

**TOXICITY OF NOVEL INSECTICIDES TO
Pieris brassicae (Linnaeus) AND THEIR
RESIDUE DYNAMICS ON CABBAGE**

THESIS

By

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(A-2020-30-025)**

Submitted to



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**in
partial fulfilment of the requirements for the degree
of
MASTER OF SCIENCE IN AGRICULTURE
(DEPARTMENT OF ENTOMOLOGY)
(ENTOMOLOGY)
2022**

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CERTIFICATE – I

This is to certify that the thesis entitled, “**Toxicity of novel insecticides to *Pieris brassicae* (Linnaeus) and their residue dynamics on cabbage**” submitted in partial fulfilment of the requirements for the award of the degree of **Master of Science (Agriculture)** in the discipline of **Entomology** of CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur is a bonafide research work carried out by **Battu Jabez Raju (Admission No. A-2020-30-025)** son of **Smt. Darla Hephzibah Beulah** and **Late Sh. Battu Solomon Raju** under my supervision and that no part of this thesis has been submitted for any other degree or diploma.

The assistance and help received during the course of this investigation have been fully acknowledged.

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Major Advisor

CERTIFICATE- II

This is to certify that the thesis entitled, “**Toxicity of novel insecticides to *Pieris brassicae* (Linnaeus) and their residue dynamics on cabbage**” submitted by **Battu Jabez Raju (Admission No. A-2020-30-025)** son of **Smt. Darla Hephzibah Beulah** and **Late Sh. Battu Solomon Raju** to the CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur in partial fulfilment of the requirements for the degree of **Master of Science (Agriculture)** in the discipline of **Entomology** has been approved by the Advisory Committee after an oral examination of the student in collaboration with an External Examiner.

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ACKNOWLEDGEMENTS

It gives me immense pleasure to present my thesis. I cannot express my gratitude to God, Almighty for his kindness, grace and favour he has shown me through the darkest of the nights.

*With the overwhelming sense of legitimate pride and exuberant pleasure I would like to express my eternal gratitude to my loving and revered advisor **Dr. Prem Chand Sharma** (Principal Scientist, Department of Entomology), Chairperson of my Advisory Committee for his excellent and praiseworthy guidance, indescribable humanitarian behavior and parental affection that circumvallated me during my hardships throughout the course of my study and particularly at the time of research and preparation of this manuscript. I will be forever indebted to him.*

*I express my sincere gratitude to the worthy members of my advisory committee, **Dr. Pawan Kumar Sharma** (Principal Scientist, Department of Entomology), **Dr. Amar Singh** (Principal Scientist, Department of Plant Pathology) and **Dr. Janardan Singh** (Dean's Nominee, Professor and Head, Department of Organic Agriculture and Natural farming) for their inspiring guidance at different stages of study and critical assessment of the manuscript.*

*I take this opportunity to convey my sincere thanks to **Dr. R.S. Chandel** (Principal Scientist and Head, Department of Entomology) and **Dr. Ajay Kumar Sood** (Principal Scientist, Department of Entomology), **Dr. K.S. Verma** (Professor, Department of Entomology), **Dr. Surjeet Kumar** (Professor, Department of Entomology), **Dr. Anjana Thakur** (Assistant Professor, Department of Entomology), **Dr. Sharmishtha Thakur** (Assistant Professor, Department of Entomology) and **Dr. Suman Sanjta** (Assistant Professor, Department of Entomology), for abetment whenever needed at various stages of this investigation and my studies. Words cannot suffice my thankfulness and obligation towards my teachers **Dr. S. Dayakar**, **Dr. B. Subrahmanyam**, **Dr. M.S. Sai Reddy** and **Mr. Kishore Raju** for their altruistic support and guidance.*

*I feel my utmost duty to express my sincere thanks to **Dr. Suresh Gautam**, the Dean, Postgraduate Studies, **Prof. H.K. Chaudhary**, Hon'ble Vice Chancellor, CSK HPKV Palampur and University authorities for providing necessary facilities.*

I wish there could have been a more befitting way than words to express deep sense of gratitude to the office, laboratory and field staff of the Department of Entomology for their assistance and co-operation provided to me during the course of investigation.

*I would like to thank my mother **D.H. Beulah** and my aunt **D.L. Kumari**, for their encouragement and inspiration throughout my life. I take this precious moment to express my deep sentiments and indebtedness to my brothers **Shalom**, **Samuel**, **Joy**, **Paul** and my sister **Akanksha** whose affection, moral support and help led me to achieve my destination successfully.*

*I owe a debt of gratitude to my seniors **Karthik Somala**, **Tony Manoj**, **Ramesh**, **Matha Venkateswara roa**, **Anil**, **Gopi Prasad**, **Mayur**, **Ilyas**, **A. Praveen Kumar**, **Kulveer**, **Vivek**, **Naresh Kumar**, **Dixit**, **Rahul Sharma**, **Vasu Mehta**, **Sukchain Singh**, **Sarwan Kumar**. I am forever grateful to my loving seniors **Pandit Praveen**, **Karthik Ramappa**, **Himanshu Thakur** and **Nitika Negi** for their continuous support and guidance throughout this degree. I would also like to extend my appreciation to my juniors **Suresh**, **Prakash**, **Ayush**, **Prashanth**, **Halesh** and **Sai Kiran** for their moral support throughout the course of study*

*I would like to take this moment to thank my friends **Ravi**, **Pavan**, **Chakradhar**, **Nageswara rao**, **Sunil Karthik**, **Harish**, **Chethan**, **Kanna**, **Pradeep**, **Venkatesh Vulli**, **Gopi**, **Prasad**, **P. Venkatesh**, **Ram Babu**, **Rajesh**, **Sudheer**, **Harsha** and **Reshma** for their moral support and encouragement. I am ineffable to express my thankfulness to my friends **Deepak**, **Gurpreet**, **Sandeep**, **Vijay**, **Gowtham**, **Swapnil**, **Sachin Saharan**, **Sachin Sharma**, **Ritesh** and **Vishrava** for their help and support during my stay at Palampur.*

One last word since it is practically impossible to list all the names who contributed to my work, it seems proper to issue a blanket of thanks for those who helped me directly or indirectly during the course of study. Any omission in this brief acknowledgement doesn't mean lack of gratitude. All mistakes and imperfections are, of course mine.

Place: Palampur

Dated: July 30, 2022

(**Battu Jabez Raju**)

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LIST OF ABBREVIATIONS USED

Sr. No.	Abbreviations	Meaning
1.	%	Per cent
2.	/	Per
3.	@	At the rate
4.	°C	Degree Celsius
5.	µl	Micro Litres
6.	a.i.	Active ingredient
7.	EC	Emulsifiable Concentrate
8.	et al.	Et alii (and other)
9.	etc.	Et cetera
10.	Fig.	Figure
11.	g	Gram
12.	ha	Hectare
13.	i.e.	Id est (that is)
14.	kg	Kilogram
15.	L	Litre
16.	m	Metre
17.	mg	Milligrams
18.	min.	Minutes
19.	mL	Millilitre
20.	OD	Oil Dispersion
21.	ppm	Parts per million
22.	SC	Suspension Concentrate
23.	SD	Standard Deviation
24.	SG	Soluble Granules
25.	sp.	Species
26.	sq. m	Square metre
27.	viz.	Vi delictet (namely)

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Title of the thesis : Toxicity of novel insecticides to *Pieris brassicae* (Linnaeus) and their residue dynamics on cabbage
Name of the student : Battu Jabez Raju
Admission number : A-2020-30-025
Major discipline : Entomology
Minor discipline : Plant Pathology
Date of thesis submission : July 30, 2022
Total pages of the thesis : 73
Major advisor : Dr. Prem Chand Sharma

ABSTRACT

Laboratory studies were conducted in the Toxicology Laboratory of Department of Entomology, CSKHPKV, Palampur to evaluate the toxicity of novel insecticides against *Pieris brassicae*. A field experiment was conducted at the Experimental Research Farm of Department of Entomology, CSKHPKV, Palampur during the *rabi* season 2021-22 to determine the insecticide residues on cabbage, variety Pusa Hybrid-18. The intrinsic toxicity of insecticides comprising cyantraniliprole, cypermethrin, emamectin benzoate and spinosad was determined against neonate and 3rd instar larvae of *P. brassicae*. The LC₅₀ values of cyantraniliprole, cypermethrin, emamectin benzoate and spinosad for neonate larvae were 0.00003, 0.00029, 0.002 and 0.061 ppm, respectively. Cyantraniliprole, cypermethrin and emamectin benzoate were 2033.33, 210.34 and 30.5 times more toxic than spinosad to the neonate larvae of *P. brassicae*. The LC₅₀ values of cyantraniliprole, cypermethrin, emamectin benzoate, and spinosad tested against 3rd instar larvae of *P. brassicae* were 0.007, 0.484, 0.017 and 0.202 ppm, respectively. Cyantraniliprole, emamectin benzoate and spinosad were 69.14, 28.47 and 2.40 times more toxic to 3rd instar larvae of *P. brassicae* when compared to cypermethrin. The average initial deposits of cyantraniliprole when applied at a dosage of 60 g and 120 g a.i./ha were 0.4515±0.015 and 1.1487±0.174 mg/kg, respectively. Emamectin benzoate had the initial deposits of 0.2991±0.009 and 0.3663±0.008 mg/kg at 10 g and 20 g a.i./ha, respectively. The mean initial deposits of spinosad were 0.5151±0.027 and 0.9713±0.128 mg/kg at the doses of 15 g and 30 g a.i./ha, respectively. The half-life values were 1.3 and 4.2 days for cyantraniliprole, 0.9 and 1.5 days for emamectin benzoate; 2.3 and 4.5 days for spinosad when applied at recommended and double the recommended doses, respectively. Removal of outer leaves and boiling the heads for 5 minutes proved most effective processing methods as they removed 100 per cent of residues.

