microorganisms and reported that the soil enzymes like dehydrogenase, urease, catalase and phosphatase activities were significantly reduced in the cypermethrin and thiamethoxam treated soil. Further they reported an inhibitory effect on metabolic processes in the soil due to the decrease in population of soil microbes more particularly the nitrifying bacterial communities.

The adverse effect of insecticidal treatment on the activity and population of beneficial soil microbial communities were investigated by Arora and Sahni (2016) at Central Soil Salinity Research Institute, Lucknow. Chemical pesticides reduced the activities of soil microbes and thus affect the nutritional quality of soils. They further reported that the field application of glyphosate increased microbial biomass carbon by 17 per cent and microbial biomass nitrogen by 76 per cent at 14 days after treatment. The soil microbial biomass carbon increased significantly up to 30 days in chlorpyrifos as well as cartap hydrochloride treated soil but thereafter decreased progressively with time.

Lalfakzuala *et al.*, (2015) carried out an experiment to study the effect of two insecticides *viz.*, monocrotophos and endosulfan on the growth of soil fungi, *viz.*, *Penicillium*, *Fusarium* and *Pythium* at Mizoram. They reported that the application of

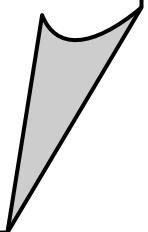
both the insecticides significantly reduced the growth of the three fungi and endosulfan showed more inhibitory effect as compared to monocrotophos.

Ghosal *et al.* (2018) studied the impact of seven new generation insecticides *viz.*, rynaxypyr, fipronil, cartap hydrochloride, carbofuran, phorate, imidacloprid and chlorpyrifos on beneficial rhizospheric microorganisms in rice maize cropping system and reported that all the insecticides had negative impact on the total bacterial and fungal population in soil up to 28 days. They further reported that the population of bacteria and fungi increased in all the treated plots during 75 days after application due to the utilization of the insecticide as well as its degraded products as carbon source.

Ataikiru *et al.* (2019) conducted a study to investigate the effect of carbofuran and paraquat on soil biochemical characteristics on certain soils in the Niger Delta region of Nigeria. This study revealed that the pesticides cause temporal impact on microbial populations and their enzymatic activities when applied at recommended field application doses.

An experiment was conducted by Al-Ani *et al.* (2019) at Alrashedia area of Mosul, Iraq to identify the effect of pesticides on soil microorganisms. Glyphosate 48% and α -cypermethrin 10% and malathion 50% WP were separately added to the soil at 0, 50, 100 and 200 ppm and incubated in the laboratory at 30 °C. They found significant reduction of microbial activities and population of soil bacteria, fungi and actinomycetes. They further reported the effect which depends upon the type and amount of pesticide as well as length of the incubation period. Soil samples treated with 200 ppm of malathion resulted the lowest microbial activities and counts of bacteria, fungi and actinomycetes.

Materials and Methods... ✍



CHAPTER III

MATERIALS AND METHODS

The present investigation entitled “Impact of certain newer insecticides on soil faunal diversity in French bean (*Phaseolus vulgaris* L.)” was carried out in Horticulture Experimental Farm; Soil Arthropod Pests Laboratory, Department of Entomology and Department of Plant Pathology of Assam Agricultural University (AAU), Jorhat during 2020-21. The details of materials used and methodology followed during the study period are presented in this chapter.

3.1 Location of the field experiment

The field experiment was conducted from December, 2020 to March, 2021 at the Horticulture Experimental Farm, AAU, Jorhat situated at latitude, longitude and altitude of 26°47' North, 94°12' East and 87 m above mean sea level, respectively.

3.2 Design and layout of experimental plot

The field experiment was conducted in Randomized Block Design (RBD) with three replications. The gross area of the experimental plot was 275 sq. m. (25 m×11 m) which was divided into three blocks. Each block was further subdivided into 7 equal plots measuring 9 sq. m. (3 m×3 m). The spacing between blocks and plots was 0.5 m and 0.5 m, respectively. The plan and layout of the experiment is shown in Fig. 3.1 and Plate 3.1(a).

3.3 Raising of French bean crop

The French bean crop (Variety: *Sunheri*) was grown as test crop by following all the standard package of practices of Assam [Plate 3.1(b)]. The seeds were sown in each plot maintaining a spacing of 45 cm between row to row and 30 cm between plant to plant, on 11th January, 2021.

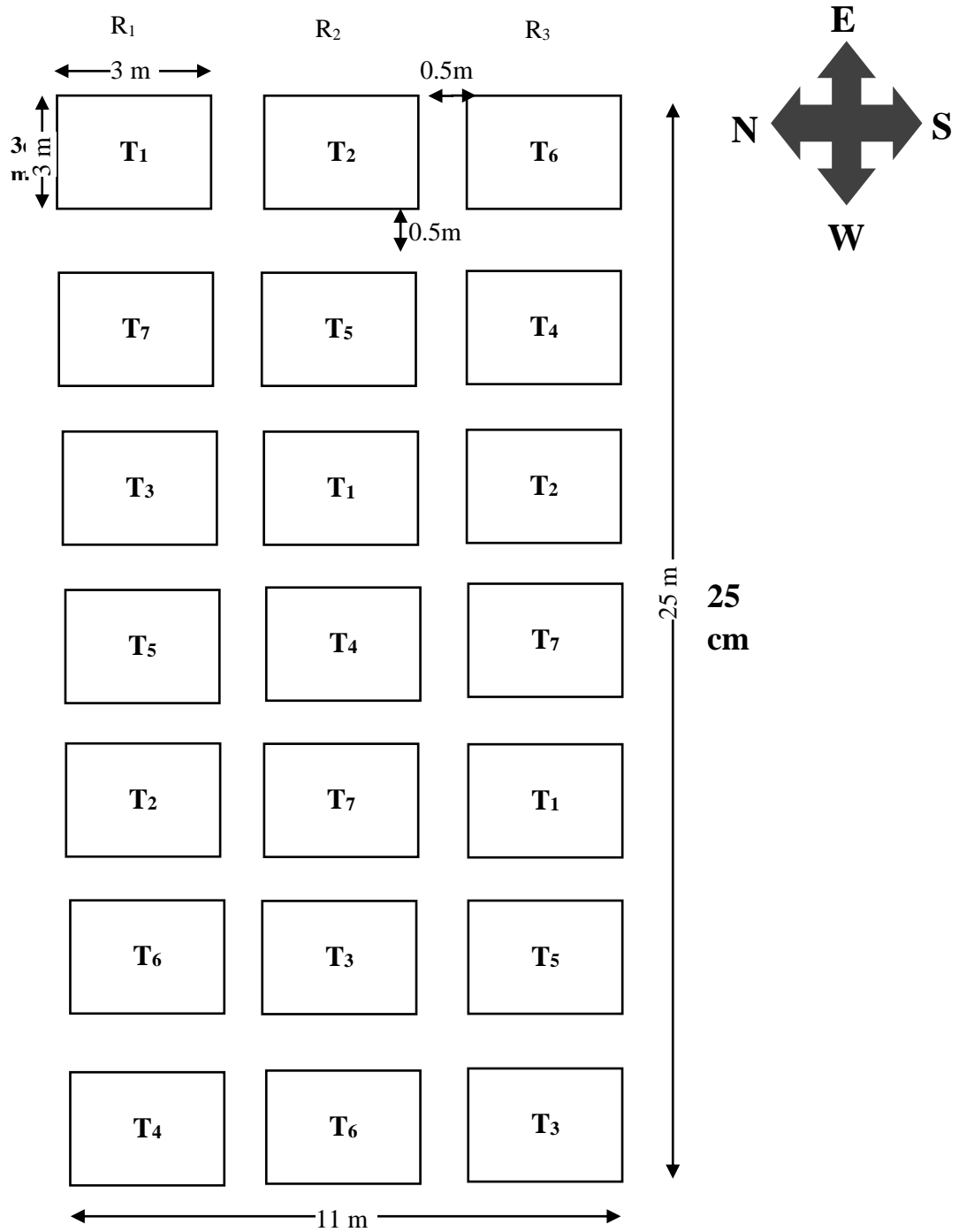


Fig. 3.1. Layout of the field experiment

Design	: Randomized Block Design		
Gross Area	: 275 sq. m.	Plot size	: 9 sq. m. (3 m×3 m)
Replication	: 3	Treatments	: 7
Total number of plots	: 21	Space between Plots	: 0.5 m



a.



b.

Plate 3.1(a-b). Layout of the experimental plot

3.4 Application of treatments

The experiment was conducted with total seven treatments including one control. Six newer insecticides *viz.*, clothianidin 50 WDG, fipronil 0.3 G, thiamethoxam 25 WG, imidacloprid 70 WG, chlorantraniliprole 0.4 GR and fipronil 40%+imidacloprid 40% WG were selected for conducting the experiment. The granular form of insecticides *i.e.* fipronil 0.3 G @ 50 g *a.i./ha* and chlorantraniliprole 0.4 GR @ 100 g *a.i./ha* were mixed with pulverized soil and applied in the furrows before sowing of seeds whereas required amount of clothianidin 50 WDG @ 120 g *a.i./ha*, thiamethoxam 25 WG @ 80 g *a.i./ha*, imidacloprid 70 WG @ 300 g *a.i./ha* and fipronil 40%+imidacloprid 40% WG @ 300 g *a.i./ha* were mixed with required amount of water and sprayed in seed furrows. After application of insecticides in respective plots, the seeds of French bean crop were sown in furrows and then covered with soil.

Table 3.1. Details of treatments used in the experiment

Treatments	Dose (g <i>a.i./ha</i>)
T ₁ : Clothianidin 50 WDG	120
T ₂ : Fipronil 0.3 G	50
T ₃ : Thiamethoxam 25 WG	80
T ₄ : Imidacloprid 70 WG	300
T ₅ : Chlorantraniliprole 0.4 GR	100
T ₆ : Fipronil 40% + Imidacloprid 40% WG	300
T ₇ : Control	-

3.5 Collection of soil macro arthropods

In the present study, soil macro arthropods were collected by using pitfall traps of 15 cm height and 10 cm diameter and four traps were installed in each plot [Plate 3.2 (a-b)] (Melbourne,1999). The traps were inserted carefully into the soil surface so that the upper rim of the traps was at the level of the soil surface. A total number of 84 pitfall traps were placed in the whole experimental area and they were

filled with conservation fluid consisting of 70 per cent ethyl alcohol. The number of soil macroarthropods captured in each trap were recorded at pre-treatment and 15, 30, 45, 60 and 75 days after application of insecticides at 8 AM in the morning (Goncalves and Pereira, 2012).

3.6 Collection and extraction of soil microarthropods

Soil samples were collected from each experimental plot at pre-treatment, 15, 30, 45, 60 and 75 days after insecticide application for sampling of soil microarthropods. Samples were collected in polyethylene zip bags at a constant depth of 0-10 cm by using a '*khurpi*' [Plate 3.3(a)] and making a quadrat of 30 cm length and 8 cm breadth (Paul *et al.*, 2011). The soil samples were sealed and tagged in the field [Plate 3.3(b)] and then, brought to the Soil Arthropod Pests Laboratory, Department of Entomology, AAU, Jorhat for the extraction of soil microarthropods.