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1. INTRODUCTION

India is the largest producer of vegetables following China. Cabbage (*Brassica oleracea* var. *capitata*) belongs to the family Brassicaceae, one of the cole crops and an important vegetable in which the economic part is the edible “head”. Globally, cabbage is grown over an area of 2.41 M ha (FAOSTAT 2020). According to the 2020 statistics, the production of cabbage along with other *Brassicaceae* was 71 million tonnes with China being the leading producer followed by India (FAOSTAT 2022).

The production scenario for the fiscal year 2021 is that the total area under cabbage cultivation in India is around 0.404 M ha and production is around 9.59 million metric tonnes (Statista 2022). The average productivity of cabbage in India is 22.7 MT/ha (Anonymous 2018). In India, West Bengal, Orissa, Gujarat, Madhya Pradesh, Assam and Bihar are the major cabbage producing states. West Bengal being the highest producer of cabbage (23.41 million tonnes), contributes about 24.4% of the total cabbage produced in India. The production in Himachal Pradesh is around 1.46 million tonnes, thus contributing about 1.53% to the total production in India (Anonymous 2022).

Raw cabbage is a rich source of vitamins K & C (USDA FoodData Central 2019) and a moderate source of vitamins B6 and B9. The phytochemicals present in cabbage such as sulforaphane and other glucosinolates are subject to investigation as they might have potential anti-disease effects (Dinkova-Kostova et al. 2012). Cabbage is a rich source of indole-3-carbinol which is being studied for its anti-carcinogenic properties (Wu et al. 2010). Off-season cabbage cultivation is of high market value. Cabbage thrives its best in a relatively cool and moist climate. It is directly consumed as a fresh vegetable and it has a shelf life of 14-20 days.

The cabbage crop is ravaged by a number of insect pests which hamper its productivity. The major pests of cabbage occurring in Indian sub-continent are tobacco caterpillar, *Spodoptera litura* Fab., diamondback moth, *Plutella xylostella* (Linnaeus), cabbage butterfly, *Pieris brassicae* (Linnaeus), cabbage leaf webber, *Crociodolomia bionotalis* (Zeller), aphids, *Brevicoryne brassicae* (Linnaeus), *Lipaphis erysimi* (Kaltenbach), painted bug, *Bagrada cruciferarum* (Kirkaldy) and flea beetle,

Phyllotreta cruciferae (Goeze) (Hasan and Ansari 2010; Bana et al. 2012). The insect pests recorded in Himachal Pradesh include *B. brassicae*, *P. xylostella*, *P. brassicae*, *P. orichalcea*, *H. armigera* and *B. cruciferarum* appearing in the descending order (Bhatia and Verma 1994). The regular pests recorded are *B. brassicae*, *P. brassicae*, *P. xylostella* and *P. orichalcea* (Bhatia and Verma 1995).

Among these pests, cabbage butterfly, *P. brassicae* is one of the most destructive insect pests causing damage in all the growth stages (Khalid 2006; Ali and Rizvi 2007). It is an oligophagous pest feeding on the crops belonging to the family Cruciferae (Hasan and Ansari 2010). It is a migratory pest which spends the winters in the plains of the Indian sub-continent (Gupta 1984). The larvae feed on the leaves as well as seeds of cabbage and cauliflower causing economic losses (Hasan 2008; Sharma and Gupta 2009). A single larvae of *P. brassicae* is capable of consuming 74-80 cm² leaf area (Younas et al. 2004). There are reports of development of resistance in *P. brassicae* to some insecticides (Sharma and Gupta 2009). Crop failure in cole crops has been reported in the case of severe infestation by *P. brassicae* (USDA, 1984; Giri et al. 2020).

For the management of these insect pests, the farmers of Himachal Pradesh use malathion, chlorpyrifos, cypermethrin, profenofos, imidacloprid, methyl demeton, dimethoate, etc. (Singh et al. 2016). Recently, many of these insecticides have been phased out for use. The insecticides approved for use on cabbage in India include many new chemistry insecticides viz., acetamiprid, chlorantraniliprole, chlorfenapyr, chlorfluazuron, cyantraniliprole, diafenthiuron, emamectin benzoate, indoxacarb, spinosad etc. (Anonymous 2021).

In order to mitigate the yield losses occurring in cabbage and cauliflower, majority of the farmers exclusively rely on the pesticides for controlling the insect pests and spray pesticides even before the incidence of pest as a preventive measure (Weinberger and Srinivasan 2009). Since cabbage heads are harvested at short intervals, these are likely to retain unavoidably high level of pesticide residues, thus posing hazards to consumers' health. About two million tonnes of pesticides are used around the world annually affecting human health and the environment due to their high toxicity, persistence, and bioaccumulation. Moreover, they are also toxic to non-target beneficial organisms like predators and parasitoids and are responsible for pest

resurgence and the development of resistance in insects (Dutta 2015; Mittal et al. 2021; Sai Reddy et al. 2022).

Keeping in view the above facts, the present investigations were carried out with following objectives:

1. To evaluate the novel insecticides for their toxicity to *Pieris brassicae* larvae
2. To determine the residues of insecticides on cabbage and the effect of processing on the removal of residues

2. REVIEW OF LITERATURE

Cabbage is an important cruciferous crop and this vegetable is consumed directly after harvesting as raw or in cooked form. The pest complex infesting cabbage causes severe yield losses and the cabbage butterfly, *Pieris brassicae* is a major pest of cabbage in the mid-hill region of Himachal Pradesh. In the present investigation, intrinsic toxicity of new chemistry insecticides was studied against *P. brassicae* larvae and residues of these insecticides on cabbage were determined. The literature related to the present study have been reviewed under following headings:

2.1 Intrinsic toxicity studies

Dhingra et al. (2008) evaluated the toxicity of some synthetic pyrethroids viz., deltamethrin, cypermethrin, λ -cyhalothrin, alphamethrin, bifenthrin, fenpropathrin, β -cyfluthrin and fenvalerate against the 3rd instar larvae of *P. brassicae* using the leaf dip method and direct spray method. The LC₅₀ value of cypermethrin was 9 mg mL⁻¹ by leaf dip method and 11.6 mg mL⁻¹ by direct method.

Dhawan et al. (2010) studied the toxicity of insecticides against the 3rd instar larvae of *P. brassicae* under laboratory conditions. Spinosad (Tracer 48 SC) @ 0.02% caused mortalities of 86.66, 93.33 and 100% after 24, 48 and 72 hours after treatment, respectively. The mean mortality due to spinosad was 93.33%. The LC₅₀ values of novaluron, indoxacarb, spinosad, chlorantraniliprole, endosulfan, azadirachtin and *Bacillus thuringiensis* were found to be 0.006, 0.003, 0.017, 0.002, 0.004, 0.574, and 0.613 per cent, respectively. It was also found that spinosad was 36.06 times more toxic than *B. thuringiensis*.

An experiment was laid out to test the efficacy of the synthetic pyrethroid insecticides deltamethrin, cypermethrin and fenvalerate along with endosulfan against the larvae of *P. brassicae* which were 1-2 days old and 8-9 days old. The LC₅₀ values of cypermethrin, deltamethrin, fenvalerate, and endosulfan against the 1-2 days old larvae were 0.0329, 0.0239, 0.258 and 0.733 ppm, respectively. The results of the experiment indicated that cypermethrin was 21.93 times more toxic to the 1-2 days

old larvae than endosulfan. The LC_{50} values of cypermethrin, deltamethrin, fenvalerate, endosulfan against 8–9 day old larvae were 0.124, 0.116, 0.757 and 2.676 ppm, respectively (Kumar et al. 2010).

Kannan et al. (2011) evaluated the toxicity of spinosad 2.5 SC, indoxacarb 14.5 SC, emamectin benzoate 5 SG, azadirachtin 0.5 EC and quinalphos 25 EC against 3rd instar larvae of *Crociodolomia binotalis*. The LC_{50} and LC_{90} values of spinosad 2.5 SC were reported as 0.0323 and 0.411 ppm, respectively. Emamectin benzoate 5 SG was more effective than spinosad, indoxacarb, azadirachtin and quinalphos against 3rd instar larvae of *C. binotalis*. The mortality assay revealed that emamectin benzoate was nearly 77 times more toxic to the larvae than quinalphos based on LC_{50} values. The LC_{50} and LC_{90} values of emamectin benzoate were 0.0221 ppm and 0.241 ppm, respectively. Verma (2012) assessed intrinsic toxicity of neem oil and spinosad against the third instar larvae of *P. brassicae*. The results indicated that spinosad was more toxic to the larvae with LC_{50} and LC_{90} values of 0.0006% and 0.003%, respectively.

The intrinsic toxicity of emamectin benzoate and chlorantraniliprole was studied against the 3rd instar larvae of *Spodoptera litura* by Parthiban et al. (2014). The results of the experiment indicated that the LC_{50} and LC_{90} values of emamectin benzoate after 24 and 48 hours were 0.0092 and 0.0341%, 0.0036 and 0.0152%, respectively. For chlorantraniliprole, the LC_{50} and LC_{90} values were 0.011 and 0.036% after 24 hours, 0.0043 and 0.017% after 48 hours, respectively.

Wang et al. (2014) conducted an experiment to determine the toxicity of cyantraniliprole and chlorantraniliprole against 3rd instar larvae of *A. ipsilon*. The findings revealed that the LC_{50} value of chlorantraniliprole against *A. ipsilon* larvae was 0.13 mg L⁻¹. Moadeli et al. (2014) reported the LC_{50} value of spinosad against 1st instar larvae of *Spodoptera exigua* as 0.096 ppm. Similarly, cyantraniliprole at the concentrations of 1.6, 0.8, 0.4, 0.2, 0.1, and 0.05 µg g⁻¹ incorporated into the artificial diets were evaluated for their toxicity on the 4th instar larvae of *A. ipsilon* and the LC_{50} value was found to be 0.354 µg g⁻¹ (Xu et al. 2016).

The toxicity of chlorantraniliprole against early (1st and 2nd) instars and late (3rd and 4th) instars of *P. rapae* was evaluated by Su et al. (2017). They reported the

LC₅₀ values of chlorantraniliprole against early and late instar larvae by leaf dip method after 24 hours to be 0.95 and 7.01 mg L⁻¹, respectively. The LC₉₀ values against early and late instar larvae of *P. rapae* were found as 4.32 and 19.65 mg L⁻¹, respectively.

Khan and Kumar (2017) tested the efficacy of imidacloprid, spinosad, cypermethrin, neem oil + cypermethrin, NSKE + cypermethrin, neem oil, NSKE, Neemarin®, *Metarhizium anisopliae*, *Beauveria bassiana*, *Bacillus thuringiensis* and NPV against the 3rd instar larvae of *P. brassicae*. Their findings revealed that the mortality caused by cypermethrin after 24, 48 and 72 hours was 94.2, 96.7 and 99.2 %, respectively. Spinosad caused 96.7, 98.3 and 100% mortality after 24, 48 and 72 hours of treatment, respectively under laboratory conditions. Sukirno et al. (2017) reported the LC₅₀ values of spinosad against 1st instar larvae of *S. littoralis* and *S. exigua* as 2.19 and 1.40 ppm, respectively.

Sharma et al. (2019) tested different insecticides against the 3rd instar larvae of *P. brassicae* and reported the LC₅₀ values of spinosad, chlorantraniliprole, flubendiamide, emamectin benzoate, indoxacarb and novaluron as 0.006, 0.002, 0.01, 0.00007, 0.00014 and 0.002 ppm, respectively. Shashni (2020) tested spinosad against the neonate larvae of *P. brassicae* under laboratory conditions. The LC₅₀ and LC₉₀ values of spinosad were 0.034 ppm and 0.190 ppm, respectively. It was also reported that spinosad was 1.09 x 10⁵ times more toxic than *Brahmastra* (based on LC₅₀ values) and 1.21 x 10⁵ times more toxic than *Agniastra* (based on LC₉₀ values).

The toxicity of emamectin benzoate, indoxacarb and chlorantraniliprole against different instars of *A. ipsilon* was evaluated. The experimental findings indicated that emamectin benzoate was having the highest toxicity amongst the tested insecticides. The LC₅₀ values of indoxacarb, emamectin benzoate and chlorantraniliprole against 1st instar larvae were 0.259, 0.0874, 0.934 mg L⁻¹ and against 3rd instar larvae, the LC₅₀ values were 0.809, 0.164, 1.284 mg L⁻¹, respectively (Ismail 2021).

2.2 Insecticide residue studies

The literature pertaining to residues of different insecticides on cabbage, the half-life values and effect of processing on the removal of residues have been described below:

2.2.1 Cyantraniliprole

Kar et al. (2012) extracted the cabbage samples with ethyl acetate following QuEChERS method. Distilled ethyl acetate was added to the sample in a 50 mL centrifuge tube and was centrifuged in a high-speed homogenizer at 1400-1500 rpm for 2-3 minutes. 10g of NaCl was added for phase separation and again centrifuged at 2500-3000 rpm for 3 min. 15 mL of supernatant was transferred in to a test tube through 10 g of activated sodium sulfate. This extract was cleaned up by dispersive solid phase extraction. An aliquot of 6 mL ethyl acetate extract was taken in a test tube containing 15 mg of PSA sorbent, 90 mg of anhydrous MgSO₄ and 5 mg of graphitic carbon black (GCB) and the test tube was vortexed and centrifuged at 2500–3000 rpm for 1 min. 4 mL aliquot of this ethyl acetate extract was evaporated to dryness using a low volume evaporator at 35°C to dryness. The final volume was made up to 2 mL with HPLC grade distilled acetonitrile. The residues were quantified by using high performance liquid chromatography (HPLC) equipped with photodiode detector.

Pokchoi (Chinese cabbage) samples were extracted with acetonitrile for the residue of cyantraniliprole. The homogenized samples were added into a PTFE centrifuge tube containing 10 mL acetonitrile and is vortexed for 2 min. After adding 6 g of anhydrous magnesium sulfate and 1.5 g of sodium acetate to the tube, it was vortexed for 2 min before centrifuging at 5000 rpm for 8 min. An aliquot of 2 mL from the upper acetonitrile layer was transferred into a tube containing 50 mg PSA sorbent and 150 mg anhydrous magnesium sulfate. This tube was vortexed 1 min and then centrifuged at 5000 rpm for 5 min. The top acetonitrile layer was filtered through a 0.22 mm filter membrane for UPLC–MS/MS analysis. The residues of cyantraniliprole in Pakchoi (Chinese cabbage) were estimated using UPLC equipped with a MS/MS detector. The average recovery of cyantraniliprole was 90.8, 88.5 and 97.6% when the samples were spiked at 0.1, 0.05 and 0.01 mg/kg levels of

fortification. The LOQ and LOD for cyantraniliprole were 0.1 ng/kg and 0.01 mg/kg respectively. The residues of cyantraniliprole ranged from 0.02-1.27 mg/kg on Pakchoi at 3-7 days after spraying when the dosage was 60-90 g a.i./ha. The retention time for cyantraniliprole was 1.82 minutes. The half-life values of cyantraniliprole at two different locations were 2.9 and 3.6 days, respectively. The safety intervals for the two locations were worked out to be 5.3 and 6.4 days, respectively (Sun et al. 2012).

Kar et al. (2017) extracted cabbage samples (15 g) with ethyl acetate which was filtered 10g pre-activated sodium sulfate. This was later cleaned up with 0.15 g PSA, 0.90g anhydrous $MgSO_4$ and 0.05 g GCB. This was reduced to dryness using a rotary vacuum evaporator which was later made up to 2 mL using acetonitrile. The recoveries obtained were 88.5, 89.4, 94.3 and 89.3% when spiked at 0.1, 0.25, 0.5 and 1 mg/kg levels of fortification. The mean initial deposit of chlorantraniliprole was 0.12 and 0.22 mg/kg when applied at the doses of 9.25 and 18.5 g a.i./ha. The half-life values of chlorantraniliprole were calculated as 1.80 (9.25 g a.i./ha) and 1.46 days (18.5 g a.i./ha).