3.7 Extraction of microarthropods from collected soil samples

The equipment "Tullgren funnel" was used to extract the soil microarthropods from collected soil samples which was operated by using 40 Watt Tungsten electric bulbs, keeping in high light intensities for 72 hours of exposure [Plate 3.4(a)] (Akoijam and Bhattacharyya, 2012). This funnel was developed by Tullgren in 1918 which is basically a dry funnel soil biota extractor designed based on the well-established behavior of soil dwelling insects which live in soil or litter and gradually move downward as the upper soil layer starts drying. The working principle of the instrument is that the test soil sample is placed in a netted bottom container mounted on a specially designed aluminum funnel top [Plate 3.4(b)]. The soil surface is exposed to a controlled intensity light which results in gradual drying of soil samples. Progressive drying of the soil samples induces migration of many soil micro arthropods from upper to lower strata and eventually they fall in to the funnel. The neck of the funnel opens in a glass vial containing alcohol or water, where the micro arthropods can be collected. The extracted soil microarthropods were collected in collecting tubes (40ml) containing 70 per cent ethyl alcohol [Plate 3.4(c)] and was transferred into a clean Petri dish (15cm diameter) for the separation of soil microarthropods. The soil microarthropods were then cleaned, categorized and counted with the help of a Stereozoom Microscope (Model: Carl Zeiss Stemi 2000-C) in the Soil Arthropod Pests Laboratory, Department of Entomology, AAU, Jorhat [Plate 3.4(d)]. The



Plate 3.2 (a-b). Installation of pitfall traps



a. Collection of soil samples



b. Tagging and sealing of soil samples

Plate 3.3 (a-b). Collection of soil samples for extraction of soil microarthropods



a. Tullgren Funnel



b. Placing of soil in the funnel



c. Collection of soil microarthropods



d. Stereozoom Microscope used for the separation of soil microarthropods

Plate 3.4 (a-d). Extraction of soil microarthropods by using Tullgren Funnel

population of extracted soil microarthropods were then estimated in numbers/ sq. m. by using the following formula as suggested by Singh *et al.* (1978).

$$P = \frac{10,000 \times X}{(B \times L) n}$$

Where, P = Population of microarthropods per sq. m.

X = Number of microarthropods extracted from the funnel

B = Breadth of the quadrat (cm)

L = Length of the quadrat (cm)

n = Number of samples

3.8 Collection of soil samples for the assessment of soil microbial population

Soil samples were collected in polyethylene zip bags from each experimental plot up to a depth of 10 cm at pre-treatment, 15, 30, 45, 60 and 75 days after insecticide application. The samples were then properly tagged, sealed and transported to the laboratory of the Department of Plant Pathology, AAU, Jorhat for the assessment of soil microbial population [Plate 3.5(a-b)].

3.9 Serial dilution method, Plating and Laboratory incubation

In laboratory, total bacterial and fungal population were enumerated on Nutrient Agar (NA) and Potato Dextrose Agar (PDA) media, respectively by following serial dilution technique and pour plate method (Sanders, 2012) [Plate 3.5(c-f)]. Serial dilution is a series of sequential dilutions that are performed to convert a dense solution into more usable concentrations. For assessment of soil microbial population, 1 g of soil from each soil sample was mixed in 10 ml of sterile water and thoroughly shook it to prepare undiluted stock solution under a laminar airflow chamber. Using sterile pipette, 1 ml of stock solution was transferred into 9 ml of sterile water to prepare 10^{-1} concentrated solution. Further dilution was done by taking 1ml from 10^{-1} concentration and mixed with 9 ml sterile water to prepare a 10^{-2} concentrated solution. The process was repeated till 10^{-3} concentration was achieved. For bacterial culture, 0.1 ml aliquot from 10^{-3} concentration was transferred to the sterilized Petri dish and then poured 15-20 ml molten Nutrient Agar media into it inside the laminar air flow chamber.



a. Collection of soil samples



b. Tagging and sealing of soil samples



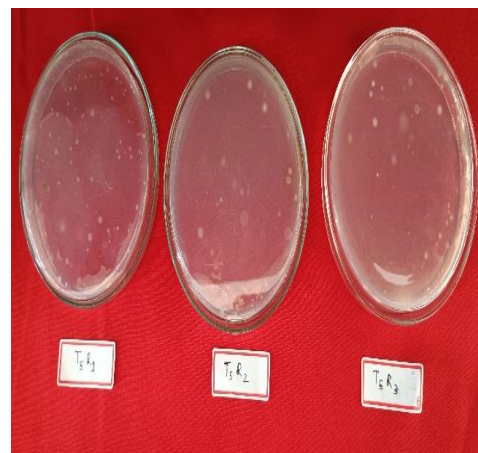
c. Prepared PDA media for fungal culture



d. Prepared NA media for bacterial culture



e. Plating under laminar air flow chamber



f. Counting of microbial population

Plate 3.5 (a-f). Assessment of soil microbial population

For fungal culture, 0.1 ml aliquot was taken from 10^{-3} concentration into the sterilized Petri dish and then poured molten Potato Dextrose Agar media into it inside the laminar air flow chamber. After closing the lid, the Petri dish was gently swirled to mix the sample and melted agar ensuring the even covering of media to get uniform distribution of cells. For both bacteria and fungi, three replication plates were prepared from 10^{-3} concentrated solution of each soil sample and allowed to solidify. Then, the Petri dishes were incubated in BOD incubator at $28 \pm 1^\circ\text{C}$ and the number of colonies were counted after 48 hours of incubation (Ghosal *et al.*, 2018).

Both the bacterial and fungal colonies were counted in terms of colony forming unit (CFU)/g or ml which was calculated by using following standard formula,

$$\text{CFU/g or ml} = \frac{n \times d}{S}$$

Where,

n = Number of colonies

d = Dilution factor

S = Volume of sample transferred to petri dish

3.10 Statistical analysis

Data obtained from the experiments were subjected to Randomized Block Design and was analyzed by Fisher's method of analysis of variance (ANOVA). The significance and non-significance of mean values among the treatments in different days intervals were ascertained at 5 per cent level of significance (Panse and Sukhatme, 1985).

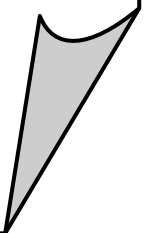
The critical difference (CD) was calculated to find out the significant mean difference between the treatments by following:

$$\text{CD}_{0.05} = \text{SEd} \times t_{0.05} \text{ (Fisher's value)}$$

The standard error of the difference was calculated by using the following formula:

$$\text{SEd}(\pm) = \sqrt{\frac{2 \times \text{Error mean square}}{\text{Number of replications}}}$$

Experimental Findings... ✎



CHAPTER IV

EXPERIMENTAL FINDINGS

Field and laboratory experiments were carried out in the Horticulture Experimental Farm; Soil Arthropod Pests Laboratory, Department of Entomology and Department of Plant Pathology, AAU, Jorhat, respectively to study the impact of certain newer insecticides on soil faunal diversity in French bean (*Phaseolus vulgaris* L.) during 2020-21. The salient findings of the experiments are presented in this chapter.

4.1 Effect of newer insecticides on soil arthropod population

4.1.1 Abundance of different soil arthropods in the pretreated plots

Pitfall traps as well as Tullgren funnels were used to determine the abundance of different soil arthropods in each plot prior to the application of insecticides. It can be seen from the results that the classes Insecta and Arachnida were abundant in the experimental area among the various soil arthropods (Table 4.1). Out of different soil macroarthropods observed prior to the application of insecticides, Hymenoptera was recorded to be the most dominant order (54.74%) followed by Coleoptera (13.68%) and Araneae (11.57%). The other orders recorded in least abundance were Hemiptera (5.26%), Orthoptera (4.21%), Neuroptera (3.16%), Isoptera (2.80%), Dermaptera (2.47%) and Lepidoptera (2.11%) (Fig. 4.1) [Plate 4.1 (a-n) & 4.2 (a-h)]. The per cent relative abundance of soil microarthropods recorded in the pretreated plots is presented in Table 4.1 and graphically represented in Fig. 4.2. An average of 4536 numbers of soil microarthropods per plot were extracted, out of which the abundance of Collembola and Oribatida were recorded to be 64.72 and 35.28 per cent, respectively [Plate 4.3 (a-e)].

4.1.2 Effect of newer insecticides on soil macroarthropod population

The impact of certain newer insecticides on the population of soil macroarthropods at fortnightly intervals is presented in Table 4.2 and graphically depicted in Fig. 4.3. Experimental results revealed that the population of soil macroarthropods in different plots prior to the application of insecticides ranged between 89.00 and 95.33 which showed statistical parity with each other. When the soil macroarthropods were sampled at 15 days after application of insecticides, the

Table 4.1. Abundance of different soil arthropods recorded in the pretreated plots

Arthropods	Order	Number of individuals	Accumulative Frequency (%)	Cumulative frequency (%)
Soil macroarthropods	Hymenoptera	50.28	54.74	54.74
	Coleoptera	12.57	13.68	68.42
	Hemiptera	4.83	5.26	73.68
	Orthoptera	3.87	4.21	77.89
	Neuroptera	2.90	3.16	81.05
	Isoptera	2.57	2.80	83.85
	Dermaptera	2.27	2.47	86.32
	Lepidoptera	1.94	2.11	88.43
	Araneae	10.63	11.57	100
Total		91.86		
Soil microarthropods	Collembola	2936.43	64.72	64.72
	Oribatida	1600.20	35.28	100
Total		4535.72		

Data are mean of 21 observations

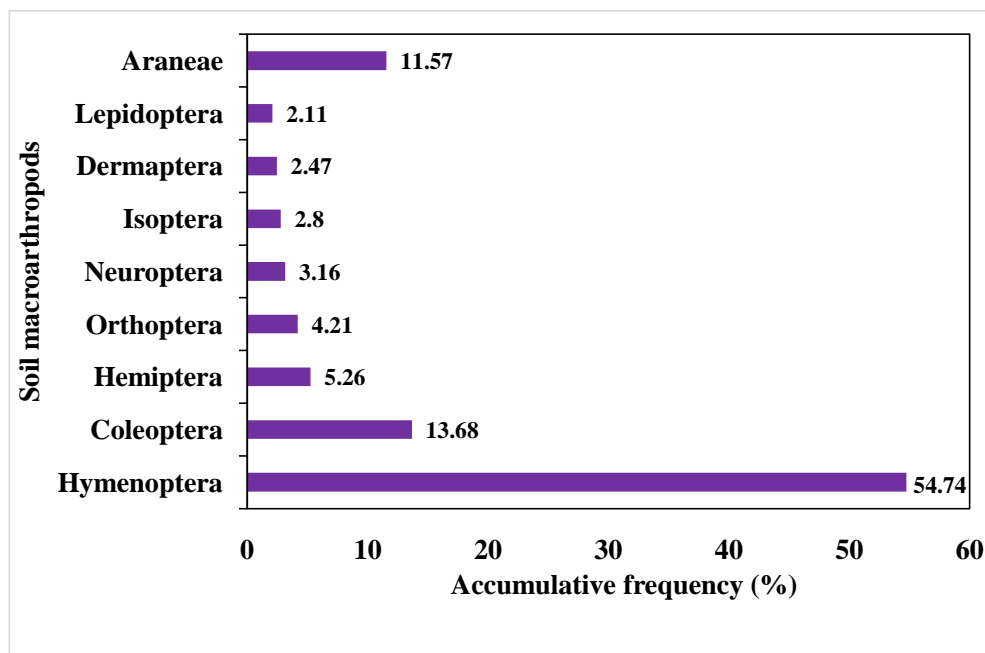


Fig. 4.1. Abundance of different soil macroarthropods recorded in the pretreated plots

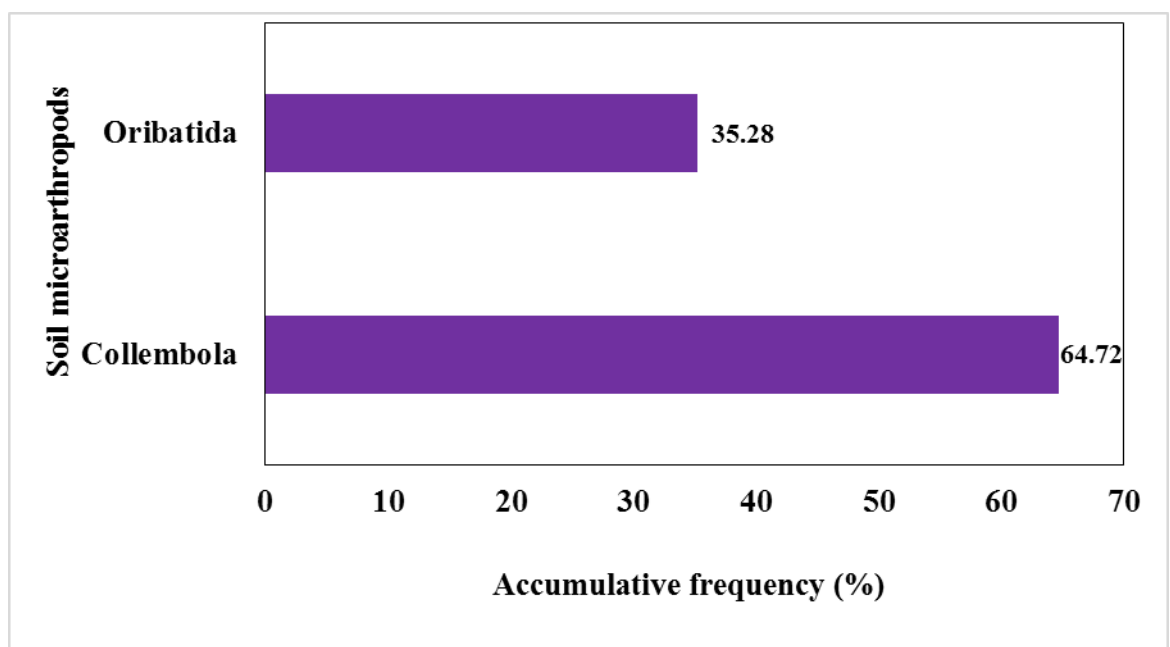


Fig. 4.2. Abundance of different soil microarthropods recorded in the pretreated plots



a.



b.



c.



d.



e.



f.



g.



h.



i.



j.



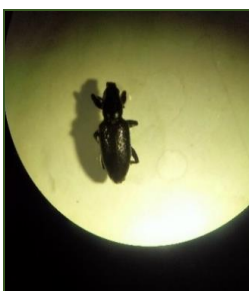
k.



l.

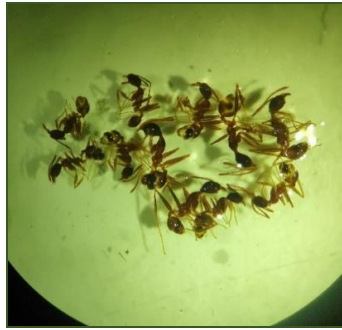


m.



n.

**Plate 4.1 (a-n). Coleopteran insects collected through pitfall traps
(a-g) Ground beetles, (h) Click beetle, (i) Darkling beetle,
(j-k) Lady bird beetle, (l) Rove beetle, (m-n) Weevil**



a. Ants



b. Red ant



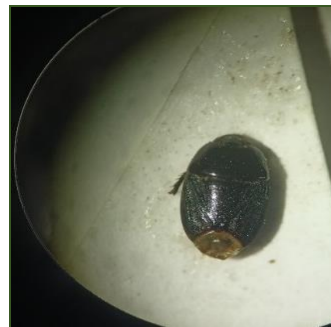
c. Termite



d. Cutworm



e. Antlion grub



f. Small black bug

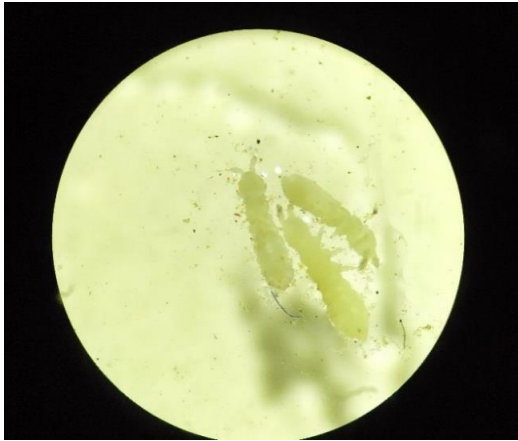


g. Mole cricket



h. Field cricket

Plate 4.2 (a-h). Other soil macroarthropods observed during the experimental period



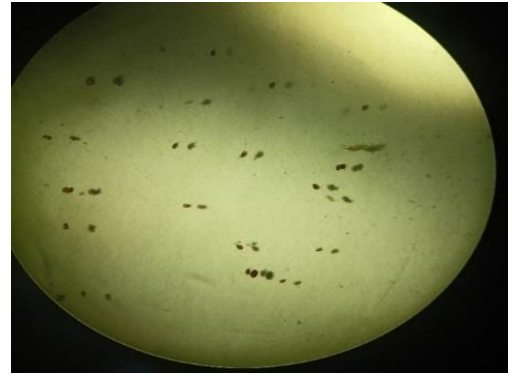
a. *Cyphoderus* sp.



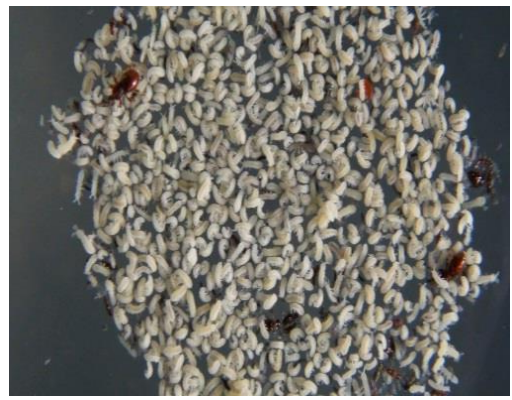
b. *Hypogastrura* sp.



c. Oribatid mites



**d. Mixed population
of collembolans**



**e. Mixed population of
collembolans and mites**

Plate 4.3 (a-e). Soil microarthropods collected during the experimental period

Table 4.2. Effect of certain newer insecticides on soil macroarthropod population (numbers/plot) at different days intervals

Treatment	Pre-treatment	15 DAT	30 DAT	45 DAT	60 DAT	75 DAT
	(Mean± SD)					
T ₁	93.67±7.57	50.33±7.23	57.67±6.81	64.00±7.21	73.67±6.66	80.67±7.57
T ₂	91.67±6.51	47.67±6.03	53.00±6.24	61.33±7.57	70.00±7.21	77.33±5.51
T ₃	89.33±7.09	49.67±6.66	56.33±7.23	62.00±6.56	72.33±6.03	79.33±7.02
T ₄	89.00±6.24	48.33±7.37	54.67±6.11	61.67±7.64	71.67±5.86	78.67±6.66
T ₅	94.33±6.03	52.67±7.57	59.00±7.21	65.67±6.81	74.33±7.09	81.33±8.08
T ₆	89.67±6.35	47.33±6.81	52.67±6.11	60.67±6.66	69.33±7.37	77.00±6.24
T ₇	95.33±7.37	89.33±7.23	97.67±7.09	101.33±6.51	99.00±6.56	106.33±7.02
SEd(±)	2.96	2.56	2.94	2.31	2.33	2.45
CD (p=0.05)	6.44	5.59	6.41	5.02	5.08	5.33

T₁= Clothianidin 50 WDG, T₂= Fipronil 0.3 G, T₃= Thiamethoxam 25 WG, T₄= Imidacloprid 70 WG, T₅= Chlorantraniliprole 0.4 GR, T₆= Fipronil 40% + Imidacloprid 40% WG, T₇= Control

Data are mean of 3 replications

DAT: Days after treatment

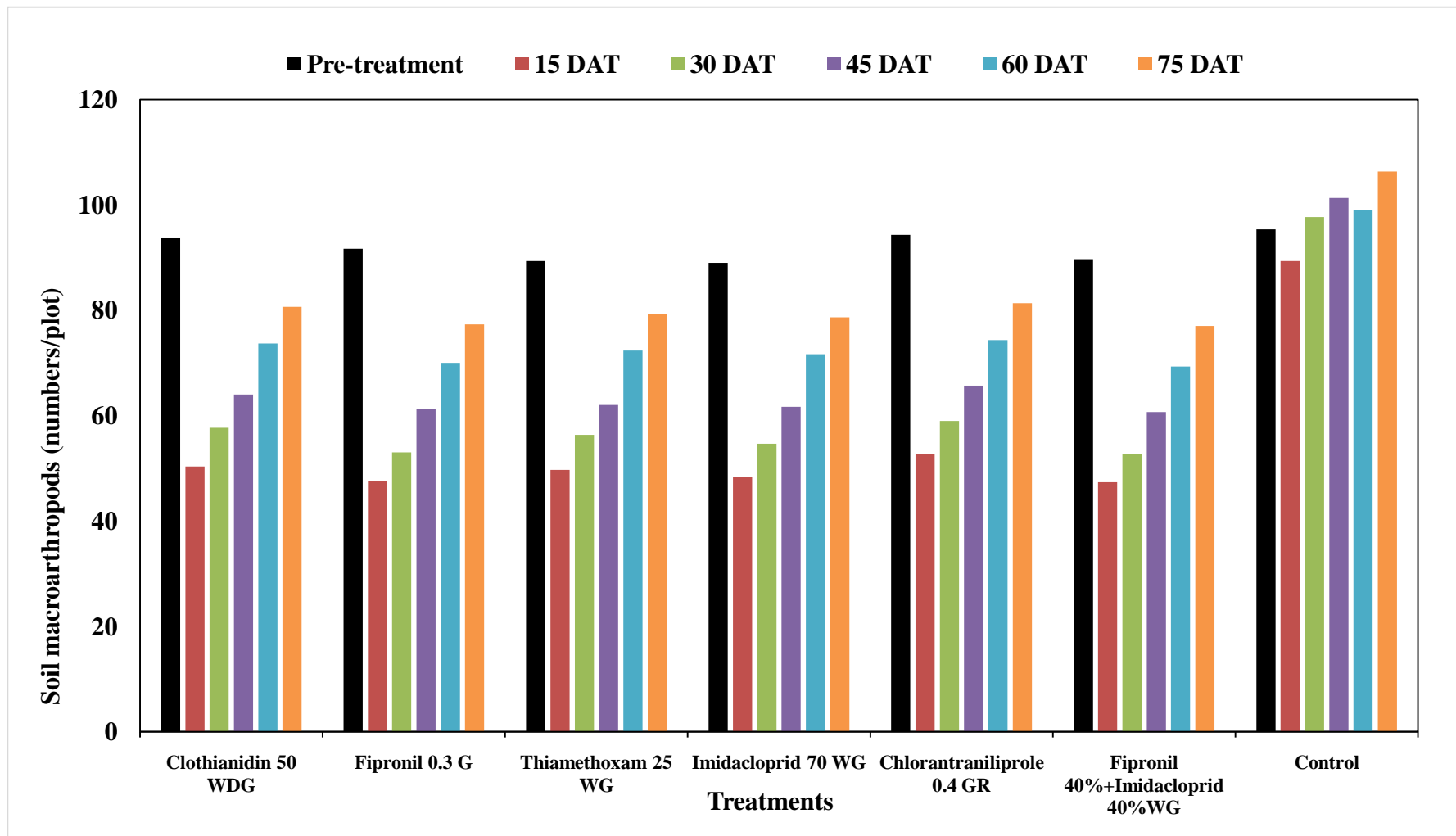


Fig. 4.3. Effect of certain newer insecticides on soil macroarthropod population (numbers/plot) at different days intervals