The residues of chlorantraniliprole on Ssam cabbage were determined using HPLC-MS/MS with a mobile phase consisting of solvent A (0.1% formic acid, 5 mM ammonium formate in water) and solvent B (0.1% formic acid, 5 mM ammonium formate in methanol). The samples were extracted in acetonitrile and vortexed at 250 rpm for 30 minutes. The extract was suction filtered using Buchner funnel and the filter cake was washed with acetonitrile (50 mL). The filtrate was evaporated to about 1mL using a rotary vacuum evaporator ($< 40^\circ C$) and collected into a separatory funnel containing saturated sodium chloride solution and water of 50 mL each. This extract was then partitioned twice with 100 mL, 50 mL dichloromethane each time and the solvent layer was passed through anhydrous sodium sulfate and evaporated to dryness in a rotary vacuum evaporator. The residue was then dissolved into 10 mL of ethyl acetate/n-hexane. The sample was then loaded into SPE cartridge washed with 15 mL of ethyl acetate/n-hexane (30:70, v/v) and was eluted with 10 mL of ethyl acetate/n-hexane (40:60, v/v). the eluent was evaporated through a stream of nitrogen and then mixed with 10 mL of acetonitrile. The average recovery of chlorantraniliprole was 70.3 and 88% when the samples were spiked at 0.1 and 0.01

ppm levels of fortification. The LOQ was worked out to be 0.01mg/kg. The residues of chlorantraniliprole ranged from 0.05-0.95 mg/kg. The residue levels dropped by less than half of the MRL *i.e.*, 2 mg/kg after 7 days (Baek Soo-jin 2018).

Preethi et al. (2019) extracted homogenized cabbage samples weighing 10 g in acetonitrile. 1g of NaCl and 4g of anhydrous MgSO₄ were added to the sample mixture and vortexed for 2 min followed by centrifugation for 10 min at 6000 rpm. A 9 mL aliquot of the supernatant was transferred into a test tube containing 4 g of Na₂SO₄ and vortexed. 6 mL of supernatant from this test tube was transferred to a 15 mL centrifuge tube containing 10 mg GCB, 100 mg PSA and 600 mg anhydrous MgSO₄ and vortexed for 1 min and followed by the centrifugation for 10 min at 3000 rpm. 4 mL aliquot of the supernatant was concentrated to dryness by passing a gentle stream of nitrogen by using the Turbovap LV set at 40°C. The final volume was made up with HPLC grade acetonitrile for UHPLC analysis. The LOQ and LOD values were worked out to be 0.05 and 0.015 mg/g, respectively. The mean recovery values of chlorantraniliprole were 94.81, 92.58 and 96.35% when the cabbage samples were spiked at 0.05, 0.25, 0.50 mg/g levels of fortification, respectively. The residues started decreasing right from the next day and were below the detection limits by the 10th day. The half-life of chlorantraniliprole on cabbage was 2.29-2.53 days.

Cabbage samples were extracted with acetonitrile and dried over 4 g of MgSO₄, and 1 g of NaCl, and instantly homogenized. The clear supernatant was then cleaned up with 30 mg of PSA and 200 mg of anhydrous MgSO₄. The contents were centrifuged at 1500 rpm for 5 min. Finally, the supernatant from the Eppendorf tube was filtered through a 0.2-µm syringe filter for analysis using HPLC-PDA. The LOQ and LOD values of cyantraniliprole on cabbage were worked out to be 0.001 and 0.005 mg/kg, respectively. The recoveries of cyantraniliprole 97.52, 97.34, 91.02 and 89.80 per cent were obtained when the samples were spiked at 0.5, 0.1, 0.05 and 0.01 mg/kg levels of fortification. The initial deposits of cyantraniliprole on cabbage at the doses of 60 and 120 g a.i./ha after 1st spray were 0.403 and 1.195 mg/kg, respectively. Similarly, the initial deposit after the 2nd spray was 0.798 and 1.286 mg/kg, respectively. The half-life of cyantraniliprole on cabbage ranged from 3.5-4.8 days (Kumar et al. 2021).

2.2.2 Emamectin benzoate

Chi et al. (2009) extracted Chinese cabbage samples with acetonitrile and cleaned with graphitic carbon black and the residues were analysed using LC-MS. The recoveries of emamectin benzoate ranged from 71.4 to 93.2% when the samples were fortified at 0.01, 0.05 and 0.5 mg/kg levels. The LOD and LOQ were 0.5 ng/kg and 0.05 mg/kg, respectively. The half-life of emamectin benzoate was worked out to be 1.3 days.

Wang et al. (2012) extracted homogenized cabbage samples (10 g) with acetonitrile. The mixture was shaken vigorously for 1 min with vortex mixer. To the resultant mixture, 1g sodium chloride and 4g anhydrous magnesium sulfate were added and it was vortexed for 30s. The extracts were then centrifuged for 5 min at 3800 rpm. The upper layer of about 1 mL was collected and placed into a 2.0 mL micro-centrifuge vial containing 50 mg Primary secondary amine (PSA) and 150 mg MgSO₄. The sample was again vortexed for 1 min and then centrifuged for 5 min at 6000 rpm with a microcentrifuge. The upper extract was filtered through a 0.22 mm pore membrane filter and is collected for further chromatographic analysis. They obtained recoveries of 85.7, 91.6 and 97% at 0.001, 0.01 and 0.1 mg/kg levels of fortification, respectively. The LOQ was worked out to be 0.001 mg/kg. The average final residues of emamectin benzoate when applied twice and thrice at a dosage of 4.5 g a.i./ha ranged from 0.001-0.052 mg/kg at 7-14 days after spraying. The half-life values of emamectin benzoate ranged from 1.34-1.72 days.

Cabbage samples were extracted in 1% acetic acetonitrile and this mixture is vortexed for 30s. To this mixture, 4 g anhydrous magnesium sulfate and 1 g anhydrous sodium acetate were added and were vortexed vigorously for 1 min. After centrifuging at 4000 rpm for 5 min, an aliquot of 1 mL of the top layer of the extract was collected into a 2 mL micro-centrifuge containing 50 mg PSA and 150 mg anhydrous magnesium sulfate. This mixture was shaken for 1 min and centrifuged at 6000 rpm for 5 min. The top layer of the mixture was filtered through a 0.45 pore membrane filter and is subjected to HPLC-MS analysis. The average recovery rates of emamectin benzoate on cabbage were 86.4, 84, and 87.4% at the fortification levels of

0.01, 0.1, and 1 mg/kg, respectively. The LOQ was worked out to be 0.01 mg/kg and the half-life was worked out to be 1 day (Liu et al. 2012).

Singh et al. (2013) soaked macerated cabbage head samples (20 g) overnight in solution containing 50 mL acetonitrile and 50 mL methanolic solution having ammonium acetate and 1 mL triethanolamine. This overnight soaked mixture was filtered out into 1 L separatory funnels along with the rinsate of acetonitrile and methanol (50:50 v/v). The contents of the separatory funnel were diluted by adding 600 mL of brine solution and were partitioned into dichloromethane (75 x 75 mL) and ethyl acetate (75 x 75 mL) and then were combined. These combined fractions were filtered through anhydrous sodium sulfate and the extract was cleaned up using 20 g silica gel mixed with sodium sulfate in a glass column. This column was eluted with 150 mL of dichloromethane and the extract obtained was concentrated. The purified and concentrated extract was then made up to 5 mL using HPLC grade methanol. The residues were analysed using HPLC (Shimadzu) with a photodiode array detector. The recovery of emamectin benzoate at 0.5, 0.25 and 0.05 mg/kg levels of fortification was consistent and above 92%. The LOQ and LOD were worked out to be 0.05 and 0.017 mg/kg. The residues of emamectin benzoate were diminishing right from the next day after the spray and were below the detection limits after by the 5th day in case of both the doses. The half-life values of emamectin benzoate were 0.88 day when applied @ 8.5 g a.i./ha and 1.25 days when applied @ 17 g a.i./ha. They also suggested a waiting period of 1 day for the safe consumption of cabbage.

Cauliflower samples were extracted in ethyl acetate for the analysis of emamectin benzoate residues. The extract was then filtered by Whatman No. 1 filter paper and the filtrate was collected. The top ethyl acetate layer was collected and separated. This was concentrated to dryness using a rotary vacuum evaporator. To this, 5 mL of the mobile phase [acetonitrile: ammonium acetate buffer (50:50)] was added and mixed well and the filtered using a 0.2m filter. The final filtrate obtained was injected into HPLC for chromatographic analysis. Emamectin benzoate was applied on cauliflower at the doses of 1.5 L and 3 L/ha. The samples were collected and analyzed for the residues after 6 hours of field application of the insecticide. The LOD and LOQ were worked out to be 0.0022 and 0.0029 mg/kg, respectively. The residues at the dosage of 1.5 L and 3 L/ha were found to be 0.064 and 0.116 mg/kg,

respectively. The recovery of emamectin benzoate at the level of 25, 50 and 75 ng/mL were found to be 100, 99.96, and 99.81%, respectively (Rajasekaran et al. 2013).

Chahil et al. (2014) soaked cauliflower samples overnight in acetonitrile and methanol mixture, and extracted them with acetonitrile and methanol mixture (50:50 v/v) along with the rinsate. This mixture was diluted with 600 mL brine solution and partitioned into dichloromethane (2 x 75 mL) and ethyl acetate (2 x 75 mL) and these fractions were combined. The mixture of these fractions was dried over anhydrous sodium sulphate. The extract was then cleaned up with 20 g silica gel (60–120 mesh) mixed with sodium sulfate (15 g), in a glass column which was eluted with dichloromethane. This eluent of cleaned extract was concentrated using a rotary vacuum evaporator and the final sample size was made up to 5mL with using HPLC grade methanol. The residues were determined using a HPLC fitted with a photodiode array detector. The results revealed that the average recoveries of emamectin benzoate were 101.4, 97.2 and 91.2% at 0.05, 0.25 and 0.50 mg/kg levels of fortification. The average initial deposits of emamectin benzoate were 0.22 and 0.30 mg/kg at the doses of 8.5 and 17 g a.i./ha, respectively. The LOQ and LOD were worked out as 0.05 and 0.017 mg/kg, respectively. The residues were below the detection limit by the 7th day at both the doses. The half-life values of emamectin benzoate applied 4 times each at an interval of 10 days at the doses of 8.5 and 17 g a.i./ha to be 1.72 and 2.26 days, respectively.

Cabbage samples extracted with 1% acetic acid were sonicated for 15 min to which 4g of MgSO₄ and 1g of NaCl were added and were shaken vigorously for a minute followed by centrifugation at 4000 rpm for 3 minutes. The top layer of supernatant (about 1 mL) was collected into a 2 mL micro-centrifuge tube containing 50 mg of PSA, 5 mg of graphitized carbon black (GCB) (40-60m) and 150 mg of MgSO₄ and then centrifuged at 4000 rpm after vortexing. The supernatant was collected and filtered through a 0.22 mm polytetrafluoroethylene (PTFE) filter and is collected into a glass vial. The residues of emamectin benzoate and lufenuron on cabbage were determined using QuEChERS method coupled with UPLC-MS/MS. The LOQ and LOD for emamectin benzoate were worked out to be 0.01 and 1.4×10^{-4} mg/kg, respectively. The recoveries of emamectin benzoate on cabbage were 91.1, 99, and 98% at 0.5, 0.1, and 0.01 mg/kg level of fortification. The half-life values of

emamectin benzoate and lufenuron were determined as 1.08–2.70 and 1.74–5.04 days, respectively in cabbage (Dong et al. 2015).

2.2.3 Spinosad

Sharma et al. (2007) extracted cabbage head samples (weighing 50g) with 100 mL of acetonitrile-water mixture (8:2 by volume) followed by partitioning with dichloromethane. After evaporating in a rotary vacuum evaporator, 1 mL of methanol + acetonitrile + 20g L⁻¹ aqueous ammonium acetate (1:1:1 by volume) was added, which was used for HPLC analysis. Cabbage samples fortified at 0.05 and 2 mg g⁻¹ resulted in the recoveries between 80-83%. The mean initial deposit of spinosad on cabbage was determined to be 2.97 and 5.85 mg g⁻¹ when applied at the doses of 17 and 35 g a.i./ha, respectively. The half-life values of spinosad were found to be 2.8 (17 g a.i./ha) and 2.0 days (35 g a.i./ha).

A 50 g representative sample soaked overnight in 100 mL methanol was filtered and diluted with 600 mL brine solution and partitioned with dichloromethane thrice and once with hexane. The combined extract was concentrated to about 5 mL and cleaned by using a mixture of silica gel, anhydrous sodium sulphate and activated charcoal (10:10:2). The cleaned-up sample was eluted with dichloromethane and was concentrated to near dryness and transferred to 5 mL of methanol which was used for HPLC analysis. Spinosad applied at a dosage of 15 and 30 g a.i./ha resulted in average initial deposits of 0.57 and 1.34 mg/kg, respectively. The recovery rates ranged from 83.3 to 86.3% when the samples were spiked at 0.2, 0.5, 1.0 mg/kg level of fortification. These residues were below the limit of quantification (LOQ) i.e., 0.02 mg/kg after 10 days at both the dosages. The half-life values of spinosad were worked out to be 1.20 and 1.58 days at the dosage of 15 and 30 g a.i. /ha, respectively on cauliflower curds. They also suggested a waiting period of about 6 days for the safe consumption of cauliflower when minimum effective dosages were applied (Mandal et al. 2009).

Akbar et al. (2010) extracted cabbage samples (30g) in acetonitrile: water mixture (8:2) and partitioned the extract once with dichloromethane and second time with methanol, sodium hydroxide and dichloromethane. After reducing to dryness, the final volume was made up with *n*-hexane. The mean initial deposit of spinosad was

found to be 0.614 and 0.414 mg/kg when applied at a dosage of 35.5 g a.i./ha on leaves and heads, respectively. The half-life values of spinosad on leaves and heads was worked out to be 3.45 and 3.50 days.

Singh and Battu (2012) extracted the cabbage samples in methanol and dried over anhydrous sodium sulfate. The extract was concentrated under vacuum and was cleaned up with silica gel mixed with primary secondary amine and MgSO_4 . The LOQ and the limit of detection (LOD) were worked out to be 0.01 mg kg^{-1} and 0.03 mg kg^{-1} , respectively. The average recovery of spinosad ranged from 80 to 88.7% when the samples were spiked at 0.01, 0.05, 0.1 and 0.2 mg/kg level of fortification. The mean initial deposit of spinosad on cabbage was worked out to be 0.33 mg kg^{-1} when applied @ 15 g a.i./ha and 0.56 mg kg^{-1} when applied @ 30 g a.i./ha. The residues of spinosad were not detected after 5 days of spraying (when applied @ 15g a.i/ha) and 10 days after spraying (when applied @ 30g a.i/ha) *i.e.*, below the LOD which is 0.03 mg kg^{-1} . The half-life values of spinosad were found to be 1.4 and 1.5 days at the dosage of 15 and 30 g a.i./ha, respectively. The residues dissipated to below MRL levels after 5.5 and 7.2 days for the doses of 15 and 30 g a.i./ha, respectively. Hence, a safe waiting period of 5 days was advised when applied at recommended dosage.

Tomato samples were extracted in acetonitrile:water (80:20) mixture and the volume of the extract was reduced under vacuum. This extract was then partitioned with dichloromethane, methanol and sodium hydroxide solution. The resultant organic phase was again concentrated, residue was dissolved with 10mL of *n*-hexane and was cleaned up with silica solid phase extraction cartridge. Spinosad was sprayed on tomato crops in a polyhouse at the doses of 15 and 30 g a.i./ha. The mean recovery of spinosad ranged from 80.6 to 90.1% when the samples were spiked at 0.02, 0.05, 0.1, and 0.2 mg/kg level of fortification. The residues of spinosad dwindled from 1 day after application and were below the quantification limit (LOQ) *i.e.*, 0.02 mg kg^{-1} after 7 days (for 15 g a.i./ha dosage) and 10 days (for 30 g a.i./ha dosage). The half-life values and safety intervals of spinosad were worked out to be 1.2 days and 1.9 days for the dosage of 15 g a.i./ha; 1.6 days and 3.9 days for the dosage of 30 g a.i./ha (Kashyap et al. 2015).

Shashni (2020) extracted the cauliflower curds in a warring mixture with 100 mL of acetonitrile-water mixture (8:2). This extractant was then homogenized and was filtered out through Whatman No.1 filter paper along with 4-5g of sodium sulphate. This filtrate was subsequently reduced to 5 mL in a rotary vacuum evaporator. The sample was then cleaned up with celite, activated charcoal and magnesium dioxide (2:2:1) through column chromatography. Final volume was made up to 5 mL with acetonitrile for HPLC analysis. Spinosad residues were analysed by using HPLC. Consistent recovery percentages from 84 to 87% were obtained when the samples were spiked at 0.05, 0.1 and 0.2 ppm of fortification level. The LOD and LOQ of spinosad were worked out to be 0.003 mg/kg and 0.011 mg/kg, respectively. The spinosad residues started dissipating from a day after the spraying and were below quantification limit i.e., 0.011 mg/kg after 7 days. The half-life of spinosad on cauliflower when applied at a concentration of 0.015% was 2.23 days and the safety interval was worked out to be 10 days.