population in all the insecticidal treated plots was reduced significantly (47.33-52.67) as compared to the control (89.33). However, the population in all the insecticidal treated plots was recorded to be statistically *at par* with each other. Out of all the insecticidal treated plots, maximum number of soil macroarthropods was recorded in chlorantraniliprole 0.4 GR treated plots (52.67) followed by clothianidin 50 WDG (50.33) and thiamethoxam 25 WG (49.67). Further, the number of soil macroarthropods obtained in the imidacloprid 70 WDG, fipronil 0.3 G and fipronil 40%+ imidacloprid 40% WG treated plots were 48.33, 47.67 and 47.33, respectively.

Significant reduction in the number of soil macroarthropods (52.67-59.00) was also recorded at 30 days after application of insecticides in all the treated plots as compared to the control (97.67). Comparatively higher population was recorded in the insecticidal treated plots at 30 days after application of insecticides than the population recorded at 15 days after treatment. The population of all the insecticidal treated plots showed statistical parity with each other, however among all the insecticide treated plots, the highest population of soil macroarthropods were recorded in chlorantraniliprole 0.4 GR treated plots (59.00) followed by clothianidin 50 WDG (57.67) and thiamethoxam 25 WG (56.33). The number of soil macroarthropods recorded in the imidacloprid 70 WDG, fipronil 0.3 G and fipronil 40% + imidacloprid 40% WG treated plots were 54.67, 53.00 and 52.67 numbers, respectively (Table 4.2 & Fig. 4.3).

Sampling of soil macroarthropods also showed a significant decrease in their abundance in the insecticidal treated plots (60.67-65.67) as compared to the control (101.33) at 45 days after application of insecticides. In addition, the population trend of soil macroarthropods in insecticidal treated plots was recorded in comparatively higher numbers as compared to the population recorded at 15 and 30 days after application of insecticides. Out of all the insecticidal treated plots, maximum number of soil macroarthropods was sampled in chlorantraniliprole 0.4 GR treated plot (65.67) followed by clothianidin 50 WDG (64.00) and thiamethoxam 25 WG (62.00). Moreover, imidacloprid 70 WDG, fipronil 0.3 G and fipronil 40%+ imidacloprid 40% WG treated plots registered 61.67, 61.33 and 60.67 number of soil macroarthropods, respectively (Table 4.2 & Fig. 4.3).

Data recorded at 60 days after application of insecticides also indicated a significant reduction in the number of soil macroarthropods in insecticidal treated plots (69.33-74.33) as compared to the control plots (99.00). Relatively higher number of

macroarthropods were recorded at 60 days after application of insecticides as compared to the macroarthropods recorded during 15, 30 and 45 days after treatment. There was no statistical difference observed among the population of soil macroarthropod in different insecticidal treatments where the chlorantraniliprole 0.4 GR treated plot recorded the highest population of soil macroarthropods (74.33) followed by clothianidin 50 WDG (73.67) and thiamethoxam 25 WG (72.33). Moreover, 71.67, 70.00 and 69.33 number of soil macroarthropods were registered in the imidacloprid 70 WDG, fipronil 0.3 G and fipronil 40%+ imidacloprid 40% WG treated plots, respectively (Table 4.2 & Fig. 4.3).

When the soil macroarthropods were sampled at 75 days after application of insecticides, the population of soil macroarthropods in all the insecticidal treated plots registered a significant reduction (77.00-81.33) as compared to the control plots (106.33). However, the population in insecticidal treated plots was comparatively higher than recorded at 15, 30, 45 and 60 days after treatment, respectively. Among the treatments, maximum number of soil macroarthropods was recorded in chlorantraniliprole 0.4 GR treated plots (81.33) followed by clothianidin 50 WDG (80.67) and thiamethoxam 25 WG (79.33). Further, the number of soil macroarthropods recorded in the imidacloprid 70 WDG, fipronil 0.3 G and fipronil 40%+ imidacloprid 40% WG treated plots were 78.67, 77.33 and 77.00, respectively (Table 4.2 & Fig. 4.3).

4.1.3 Effect of newer insecticides on soil microarthropod population

Data presented in Table 4.3 and graphical representation of Fig. 4.4 described the impact of tested insecticides on the population of soil microarthropods at fortnightly intervals. It is prominent from the results that there was no any significant difference in the number of soil microarthropods in different plots prior to the application of insecticides which was ranged between 458.33 to 555.56/sq. m. The number of soil microarthropods recorded at 15, 30, 45, 60 and 75 days after treatment of insecticides in different treatments were ranged between 597.22-694.44, 666.67-763.89, 708.33-791.67, 958.33-1041.67 and 1027.78-1111.11 numbers/sq. m., respectively (Table 4.3 & Fig. 4.4). At each fortnight intervals, the population of soil microarthropods registered statistical parity among all the treatments and did not exhibit any significant negative impact of the tested insecticides on the soil microarthropod population during the experimental period. In addition, a gradual increase in the total number of soil microarthropods was observed from 15 to 75 days as compared to the pretreated plots.

Table 4.3. Effect of certain newer insecticides on soil microarthropod population (numbers/sq. m.) at different days intervals

Treatment	Pre-treatment	15 DAT	30 DAT	45 DAT	60 DAT	75 DAT
	(Mean± SD)					
T ₁	541.67±125.00	680.56±104.86	694.44±127.29	750.00±110.24	1041.67±110.24	1097.22±104.86
T ₂	513.89±120.28	611.11±63.65	666.67±110.24	736.11±127.29	958.33±83.33	1041.67±125.00
T ₃	486.11±63.65	625.00±83.33	736.11±96.23	763.89±86.74	986.11±120.28	1055.56±96.23
T ₄	458.33±110.24	638.89±127.29	680.56±127.29	708.33±83.33	1027.78±127.29	1069.44±63.65
T ₅	472.22±127.29	694.44±104.86	750.00±83.33	777.78±104.86	972.22±104.86	1111.11±104.86
T ₆	555.56±86.74	597.22±96.23	722.22±104.86	722.22±48.11	1000.00±72.17	1027.78±127.29
T ₇	500.00±83.33	652.78±63.65	763.89±86.74	791.67±72.17	1013.89±86.74	1083.33±83.33
SEd(±)	45.36	45.66	44.78	40.02	40.59	40.47
CD (p=0.05)	98.84	99.50	97.58	87.20	88.44	88.19

T₁= Clothianidin 50 WDG, T₂= Fipronil 0.3 G, T₃= Thiamethoxam 25 WG, T₄= Imidacloprid 70 WG, T₅= Chlorantraniliprole 0.4 GR, T₆= Fipronil 40% + Imidacloprid 40% WG, T₇= Control

Data are mean of 3 replications

DAT: Days after treatment

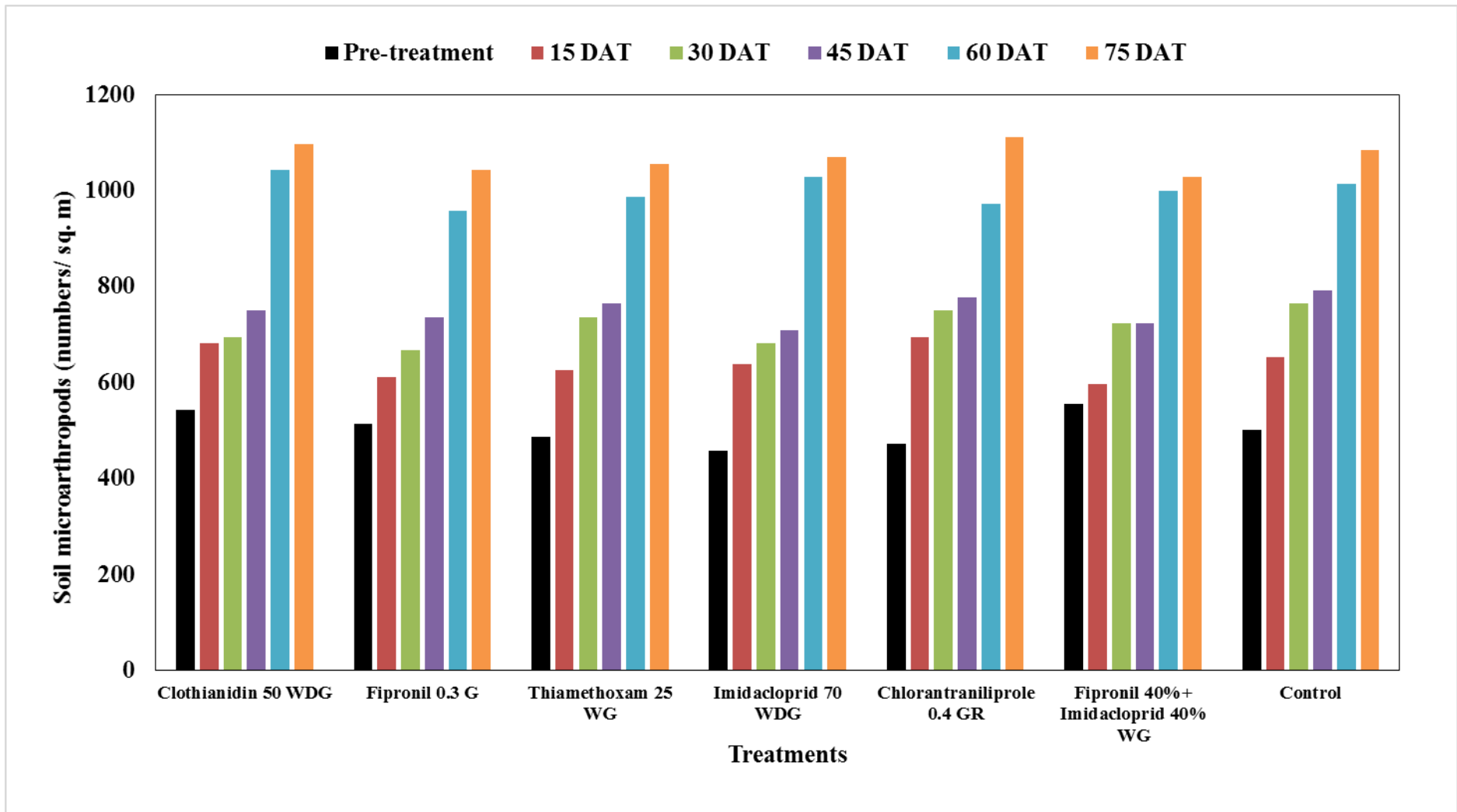


Fig. 4.4. Effect of certain newer insecticides on soil microarthropod population (numbers/sq. m.) at different days intervals

4.2 Effect of newer insecticides on soil microbial population

4.2.1 Effect of newer insecticides on total bacterial population

The effect of tested insecticides on the total soil bacterial population in terms of colony forming unit (CFU) per gram of soil was presented in Table 4.4 and graphically depicted in Fig. 4.5. The CFU count of total bacteria per gram of soil in all the treatments prior to the application of insecticides ranged between 95.67×10^4 and 104.67×10^4 which showed statistical parity with each other.

It is clear from the experimental results that the population of bacteria (cfu/g of soil) in all the insecticidal treated plots at 15 days after application of insecticides was significantly reduced (58.67 - 62.67×10^4) as compared to the control plots (98.67×10^4). The results of the insecticidal treated plots were statistically *at par* with each other where the highest CFU count of bacteria per gram of soil was recorded in chlorantraniliprole 0.4 GR treated plot (62.67×10^4) followed by clothianidin 50 WDG (62.00×10^4) and thiamethoxam 25 WG (61.33×10^4). The population of total bacteria (cfu/g of soil) obtained in the imidacloprid 70 WDG, fipronil 0.3 G and fipronil 40%+ imidacloprid 40% WG treated plots were 60.00×10^4 , 59.33×10^4 and 58.67×10^4 , respectively (Table 4.4 & Fig. 4.5).

At 30 days after application of insecticides, the population of bacteria (cfu/g of soil) in all the insecticidal treated plots ranged between 68.00×10^4 to 71.67×10^4 which showed significant reduction in the abundance of bacteria compared to the control (89.33×10^4), however, the results showed statistical parity among the insecticidal treatments. Chlorantraniliprole 0.4 GR treated plots recorded maximum population of bacteria (71.67×10^4 cfu/g of soil) followed by clothianidin 50 WDG (71.00×10^4) and thiamethoxam 25 WG (70.33×10^4). Rest of the treatments *viz.*, imidacloprid 70 WDG, fipronil 0.3 G and fipronil 40%+ imidacloprid 40% WG treated plots recorded bacterial population of 69.67×10^4 , 68.67×10^4 and 68.00×10^4 (cfu/g of soil), respectively. Moreover, the total bacterial population in insecticidal treated plots recorded at 30 days after application of insecticides was found to be comparatively higher than the data recorded at 15 days after treatment (Table 4.4 & Fig. 4.5).

When the soil bacteria were assessed at 45 days after application of insecticides, the bacterial population (cfu/g of soil) in all the insecticide treated plots indicated significant reduction (75.33 - 78.33×10^4) as compared to the control (96.00×10^4).

Table 4.4. Effect of certain newer insecticides on total bacterial population (cfu×10⁴/g of soil) at different days intervals

Treatment	Pre-treatment	15 DAT	30 DAT	45 DAT	60 DAT	75 DAT
	(Mean± SD)					
T ₁	102.33±9.29	62.00±8.66	71.00±5.20	77.67±5.77	82.00±5.57	90.33±6.43
T ₂	98.67±7.51	59.33±10.21	68.67±8.33	75.67±8.50	79.67±6.11	88.00±4.36
T ₃	103.00±8.54	61.33±7.37	70.33±7.23	77.00±5.29	81.67±6.81	89.33±6.81
T ₄	95.67±8.50	60.00±9.64	69.67±3.21	76.33±4.62	81.00±7.81	88.67±7.23
T ₅	97.00±9.85	62.67±8.50	71.67±5.51	78.33±8.39	82.67±5.03	90.67±8.39
T ₆	104.67±9.71	58.67±9.87	68.00±8.66	75.33±4.73	79.00±7.55	87.33±6.66
T ₇	101.33±9.07	98.67±7.23	89.33±4.62	96.00±7.81	100.33±7.57	106.33±5.51
SEd(±)	4.16	1.85	1.82	1.52	1.71	1.60
CD (p=0.05)	9.06	4.02	3.96	3.30	3.73	3.49

T₁= Clothianidin 50 WDG, T₂= Fipronil 0.3 G, T₃= Thiamethoxam 25 WG, T₄= Imidacloprid 70 WG, T₅= Chlorantraniliprole 0.4 GR, T₆= Fipronil 40% + Imidacloprid 40% WG, T₇= Control

Data are mean of 3 replications

DAT: Days after treatment

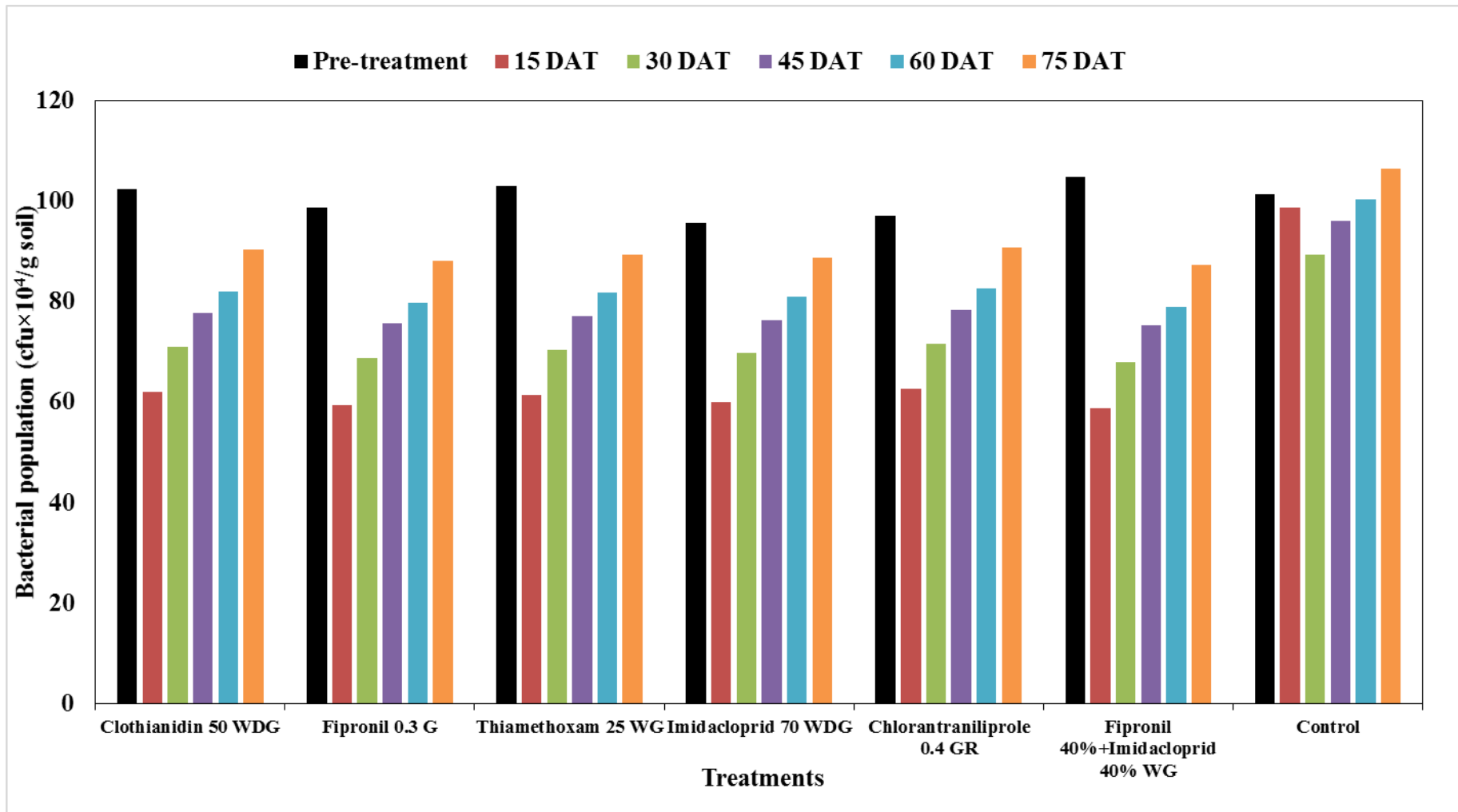


Fig. 4.5. Effect of certain newer insecticides on total bacterial population (cfu $\times 10^4$ /g of soil) at different days intervals

Among the insecticidal treatments, the population of bacteria were statistically *at par* with each other, however, the highest CFU count of bacteria was recorded in chlorantraniliprole 0.4 GR treated plots (78.33×10^4) followed by clothianidin 50 WDG (77.67×10^4) and thiamethoxam 25 WG (77.00×10^4). Besides, 76.33×10^4 , 75.67×10^4 and 75.33×10^4 colony forming unit per gram of soil were obtained from the imidacloprid 70 WDG, fipronil 0.3 G and fipronil 40% + imidacloprid 40% WG treated plot, respectively. It was found that, the total bacterial population in insecticidal treated plots at 45 days after treatment was relatively higher than 30 days after treatment (Table 4.4 & Fig. 4.5).

The assessment of soil bacteria (cfu/g of soil) registered a significant decrease in their population in all the insecticidal treated plots (79.00 - 82.67×10^4) as compared to the control (100.33×10^4) at 60 days after application of insecticides. However, all the insecticidal treated plots showed bacterial population *at par* with each other, of which, chlorantraniliprole 0.4 GR treated plots (82.67×10^4) reported the highest population of bacteria followed by clothianidin 50 WDG (82.00×10^4) and thiamethoxam 25 WG (81.67×10^4). Further, the population of total bacteria (cfu/g of soil) obtained in the imidacloprid 70 WDG, fipronil 0.3 G and fipronil 40%+ imidacloprid 40% WG treated plots were 81.00×10^4 , 79.67×10^4 and 79.00×10^4 , respectively (Table 4.4 & Fig. 4.5).

The population of bacteria (cfu/g of soil) in all the insecticidal treated plots at 75 days after treatment ranged between 87.33×10^4 to 90.67×10^4 which showed significant reduction in the abundance of bacterial population as compared to the control (106.33×10^4). However, all the insecticidal treated plots showed bacterial population *at par* with each other. In addition, chlorantraniliprole 0.4 GR treated plot (90.67×10^4) recorded maximum population followed by clothianidin 50 WDG (90.33×10^4) and thiamethoxam 25 WG (89.33×10^4). Rest of the treatments *viz.*, imidacloprid 70 WDG, fipronil 0.3 G and fipronil 40%+ imidacloprid 40% WG treated plot recorded 88.67×10^4 , 88.00×10^4 and 87.33×10^4 cfu/g of soil, respectively (Table 4.4 & Fig. 4.5). The total bacterial population in all the insecticidal treated plots at 30, 45, 60 and 75 days after application of insecticides revealed a gradual recovery as compared to the data recorded at 15 days after treatment.