2.3. Effect of processing on the removal of insecticide residues

Zhang et al. (2007) reported that washing cabbages with tap water for about 20 minutes reduced the residues of chlorpyrifos, p, p-DDT, cypermethrin, chlorothalonil by 17.6, 17.1, 19.1 and 15.2%, respectively. Kumari (2008) found that boiling the vegetables was more effective in the removal of residues when compared to washing with water. Similarly, it was reported that washing and blanching of okra reduced the residues of emamectin benzoate (38 mL/acre) by 24.13 and 41.70%, respectively (Sheikh et al. 2012).

An experiment was conducted at Ludhiana (Punjab) to determine the effect of household processing on the residues of chlorantraniliprole in cabbage and cauliflower. The household processing methods like washing, boiling and removal of outer leaves were performed to see their effect on the pesticide residues. The experimental findings indicated that washing with tap water for 2-3 minutes, boiling in water for five minutes, washing with water for 2-3 minutes followed by boiling for five minutes, removal of outer leaves removed the residues of chlorantraniliprole by 16.70, 100, 100 and 100% when applied at a dosage of 9.25 g a.i./ha and 40, 100, 100 and 100% when applied at a dosage of 18.50 g a.i. /ha 1 hour after spraying,

respectively. Washing with tap water for 2-3 minutes, boiling in water for five minutes, washing with water for 2-3 minutes and boiling for five minutes, removal of outer leaves removed the residues of chlorantraniliprole applied at a dosage of 9.25 and 18.50 g a.i./ha removed its residues by 100, 100, 100, 100% and 23.08, 100, 100 and 100%, respectively (Kar et al. 2012).

Panhwar and Sheikh (2013) carried out a study in Pakistan to analyze the effect of traditional food processing on the reduction of pesticide residues in cauliflower. Their results revealed that the initial residues of emamectin benzoate (1.04 ppm) in unwashed cauliflower samples reduced to 0.891 ppm (14.32 % reduction) upon washing. Vijayasree et al. (2014) reported that washing with plain water for 5 minutes reduced the residues of emamectin benzoate (11 g a.i./ha) and spinosad (73 g a.i./ha) on cow pea fruits by 79.39 and 72.50%, respectively 2 hours after spraying, 100 and 57.48%, respectively 3 days after spraying. In a similar fashion, it was reported that the residues of emamectin benzoate were reduced by 65% by washing and 78% by blanching when applied at a dosage of 38 mL/acre (Panhwar et al. 2014).

Wanwimolruk et al. (2017) suggested that washing cabbages and tomatoes under running water effectively removed the pesticide residues. Based on literature, Chung (2018) concluded that boiling cabbages was the most effective method for removal of residues, followed by washing with water and soaking in water. Kaushik et al. (2019) reported that washing of tomato fruits by tap, lukewarm, and saline water reduced tetraniliprole residues up to 37.6, 44.7, and 61.5%, respectively. Athulya et al. (2019) observed that washing cabbages removed 66.7% of diafenthiuron residues and boiling was very effective in removing residues up to 100%. A total of 74.6% residues were removed after boiling the cabbage heads with water for 10 min. at 100°C (Preethi et al. 2019).

2.4 Maximum residue limits (MRLs)

The maximum residue limits (MRLs) of cyantraniliprole and spinosad on cabbage as prescribed by Codex Alimentarius Commission is 2.0 mg/kg (FAO 2022; FSSAI 2020). However, the MRL value of emamectin benzoate has not been prescribed on cabbage but it has been on other vegetables as 0.02 mg/kg (FAO 2022).

3. MATERIALS AND METHODS

The present investigations on “Toxicity of novel insecticides to *Pieris brassicae* (Linnaeus) and their residue dynamics on cabbage” were carried out at the Department of Entomology, College of Agriculture, Chaudhary Sarwan Kumar Himachal Pradesh Krishi Vishvavidyalaya, Palampur situated at an altitude of 1290 meters above sea level. The investigation aimed at evaluating the toxicity of novel insecticides against cabbage butterfly, *Pieris brassicae* and their residues on cabbage which were conducted during *rabi* season 2021-22. The different materials used in conducting the experiment and the various methods employed during the course of investigations were given below:

3.1 Intrinsic toxicity studies

Intrinsic toxicity studies were carried out in the Toxicology laboratory of the Department of Entomology. The stock solutions of the novel insecticides and cypermethrin (check) as given in Table 3.1 were prepared in distilled water. Before every experiment, graded concentrations were made just prior to the experiment by using distilled water through serial dilutions. In preliminary experiments, the concentrations of insecticides giving mortality between 20 and 80 per cent were ascertained.

Freshly laid eggs of *P. brassicae* (Plate 3.1) were collected from the untreated plots of the experimental trial. These eggs were kept in petri dishes on moist filter paper for hatching (Plate 3.1). The neonate and the 3rd instar larvae were used for testing the toxicity of insecticides (Plate 3.2). The cabbage leaf discs of 25cm² area were dipped in different concentrations for 30 seconds. The treated leaf discs were allowed to dry at room temperature for 15 minutes. The treated leaf discs were kept in petri dishes on a wet filter paper. In each treatment, 30 larvae were used for testing the toxicity of insecticides. Larvae were pre-starved for 2 hours before releasing on to the treated leaf discs. The data were recorded after 24 hours and the moribund larvae were treated as dead. The corrected per cent mortality was calculated as per Abbott's (1925) formula.



Plate 3.1 Eggs of *P. brassicae* on cabbage in the field

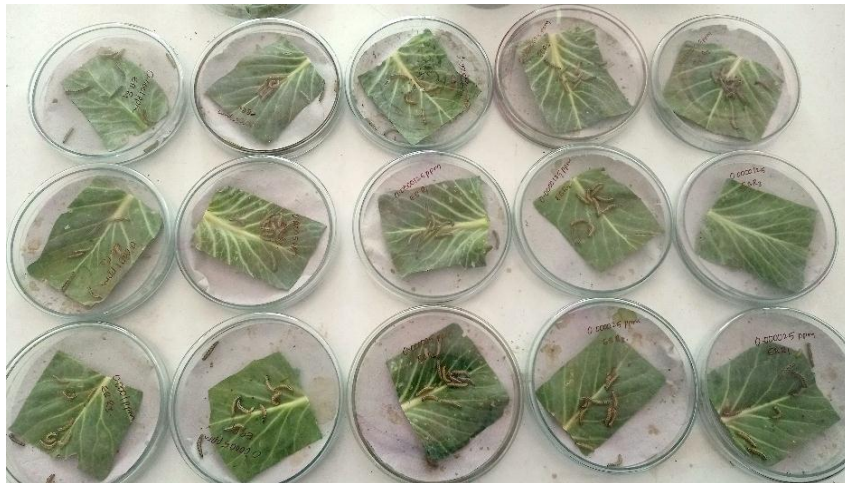


Plate 3.2 Laboratory experiment on intrinsic toxicity of different insecticides to 3rd instar larvae of *P. brassicae*

$$\text{Corrected per cent mortality} = \frac{\% \text{ mortality in treatment} - \% \text{ mortality in control}}{100 - \% \text{ mortality in control}}$$

The data so obtained were subjected to Probit analysis to work out LC₅₀ values of insecticides for neonate and 3rd instar larvae of *P. brassicae* (Finney, 1971).

Table 3.1 Insecticides used in the experiment

Sr. No.	Common name	Trade name and Formulation	Manufacturer
1	Cyantraniliprole	Benevia 10.26 OD	FMC India Pvt. Ltd.
2	Cypermethrin	Inquiry 25 EC	Gujarat Pesticides Company
3	Emamectin benzoate	Rudra 5 SG	Anu Products Ltd.
4	Spinosad	Tracer 45 SC	Dow AgroSciences India Pvt. Ltd.

3.2 Field Experiment

3.2.1 Raising of cabbage crop

Cabbage seedlings of the variety Pusa Hybrid-81 (30 days old) were transplanted at the Experimental Farm of Department of Entomology on 28th October, 2021. The details of insecticides applied in different concentrations are given in Table 3.2. The crop was raised by following all the recommended agronomic practices of CSKHPKV, Palampur (Anon. 2018).

No. of treatments	:	7
No. of replications	:	3
Plot size	:	3m × 1.8m
No. of plots	:	21
Spacing	:	45cm × 60cm
No. of plants in each plot	:	18

Table 3.2 Details of insecticides

Treatment	Insecticide	Dose (g a.i./ha)	Volume of commercial formulation per litre of water
T1	Cyantraniliprole 10.26 OD	60	1.8 mL
T2	Cyantraniliprole 10.26 OD	120	3.6 mL
T3	Emamectin benzoate 5 SG	10	0.4 g
T4	Emamectin benzoate 5 SG	20	0.8 g
T5	Spinosad 45 SC	15	0.3 mL
T6	Spinosad 45 SC	30	0.6 mL
T7	Untreated check	-	-

3.2.2 Residue studies

a. Chemicals and working standards

For the preparation of working standards, standard solutions of known concentration of pesticides were made by using the HPLC grade acetonitrile (Table 3.3 and 3.4). The standard of given purity was first dissolved in acetonitrile and different concentrations were derived from the stock solution by serial dilutions.