4.2.2 Effect of newer insecticides on total fungal population

The impact of newer insecticides on the population of total soil dwelling fungi in terms of colony forming unit (CFU) per gram of soil at fortnightly intervals was presented in Table 4.5 and graphically depicted in Fig. 4.6. The CFU count of fungi per gram of soil in all the treatments prior to the application of insecticides ranged between 78.00×10^4 and 85.33×10^4 which were statistically *at par* with each other. Data presented in Table 4.5 revealed that all the six tested insecticides significantly reduced the population of total soil fungi (48.00 - 51.67×10^4 cfu/g of soil) at 15 days after application of insecticides as compared to control (91.67×10^4). However, the results of insecticidal treated plots were statistically *at par* with each other. The highest population of fungi per gram of soil among the insecticidal treated plots was recorded in chlorantraniliprole 0.4 GR treated plots (51.67×10^4) followed by clothianidin 50 WDG (51.00×10^4) and thiamethoxam 25 WG (50.33×10^4). Besides, imidacloprid 70 WDG, fipronil 0.3 G and fipronil 40%+ imidacloprid 40% WG treated plots recorded 49.67×10^4 , 48.67×10^4 and 48.00×10^4 fungal cfu/g of soil, respectively.

The assessment of soil fungi (cfu/g of soil) at 30 days after application of insecticides showed a significant decrease (51.33 - 55.00×10^4) in their population in all the insecticidal treated plots as compared to the control (102.33×10^4). However, the data registered in all the insecticidal treated plots indicated statistical parity with each other. Out of all the treatments, chlorantraniliprole 0.4 GR treated plots recorded the highest fungal population (55.00×10^4) followed by clothianidin 50 WDG (53.67×10^4) and thiamethoxam 25 WG (53.00×10^4). Further, the population of total fungi (cfu/g of soil) obtained in the imidacloprid 70 WDG, fipronil 0.3 G and fipronil 40%+ imidacloprid 40% WG treated plots were 52.67×10^4 , 52.00×10^4 and 51.33×10^4 , respectively (Table 4.5 & Fig. 4.6).

At 45 days after application of insecticides, the population of fungi (cfu/g of soil) in all the insecticidal treated plots ranged between 57.33×10^4 to 62.67×10^4 which showed significant reduction as compared to the control (96.33×10^4). However, the results showed statistical parity among the insecticidal treatments. In addition, the maximum fungal population was recorded in chlorantraniliprole 0.4 GR treated plots (62.67×10^4) followed by clothianidin 50 WDG (62.00×10^4) and thiamethoxam 25 WG (61.33×10^4). Besides, imidacloprid 70 WDG, fipronil 0.3 G and

Table 4.5. Effect of certain newer insecticides on total fungal population (cfu×10⁴/g of soil) at different days intervals

Treatment	Pre-treatment	15 DAT	30 DAT	45 DAT	60 DAT	75 DAT
	(Mean± SD)					
T ₁	83.33±6.11	51.00±5.57	53.67±5.13	62.00±6.56	68.33±8.50	75.00±6.56
T ₂	85.33±4.93	48.67±4.04	52.00±7.00	58.33±5.86	66.00±6.56	72.67±8.14
T ₃	78.00±7.81	50.33±8.02	53.00±4.58	61.33±5.13	67.33±9.29	74.67±6.66
T ₄	79.33±9.29	49.67±8.62	52.67±6.66	59.67±6.66	67.00±5.57	73.67±5.69
T ₅	80.67±7.77	51.67±4.04	55.00±7.81	62.67±4.73	69.00±5.29	75.33±8.50
T ₆	78.33±8.50	48.00±7.21	51.33±9.45	57.33±8.50	65.67±4.04	71.00±5.29
T ₇	84.00±4.58	91.67±6.51	102.33±6.66	96.33±7.57	104.33±4.73	99.00±7.81
SEd(±)	3.43	2.00	1.88	2.64	1.81	2.02
CD (p=0.05)	7.48	4.35	4.10	5.74	3.95	4.41

T₁= Clothianidin 50 WDG, T₂= Fipronil 0.3 G, T₃= Thiamethoxam 25 WG, T₄= Imidacloprid 70 WG, T₅= Chlorantraniliprole 0.4 GR, T₆= Fipronil 40% + Imidacloprid 40% WG, T₇= Control

Data are mean of 3 replications

DAT: Days after treatment

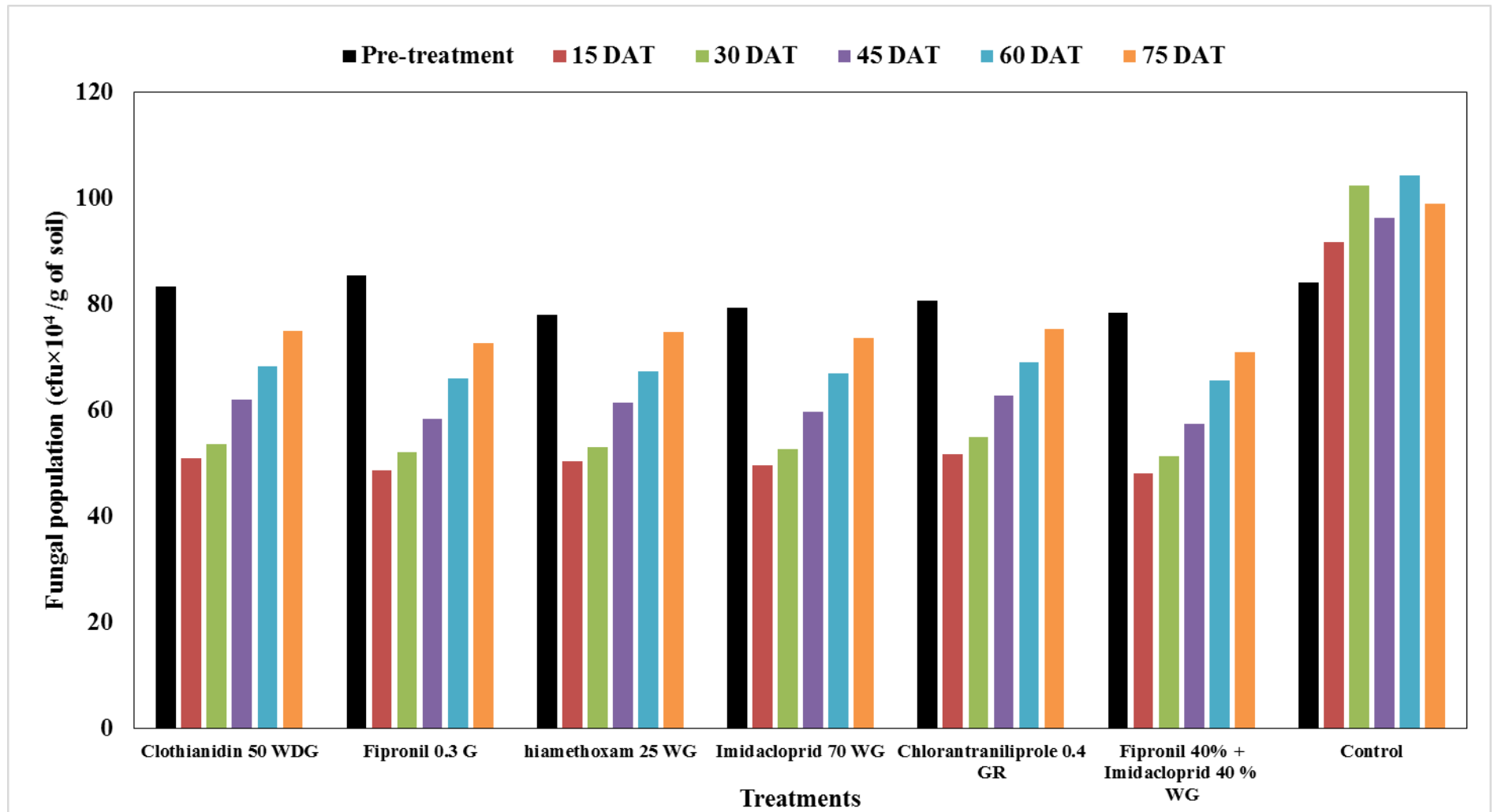


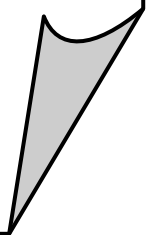
Fig. 4.6. Effect of certain newer insecticides on total fungal population ($\text{cfu} \times 10^4/\text{g}$ of soil) at different days intervals

fipronil 40%+ imidacloprid 40% WG treated plot recorded 59.67×10^4 , 58.33×10^4 and 57.33×10^4 colony forming units per gram of soil, respectively (Table 4.5 & Fig. 4.6).

When assessed at 60 days after application of insecticides, the fungal population (cfu/g of soil) in all the insecticidal treated plots indicated significant reduction (65.67 - 69.00×10^4) as compared to the control (104.33×10^4). Among the insecticidal treatments, the population of fungi were statistically *at par* with each other. However, the highest CFU count of fungi was recorded in chlorantraniliprole 0.4 GR treated plots (69.00×10^4) followed by clothianidin 50 WDG (68.33×10^4) and thiamethoxam 25 WG (67.33×10^4). Rest of the insecticidal treatments *viz.*, imidacloprid 70 WDG, fipronil 0.3 G and fipronil 40%+ imidacloprid 40% WG registered 67.00×10^4 , 66.00×10^4 and 65.67×10^4 colony forming unit of fungi per gram of soil, respectively (Table 4.5 & Fig. 4.6).

The population of fungi (cfu/g of soil) in all the insecticide treated plots at 75 days after treatment ranged between 71.00×10^4 to 75.33×10^4 which showed significant reduction in their abundance as compared to the control (99.00×10^4). However, the results showed statistical parity among the insecticidal treatments. In addition, chlorantraniliprole 0.4 GR treated plots recorded maximum population (75.33×10^4) followed by clothianidin 50 WDG (75.00×10^4) and thiamethoxam 25 WG (74.67×10^4). The plots treated with imidacloprid 70 WDG, fipronil 0.3 G and fipronil 40%+ imidacloprid 40% WG registered 73.67×10^4 , 72.67×10^4 and 71.00×10^4 colony forming unit of fungi per gram of soil, respectively (Table 4.5 & Fig. 4.6). Perusal of data in respect of 30, 45, 60 and 75 days after application of insecticides also revealed that the population of soil fungi gradually recovered in each insecticidal treated plot over time when compared with the data recorded at 15 days after application of insecticides.

Discussion... 



CHAPTER V

DISCUSSION

An investigation entitled “Impact of certain newer insecticides on soil faunal diversity in French bean (*Phaseolus vulgaris* L.)” was carried out during 2020-21. The results obtained from the investigation have been presented in the previous chapter. The salient findings of the investigation are being discussed in this chapter along with the information available on the similar lines of work.

5.1 Effect of newer insecticides on soil arthropod population

5.1.1 Abundance of different soil arthropods in the pretreated plots

The abundance of different soil macro and microarthropods in the pretreated plots was determined by using pitfall traps as well as Tullgren funnels, respectively. It is vivid from the results presented in Table 4.1 that among the various soil arthropods, the class Insecta and Arachnida were abundant in the experimental area. In the pretreated plots, Hymenoptera was the most dominant order (54.74 %) of soil macroarthropods in the pre-treated plots followed by Coleoptera (13.68 %) and Araneae (11.57 %). The other orders recorded in least abundance were Hemiptera (5.26%), Orthoptera (4.21%), Neuroptera (3.16%), Isoptera (2.80%), Dermaptera (2.47%) and Lepidoptera (2.11%) (Table 4.1 and Fig. 4.1). The present findings are in line with the results of Santos *et al.* (2007), who reported that the soil fauna in the olive grove ecosystem was numerically dominated by Formicidae groups comprising 56.60 per cent of all the soil macroarthropods captured in the pitfall traps.

Results presented in Table 4.1 also revealed the per cent relative abundance of soil microarthropods before initiating the application of insecticides in the experimental plots. An average of 4536 numbers of soil microarthropods per plot were extracted prior to the application of insecticides, out of which the abundance of Collembola and Oribatida were recorded to be 64.72 and 35.28 per cent, respectively (Fig. 4.2). In a similar line of work conducted by Neher and Barbercheck (2019) also reported that the springtails (Collembola) as well as the soil dwelling mites were the most commonly found soil microarthropods found in the top layers of the soil due to its

richness in the litter and humus content, making the habitat congenial for the soil microarthropods.

5.1.2 Effect of newer insecticides on soil macroarthropod population

The results presented in Table 4.2 and Fig. 4.3 showed the effect of tested insecticides on soil macroarthropod population at fortnightly intervals. Soil macroarthropods obtained in different plots before initiating the application of insecticides ranged between 89.00 to 95.33 numbers which were statistically *at par* with each other. However, the data pertaining to the number of soil macroarthropods per plot recorded at each fortnightly interval after application of different insecticides (47.33-52.67 at 15 DAT, 52.67-59.00 at 30 DAT, 60.67-65.67 at 45 DAT, 69.33-74.33 at 60 DAT and 77.00-81.33 at 75 DAT) revealed a significant decrease in the population as compared to the untreated plots (89.33, 97.67, 101.33, 99.00 and 106.33 at 15, 30, 45, 60 and 75 DAT, respectively). The significant reduction in the soil macroarthropod population in all the insecticidal treated plots may be due to the quick knockdown effects of the synthetic insecticides which often come in contact with the macroarthropod fauna during the time of their foraging. The present results corroborate the findings of El-Naggar and Zidan (2013), who explained that the toxicity of synthetic pesticides which could lead to the death of different soil arthropods either through consumption of the toxic particles along with their food or through contact during their grazing period. A similar kind of study was also conducted by Shar *et al.* (2016), who also reported that the application of some insecticides like bifenthrin, chlorpyrifos, endosulfan and imidacloprid severely reduced the population of spiders, ants, field cricket, snow bug and silverfish in the soil just after 1 day of treatment even at their recommended doses.

The data registered in Table 4.2 also revealed that the population of soil macroarthropods in all the insecticidal treated plots registered statistical parity with each other at each fortnight interval which provide an insight of comparatively similar effects of the tested insecticides on the soil macroarthropod population. However, among the different treatments, the maximum number of soil macroarthropods was recorded in chlorantraniliprole 0.4 GR treated plots (52.67, 59.00, 65.67, 74.33 and 81.33) followed by clothianidin 50 WDG (50.33, 57.67, 64.00, 73.67 and 80.67) and thiamethoxam 25 WG (49.67, 56.33, 62.00, 72.33 and 79.33) at 15, 30, 45, 60 and 75 days after treatment, respectively. The present finding is in line with the results of Ghosal and Hati (2019),

who reported that the highest population of minute arthropods in rynaxypyr (also known as chlorantraniliprole) treated plots among the seven different insecticidal treatments viz., rynaxypyr, fipronil, cartap hydrochloride, carbofuran, phorate, imidacloprid and chlorpyrifos. Similarly, Rahaman and Stout (2019) conducted an experiment with chlorantraniliprole 0.4 G, carbofuran 5 G, dinotefuran 20 SG, methoxyfenozide 24 SC and quinalphos 25 EC and reported a significant reduction in the numbers of predators in the rice ecosystem, however, chlorantraniliprole 0.4 G recorded the lowest toxicity among the tested insecticides.

Perusal of data presented in Table 4.2 also revealed a gradual recovery of the soil macroarthropod population in each insecticidal treated plots from 30 days onwards up to 75 days after treatment. Probable reason for the gradual recovery of the soil macroarthropod population in the insecticidal treated plots may be due to the loss of effectiveness of the insecticides after a particular time period. The present outcomes are comparable with the findings of Karam (2011), who reported that the insecticides like rynaxypyr, cartap hydrochloride, fipronil and carbofuran have no significant effect on the macro and microarthropods present in the soil at the end of the experimental period. Furthermore, the increase in the catches of soil macroarthropods in the pitfall traps in the subsequent period of the experiments can be explained by the concept of half-life of the insecticides. All the tested insecticides in the present investigation possess very short half-life period viz., 8.36, 15.18, 20.1-21.5, 28.7-35.8 and 43 days for chlorantraniliprole 0.4 GR, clothianidin 50 WDG, thiamethoxam 25 WG, imidacloprid 70 WDG and fipronil 0.3 G, respectively as reported by Sarkar *et al.* (2001); Mandal and Singh (2013); Kumar *et al.* (2014); Sharma *et al.* (2014) and Bansal *et al.* (2019).

5.1.3 Effect of newer insecticides on soil microarthropod population

Experimental results revealed the number of soil microarthropods per square metre of soil prior to the application of insecticides in different plots ranged between 458.33 to 555.56 which were statistically *at par* with each other (Table 4.3 and Fig. 4.4). When the soil microarthropods were extracted at 15, 30, 45, 60 and 75 days after insecticidal treatments, the population of soil microarthropod (numbers/sq. m.) in different plots were ranged between 597.22-694.44, 666.67-763.89, 708.33-791.67, 958.33-1041.67 and 1027.78-1111.11, respectively which showed statistical parity among all the treatments. It is vivid from the experimental results that all the tested insecticides in the present study did not exhibit any significant negative impact on the

soil microarthropod population during the experimental period. Ghosal and Hati (2019), who also tested the effectiveness of some insecticides viz., rynaxypyr, fipronil, cartap hydrochloride, carbofuran, phorate, imidacloprid and chlorpyrifos against collembolans and reported no negative effect on collembolan population compared to the pretreatment. Additionally, a progressive rise in the total number of soil microarthropods per square meter was recorded from 15 DAT onwards up to 75 DAT as compared to the pretreated plots. In the present study, soil samples were collected from a constant depth of 0-10 cm where the availability of soil microarthropods was lower during the initial period which may be due to their vertical migration in search of moisture. However in the subsequent period of the experiment, the population of soil microarthropods gradually increased which might be related to the availability of moisture content in the surface layer of the soil as a result of regular irrigation and continuous rain. The present finding corroborate the results of Moitra (2017), who reported that the abundance of collembolans and oribatid mites were significantly affected by the depth of collection and moisture availability in soil. Bhagawati *et al.* (2021) also reported a significant positive correlation of collembolans with soil moisture while studying the diversity of collembolans in three different ecosystems. Hence, the applied insecticides might not reach to the microarthropods in lethal concentrations as in case of the soil dwelling macroarthropods having larger body size and above ground foraging activity due to which an insignificant effect of the tested insecticides against soil microarthropods was observed. Moreover in the present study, applied insecticides also affected the potential predatory macroarthropods (carabids, rove beetles, ants, wasps etc.) of soil microarthropods which also can be cited as one of the possible reason for the insignificant impact of the tested insecticides on soil microarthropod fauna. The increase in the abundance of soil dwelling microarthropods following the use of synthetic insecticides due to the knockdown effect on the potential predators of soil microarthropods has already been well established (Frampton and Van den Brink, 2007; El-Naggar and Zidan, 2013).

5.2 Effect of newer insecticides on soil microbial population

5.2.1 Effect of newer insecticides on total bacterial population

The population of total soil bacteria (cfu $\times 10^4$ /g of soil) in all the treatments prior to the application of insecticides ranged between 95.67 to 104.67, which were statistically *at par* with each other (Table 4.4 and Fig. 4.5). It is vivid from

the results that all the six tested insecticides significantly reduced (58.67-62.67, 68.00-71.67, 75.33-78.33, 79.00-82.67 and 87.33-90.67) the population of total bacteria (cfu $\times 10^4$ /g of soil) in soil at 15, 30, 45, 60 and 75 days after application of insecticides, respectively as compared to the control (98.67, 89.33, 96.00, 100.33 and 106.33 at 15, 30, 45, 60 and 75 DAT, respectively). The possible reason for significant reduction in the total bacterial population might be due to the direct toxicity of insecticides or due to the indirect effect by changing the soil physicochemical properties and soil key enzymes after application of insecticides. The present finding is supported by the reports of Filimon *et al.* (2015), who conducted an enzymatic and bacteriological analysis and reported that the soil enzymes such as dehydrogenase, urease, catalase and phosphatase activities were significantly reduced in cypermethrin and thiamethoxam treated soil indicating an inhibitory effect on the metabolic processes of the soil microbial population, especially the nitrifying bacterial communities.

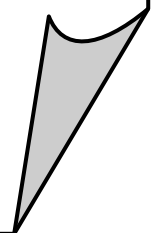
Results presented in Table 4.4 also showed that the population of bacteria found in all the insecticidal treated plots were statistically *at par* with each other at each fortnight observation period, indicating an almost similar impact of all the six tested insecticides on the total soil bacteria. It is vivid from the results that the total bacterial population in all the insecticidal treated plots registered a gradual increase at 30 days after treatments up to 75 days as compared to the population recorded at 15 days after treatment. This might be due to the toxic effects of insecticides during the early days of sampling which was overcome by the existing bacterial population in the subsequent periods due to the use of the insecticides and its degraded products as a sole source of carbon and nitrogen as nutrient source. In a similar line of study, Tang *et al.* (2018) explained that the degradation of pesticides by microbes involves a series of physiological and biochemical reactions mediated by various enzymes which ultimately results in the development of non-toxic or less toxic products. The present findings are in consistent with the results of Pandey and Singh (2003), who also reported that the chlorpyrifos and quinalphos significantly reduced the total bacterial population but recovered during 45 days after soil treatment and 60 days after seed treatment. According to Ahmed and Ahmed (2006) chlorpyrifos caused a significant reduction in the number of soil bacteria whereas the effect completely disappeared at 21 days after application. The present findings are also comparable to the results of Shahi *et al.* (2007), who explored the use of insecticides by the soil bacteria as their nutrient source. In a similar way Ghosal *et al.* (2018) reported a significant negative effect of tested

insecticides *viz.*, rynaxypyr, fipronil, cartap hydrochloride, carbofuran, phorate, imidacloprid and chlorpyrifos on the population of soil bacteria for up to 28 days followed by a gradual rise in their population till the end of the experiment.