Table 3.3 Insecticide standards used

Insecticide	Purity (%)	Manufacturer
Cyantraniliprole	98.9	Dr. Ehrenstorfer GmbH
Emamectin benzoate	90.0	Dr. Ehrenstorfer GmbH
Spinosad	94.0	Dr. Ehrenstorfer GmbH

Table 3.4 Details of chemicals used for extraction and cleanup of samples

Insecticide	Chemicals used for extraction	Chemicals used for cleanup
Cyantraniliprole	Ethyl acetate (HPLC grade)	Acetonitrile (HPLC grade)
	Sodium chloride	Anhydrous magnesium sulfate
	Anhydrous sodium sulfate	Activated charcoal
Emamectin benzoate	Acetonitrile (HPLC grade)	Acetonitrile (HPLC grade)
	Sodium chloride	Anhydrous magnesium sulfate
	Anhydrous magnesium sulfate	
Spinosad	Acetonitrile (HPLC grade)	Acetonitrile (HPLC grade)
	Anhydrous sodium sulphate	Activated charcoal: celite: magnesium oxide (2:2:1)

b. Instruments

The apparatus used in the residue studies were Rotary vacuum evaporator, muffle furnace, electronic balance, warring blender, refrigerator, ultrapure water purification system, sonicator (Plate 3.3).

c. Others

The glassware and other apparatus used were chromatography columns, separatory funnels, measuring cylinder, Whatman filter paper No. 1 *etc.*

d. Cleaning of glassware

The standard procedure as proposed by EPA (1980) was followed for thorough cleaning of the glassware. The contaminated glassware was firstly cleaned with water and then were dipped for two hours in a concentrated solution of chromic acid (20 per cent). The glassware was further cleaned with teepol and again rinsed under running water and finally cleaned with distilled water. After that, these were rinsed in acetone and then lastly the glassware was kept in an oven at 100°C for one

hour before use. Prior to usage, the glassware was rinsed with the same solvent to be used in the analysis.

3.2.2.1 Standard calibration curves

After the preparation of standard insecticide solutions, these were analyzed by injecting into HPLC equipped with UV-VIS detector. The sampling was automatic and the data were analyzed using LC Solution[®] software. The data were obtained in the form of retention time (min.) and peak area (mV). The peak areas of all the working concentrations were simultaneously compared and plotted on the graph as standard calibration curves.

3.2.2.2 Parameters of HPLC instrument

The different operational parameters of HPLC instrument used for residue estimation of insecticides are given in Table 3.5.

Table 3.5 Operational parameters of HPLC instrument

Parameters		Cyantranilprole	Emamectin benzoate	Spinosad
Mobile phase (v/v)		Acetonitrile:water (98:2)	Acetonitrile:water (80:20)	Acetonitrile:water (98:2)
Flow rate (mL/min)		Isocratic flow 1.2	Isocratic flow 0.6	Isocratic flow 2.0
Column		RP-18 end capped	RP-18 end capped	RP-18 end capped
Column length (mm)		250	250	250
Diameter (µm)		5	5	5
Detector		SPD-20A UV/VIS	SPD-20A UV/VIS	SPD-20A UV/VIS
Injection volume (µl)		20	30	5
Wavelength for detection (nm)		280	246	245
Start time (min)		0.00	0.00	0.00
End time (min)		10.00	15.00	15.00
Retention time (min)		5.649	6.504	5.179



Electronic weighing balance



Sonicator

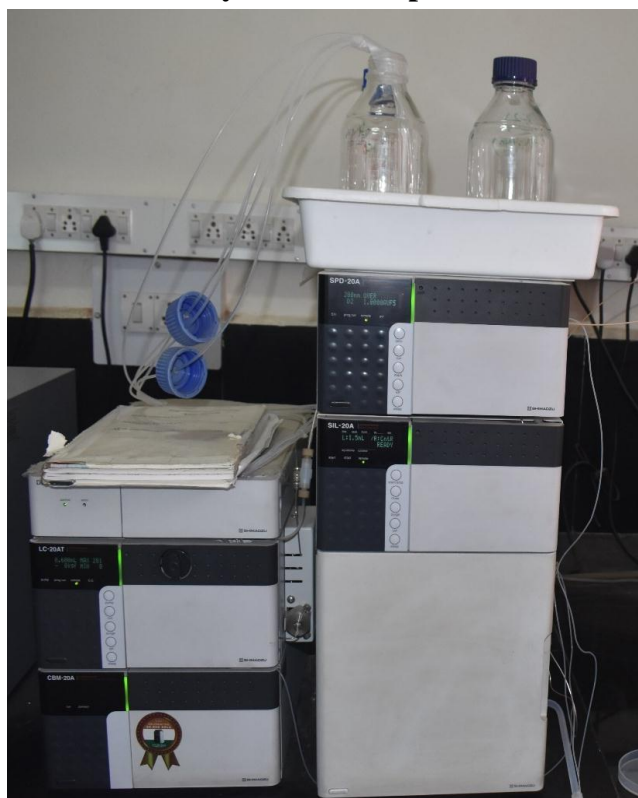


Ultrapure water purification system

Plate 3.3a Instruments used in insecticide residue analysis



Rotary vacuum evaporator



HPLC

Plate 3.3b Instruments used in insecticide residue analysis

Sampling of cabbage heads

The first and second application of insecticides were given on 11th and 25th April, 2022, respectively. After the second spray, cabbage head samples from the plots treated with cyantraniliprole, emamectin benzoate and spinosad along with the control were taken on 0 (2 hours), 1, 3, 5, 7 and 10 days of spray. Since each treatment was replicated thrice, samples from all three replications were brought accordingly. The samples were separately packed in polythene bags, labelled and brought to laboratory. Samples of each treatment were finely chopped into small pieces and were thoroughly mixed. After splitting it up into three quarters of 50 g each, samples were homogenized in a warring blender. Further, the processes involving extraction, cleanup and estimation of pesticide residues were followed.

3.2.2.3 Recovery studies

Before taking up analysis of test samples, the efficiency of the analytical method used in the study and the quality of the process being followed has to be ascertained. For this, 50 g sample of cabbage head taken from an untreated control plot was fortified with 1 mL of 100 mg/kg standard insecticide solution to get 2 mg/kg concentration. Untreated samples were fortified to get 0.5, 1, 2 mg/kg concentrations in case of emamectin benzoate and spinosad, and 0.1, 1, 2 mg/kg concentrations in case of cyantraniliprole. Residues were extracted and cleaned up as per method described under section 3.3. The blank samples were also processed in the same way to find out the interferences, if any. The cleaned-up samples were injected to high performance liquid chromatography and the recovery was calculated as per the formula given below:

$$\text{Recovery percentage} = \frac{\text{Average peak area of 2 mg/kg equivalent sample}}{\text{Average peak area of 2 mg/kg standard solution}} \times 100$$

3.3 Extraction and cleanup procedures

Cabbage samples (50g) were extracted by procedure as described by Kar et al. (2012) with slight modifications for analysis of cyantraniliprole residues. The cabbage samples were extracted in ethyl acetate and 10 g of NaCl was added for phase separation. The supernatant was filtered using 10 g of activated sodium sulfate

and hand shaken for 1 minute. This ethyl acetate extract was evaporated to 5 mL using a rotary vacuum evaporator at 35°C and this was cleaned up with 90 mg of anhydrous MgSO₄ and 5 mg of activated charcoal. The final volume was made up to 5 mL with HPLC grade distilled acetonitrile. The residues were determined by using HPLC (Shimadzu) as described in 3.2.2.2.

Emamectin benzoate residues were analyzed following the procedure of Wang et al. (2012). The homogenized samples (50 g) were extracted with acetonitrile and this extract was filtered through 1g sodium chloride and 4 g anhydrous magnesium sulfate. The volume of this extract was reduced to 5 mL using a rotary vacuum evaporator. This was cleaned up with 150 mg MgSO₄. The final volume was made up to 5 mL with acetonitrile. The samples were filtered through 0.22 µm pore membrane filter before analyzed using HPLC.

The extraction of the residues was done following the procedure described by Sharma et al. (2007) with slight modifications. Spinosad residues from cabbage heads (50 g) were extracted in a warring blender with 80 mL : 20 mL acetonitrile-water mixture (8:2). The homogenized mixture was then filtered out through Whatman No. 1 filter paper with 4-5 grams of sodium sulphate. The filtrate was reduced to 5 mL in a rotary vacuum evaporator. Samples were cleaned up with activated charcoal, celite and magnesium dioxide (2:2:1) through column chromatography as followed by Shashni (2020). Final volume was made up to 5 mL with acetonitrile. The residues were determined by using HPLC (Shimadzu) as described in 3.2.2.2.