5.2.2 Effect of newer insecticides on total fungal population

The results displayed in Table 4.5 and Fig. 4.6 showed that population of fungi (cfu/g of soil) in all the plots before initiating the application of insecticides ranged between 78.00×10^4 to 85.33×10^4 which were statistically *at par* with each other. The present investigation showed that the fungal population (cfu $\times 10^4$ /g of soil) was significantly reduced (48.00-51.67, 51.33-55.00, 57.33-62.67, 65.67-69.00 and 71.00-75.33) in all the insecticidal treated plots at 15, 30, 45, 60 and 75 days after application of insecticides, respectively as compared to control (91.67, 102.33, 96.33, 104.33 and 99.00 at 15, 30, 45, 60 and 75 DAT, respectively) which might be due to the toxic chemical nature of the tested insecticides. Furthermore, the population of total soil fungi registered a gradual recovery in each insecticidal treated plots in the subsequent period of the experiment which might be due to the utilization of the concerned insecticides or their by-products for their cellular nutrients. The present finding is in conformity with the findings of Ghosal *et al.* (2018), who reported a fall in the proliferation of fungi up to 28th days of insecticide application followed by a progressive rise in the population of total fungi up to the end of the experiment. Similar observations were also made by Pandey and Singh (2003), who reported that the population of total fungi was significantly enhanced after chlorpyrifos application, whereas quinalphos inhibited the fungal population during the initial days of treatment but no effect was observed after 60 days. In the present study, all the insecticidal treatments showed statistical parity with each other in respect of the total fungi population at each fortnight interval, however among the insecticides, chlorantraniliprole 0.4 GR treated plot recorded the highest fungal population *i.e.* 51.67, 55.00, 62.67, 69.00 and 75.33 cfu $\times 10^4$ /g of soil at 15, 30, 45, 60 and 75 days after application of insecticides, respectively. In a similar line of study, Ghosal *et al.* (2018) also reported the maximum fungal population in rynaxypyr treated plots showing its least affect to the non-targeted soil fauna.

Summary and Conclusion... 



CHAPTER VI

SUMMARY AND CONCLUSION

Field and laboratory studies were carried out in the Horticulture Experimental Farm; Department of Entomology and Department of Plant Pathology, Assam Agricultural University, Jorhat with a view to study the impact of certain newer insecticides on soil faunal diversity in French bean, *Phaseolus vulgaris* L. during 2020-21. The prominent findings of the present investigation (objective-wise) are summarized below:

Objective 1. To study the effect of certain newer insecticides on some soil arthropods in French bean

- Abundance of different soil arthropods in the pretreated plots revealed Hymenoptera as the most dominant order (54.74%) followed by Coleoptera (13.68%) and Araneae (11.57%). The other orders pertaining to soil macroarthropods recorded in least abundance were Hemiptera (5.26%), Orthoptera (4.21%), Neuroptera (3.16%), Isoptera (2.80%), Dermaptera (2.47%) and Lepidoptera (2.11%). Out of the total soil microarthropods recovered, Collembola and Oribatida registered 64.72 and 35.28 per cent of abundance, respectively before initiating the application of insecticides.
- The population of soil macroarthropods in different plots prior to the application of insecticides was ranged between 89.00 and 95.33 which were statistically *at par* with each other.
- The number of soil macroarthropods recorded in all the insecticidal treated plots *viz.*, clothianidin 50 WDG, fipronil 0.3 G, thiamethoxam 25 WG, imidacloprid 70 WG, chlorantraniliprole 0.4 GR and fipronil 40%+ imidacloprid 40% WG at 15, 30, 45, 60 and 75 days after application of insecticides was ranged between 47.33-52.67, 52.67-59.00, 60.67-65.67, 69.33-74.33 and 77.00-81.33, respectively which showed significant reduction in their population as compared

to the control (89.33, 97.67, 101.33, 99.00 and 106.33 at 15, 30, 45, 60 and 75 DAT, respectively).

- Soil macroarthropods registered in all the insecticidal treated plots at each fortnightly interval showed statistical parity with each other, however, among the insecticides the maximum number of soil macroarthropods were registered in chlorantraniliprole 0.4 GR treated plot followed by clothianidin 50 WDG and thiamethoxam 25 WG.
- The population of soil macroarthropods in all the insecticidal treated plots at 30, 45, 60 and 75 days after application of insecticides revealed a gradual recovery as compared to the population recorded at 15 days after treatment.
- The number of soil microarthropods in different plots prior to the application of insecticides ranged between 458.33 to 555.56/sq. m. which were *at par* with each other.
- Soil microarthropods population (numbers/sq. m.) at 15, 30, 45, 60 and 75 days after treatment of insecticides in different plots were ranged between 597.22-694.44, 666.67-763.89, 708.33-791.67, 958.33-1041.67 and 1027.78-1111.11, respectively.
- At each fortnight intervals, the population of soil microarthropods showed statistical parity among all the treatments and all the tested insecticides did not exhibit any significant negative impact on the soil microarthropod population during the experimental period.
- A progressive rise in the total number of soil microarthropods was obtained from 15 days after treatment onwards up to end of the experiment as compared to the pretreated plots.

Objective 2. To know the effect of certain newer insecticides on soil microbial population

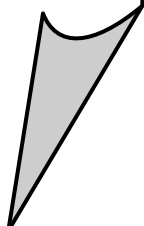
- The population of total soil bacteria ($\text{cfu} \times 10^4/\text{g}$ of soil) in all the treatments prior to the application of insecticides ranged between 95.67 to 104.67 which showed statistical parity with each other.

- The total bacterial population ($\text{cfu} \times 10^4/\text{g}$ of soil) in all the insecticidal treated plots at 15, 30, 45, 60 and 75 days after application ranged between 58.67-62.67, 68.00-71.67, 75.33-78.33, 79.00-82.67 and 87.33-90.67, respectively which showed significant decrease in their population as compared to the control (98.67, 89.33, 96.00, 100.33 and 106.33 at 15, 30, 45, 60 and 75 DAT, respectively).
- Total bacterial population in all the insecticidal treated plots were found statistically *at par* with each other, however, the highest bacterial population was recorded in chlorantraniliprole 0.4 GR treated plot followed by clothianidin 50 WDG and thiamethoxam 25 WG in each fortnight intervals.
- Total population of bacteria in soil gradually recovered in each insecticidal treated plot at 15, 30, 45, 60 and 75 days after treatment when compared with the data recorded at 15 days after application of insecticides.
- The CFU count of fungi per gram of soil before initiating the application of insecticides was ranged between 78.00×10^4 to 85.33×10^4 which were statistically *at par* with each other.
- Data registered on the total fungal population ($\text{cfu} \times 10^4/\text{g}$ of soil) in all the insecticide treated plots at 15, 30, 45, 60 and 75 days after insecticide application ranged between 48.00-51.67, 51.33-55.00, 57.33-62.67, 65.67-69.00 and 71.00-75.33, respectively which showed significant reduction in their population as compared to the control (91.67, 102.33, 96.33, 104.33 and 99.00 at 15, 30, 45, 60 and 75 DAT, respectively).
- Chlorantraniliprole 0.4 GR treated plot recorded maximum fungal population followed by clothianidin 50 WDG and thiamethoxam 25 WG, however, the total fungal population revealed statistical parity among the different insecticidal treatments.
- A significant increase in the population of total soil fungi was registered at 30, 45, 60 and 75 days after application of insecticides as compared to their population at 15 days after insecticide application.

CONCLUSION

In an effort to understand the ecotoxicological effects of newer insecticides on soil faunal diversity, it may be concluded that the tested insecticides showed a negative effect on soil macroarthropod population along with the total soil dwelling bacteria and fungi. However, this impact was significantly observed during the initial period of the experimentation which gradually decreased during the subsequent period enabling different groups of soil fauna to gradually recover. There was no any significant impact of the tested insecticides observed against the soil microarthropod population in the current study. The results so obtained from the present study will be helpful for the selection of suitable insecticides to formulate sustainable Integrated Pest Management (IPM) strategies against various soil dwelling insect pests. Further, there is a need to conduct residual analysis on insecticidal treated plots which will provide more supplementary information on the insecticide persistence in soil as well as in crops. Furthermore, analysis of soil physico-chemical properties and estimation of key soil enzyme activities after application of these insecticides will give more insights about their possible impact on soil macro, meso and microfauna.

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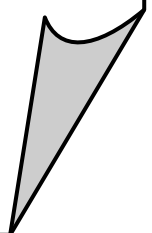
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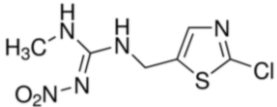
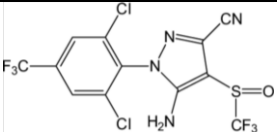
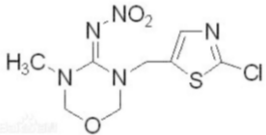
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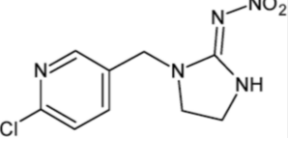
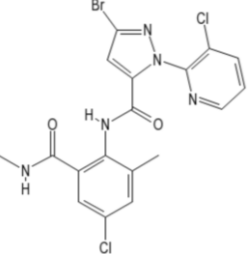
Appendix... 



APPENDIX I

Details of insecticides used in the experiment

Chemical name	Structure	IUPAC name	Trade name	Chemical group and Mode of action
Clothianidin 50 WDG		1-(2-Chloro-1,3-thiazol-5-ylmethyl)-3-methyl-2-nitroguanidine	Dantotsu	Neonicotinoid Antagonist to the nicotinic acetyl choline receptor (nAChR) in the central nervous system
Fipronil 0.3 G		5-amino-1-[2,6-dichloro-4-(trifluoromethyl)phenyl]-4-(trifluoromethylsulfinyl)-1H-pyrazole-3-carbonitrile	Regent	Phenylpyrazole Blocks GABA (Gamma Aminobutyric acid) gated chloride channels in the central nervous system
Thiamethoxam 25 WG		3-[(2-Chloro-1,3-thiazol-5-yl)methyl]-5-methyl-1,3,5-oxadiazinan-4-ylidene} nitramide	Actara	Neonicotinoid Antagonist to the nicotinic acetyl choline receptor (nAChR) in the central nervous system

Chemical name	Structure	IUPAC name	Trade name	Chemical group and Mode of action
Imidacloprid 70 WG		1-(6-chloro-3-pyridylmethyl)-N-nitroimidazolidin-2-ylideneamine	Admire	Neonicotinoid Antagonist to the nicotinic acetyl choline receptor (nAChR) in the central nervous system
Chlorantraniliprole 0.4 GR		3-Bromo-N-[4-chloro-2-methyl-6-(methylcarbamoyl)phenyl]-1-(3-chloropyridin-2-yl)-1H-pyrazole-5-carboxamide	Fertera	Diamide Opens muscular calcium channels (in particular the ryanodine receptor)
Fipronil 40%+ Imidacloprid 40% WG	-	-	Lesenta	Having dual mode of action viz., imidacloprid disturbs the proper signal transmission system by blocking nAChR and fipronil blocks GABA-gated chloride channels