3.4 Storage of samples

The final extracts and cleaned up samples of both the insecticides were stored in clean and labelled volumetric flasks and glass vials, completely airtight in deep freezer at -40°C temperature.

3.5 Estimation of insecticide residues

For the analysis of residues of cyantraniliprole, emamectin benzoate and spinosad, samples were injected into HPLC having parameters as given in table 3.5. Formula applied for converting peak area into insecticide concentration was:

$$C_2 = \frac{A_2 \times C_1 \times V \times V_1}{A_1 \times V_2 \times W}$$

Where,

A_2 = Peak area of unknown sample

C_1 = Concentration of standard insecticide in mg/kg

C_2 = Concentration of insecticide in unknown sample in mg/kg

V = Total volume of final extract in ml

V_1 = Volume of standard sample injected in μ l

A_1 = Peak area of standard insecticide

V_2 = Volume of unknown sample injected in μ l

W = Weight of sample taken in grams.

3.6 Half-life values

The formula given by Hoskins (1961) was used to calculate the half-life values of insecticides.

$$t_{1/2} = \frac{\log 2}{k_i} = \frac{0.3010}{k_i}$$

3.7 Safety interval

For calculating safety interval, the formula given by Hoskins (1961) was used:

$$t_{si} = \frac{(\log k_2 - \log tol)}{k_i}$$

Where,

t_{si} = minimum number of days to be elapsed before the insecticide reached tolerance limit

$\log k_2$ = log of initial deposit

$\log tol$ = log of tolerance limit

k_i = regression coefficient

3.8 Dissipation rates

By calculating the concentration of the given insecticide at different intervals, it was possible to determine the dissipation rate of that insecticide by using the formula:

$$\text{Per cent dissipation} = \left[100 - \frac{\text{Residues (mg/kg)}}{\text{Initial residue (mg/kg)}} \right] \times 100$$

3.9 Statistical analysis

The data on intrinsic toxicity studies were analyzed using Polo Plus[®] (v2.0) software. The residue finalized were the mean values of three replications of each treatment and all data were analyzed applying simple descriptive statistics such as the mean and standard deviation using IBM SPSS[®] Statistics software (v22).

4. RESULTS AND DISCUSSION

The results of the present investigations on “**Toxicity of novel insecticides to *Pieris brassicae* (Linnaeus) and their residue dynamics on cabbage**” are presented and discussed here. Different novel insecticides were tested against neonate and 3rd instar larvae of *P. brassicae*. These novel insecticides were also sprayed in the main field at recommended and double the recommended doses and their residues were estimated. The findings of the study are presented under the following sub-heads:

4.1 Intrinsic toxicity of novel insecticides against neonate larvae of *P. brassicae*

4.2 Intrinsic toxicity of novel insecticides against 3rd instar larvae of *P. brassicae*

4.3 Residue studies of cyantraniliprole, emamectin benzoate and spinosad on cabbage

4.4 Effect of processing on the removal of residues

4.1 Intrinsic toxicity of novel insecticides against neonate larvae of *P. brassicae*

The intrinsic toxicity of three novel insecticides namely cyantraniliprole, emamectin benzoate and spinosad was determined against neonate larvae of *P. brassicae*. Cypermethrin was taken as a check treatment. The LC₅₀ and LC₉₀ values were calculated and the experimental findings are presented in the table 4.1 to 4.4 and depicted in the figure 4.1 to 4.4.

4.1.1 Cyantraniliprole

The corrected mortalities of neonate larvae of *P. brassicae* for cyantraniliprole are presented in table 4.3 and the regression line is given in fig. 4.3. Cyantraniliprole tested at 0.000006 to 0.0001 ppm gave the mortality of 13.3 to 83.3 per cent. The LC₅₀ and LC₉₀ values were worked out to be 0.00003 and 0.00019 ppm, respectively. The fiducial limits of LC₅₀ were 0.00002 and 0.00004 ppm. The fiducial limits of LC₉₀ were 0.00011 and 0.00058 ppm. The data were homogenous ($\chi^2 = 1.077$) at 5 per cent level of significance and 3 degrees of freedom (df). The slope was calculated to be 1.546.

4.1.2 Emamectin benzoate

Emamectin benzoate when tested against neonate larvae *P. brassicae* at the concentrations of 0.00049 to 0.00781 ppm gave the mortality of 16.7 to 86.7 per cent. The corrected mortalities of neonate larvae of *P. brassicae* for emamectin benzoate are presented in table 4.2 and the regression line is given in fig. 4.2. The LC₅₀ and LC₉₀ values were worked out to be 0.0020 ppm and 0.0111 ppm, respectively. The fiducial limits of LC₅₀ were 0.0014 and 0.0023 ppm. The fiducial limits of LC₉₀ were 0.0071 and 0.0240 ppm. The slope was calculated to be 1.614. The data were homogenous ($\chi^2 = 0.498$) at 5 per cent level of significance and 3 degrees of freedom (df).

4.1.3 Spinosad

When spinosad was tested against neonate larvae of *P. brassicae* at the concentrations ranging from 0.016 to 0.25 ppm gave the mortality of 20.0 to 86.7% (Table 4.1). The regression line is given in fig. 4.1. The LC₅₀ was worked out to be 0.061 ppm with the fiducial limits of 0.045 and 0.081 ppm. The LC₉₀ value was worked out to be 0.460 ppm with the fiducial limits of 0.270 and 1.197 ppm. The data were homogenous ($\chi^2 = 0.414$) at 5 per cent level of significance and 3 degrees of freedom (df). The slope was calculated to be 1.454.

4.1.4 Cypermethrin

The corrected mortalities of neonate larvae of *P. brassicae* for cypermethrin are presented in table 4.4 and the regression line is given in fig. 4.4. Cypermethrin at 0.000062 to 0.001000 ppm gave the mortality of 16.7 to 80.0 per cent. The LC₅₀ and LC₉₀ values were worked out to be 0.00029 ppm and 0.00224 ppm, respectively. The fiducial limits of LC₅₀ were 0.00022 to 0.00040 ppm. The fiducial limits of LC₉₀ were 0.00127 to 0.00618 ppm. The data were homogenous ($\chi^2 = 0.207$) at 5 per cent level of significance and 3 degrees of freedom (df). The slope was calculated to be 1.450.

Table 4.1 Concentration mortality response of cyantraniliprole to neonate larvae of *P. brassicae*

Conc. (ppm)	Log Conc. (ppm x 10 ⁶)	Number of insects treated	Observed mortality (%)	Corrected mortality (%)	Empirical probit	Expected probit (Y)
0.000006	0.77	30	13.3	10.3	3.72	3.8
0.000012	1.07	30	33.3	31.0	4.50	4.3
0.000025	1.39	30	43.3	41.4	4.77	4.8
0.000050	1.69	30	60.0	58.6	5.23	5.3
0.000100	2.00	30	83.3	82.8	5.95	5.8
Control	-	30	3.3	0	-	-

Regression equation = 1.546X-2.253

LC₅₀ = 0.00003 ppm

Fiducial limits = 0.00002 and 0.00004 ppm

LC₉₀ = 0.00019 ppm

Fiducial limits = 0.00011 and 0.00058 ppm

$\chi^2_{cal}(p=0.05) = 1.077$

$\chi^2_{tab}(p=0.05) = 7.814$

Table 4.2 Concentration mortality response of emamectin benzoate to neonate larvae of *P. brassicae*

Conc. (ppm)	Log Conc. (ppm x 10 ⁴)	Number of insects treated	Observed mortality (%)	Corrected mortality (%)	Empirical probit	Expected probit (Y)
0.00049	0.69	30	16.7	13.8	3.92	3.9
0.00098	0.99	30	36.7	34.5	4.61	4.5
0.00196	1.29	30	53.3	51.7	5.03	5.0
0.00391	1.59	30	66.7	65.5	5.41	5.5
0.00781	1.89	30	86.7	86.2	6.08	6.0
Control	-	30	3.3	0.0	-	-

Regression equation = 1.614X-2.023

LC₅₀ = 0.0020 ppm

Fiducial limits = 0.0014 and 0.0023 ppm

LC₉₀ = 0.0111 ppm

Fiducial limits = 0.0071 and 0.0240 ppm

$\chi^2_{cal}(p=0.05) = 0.498$

$\chi^2_{tab}(p=0.05) = 7.814$

Table 4.3 Concentration mortality response of spinosad to neonate larvae of *P. brassicae*

Conc. (ppm)	Log Conc. (ppm x 10 ³)	Number of insects treated	Observed mortality (%)	Corrected mortality (%)	Empirical probit	Expected probit
0.016	1.20	30	20.0	17.2	4.05	4.0
0.031	1.49	30	33.3	31.0	4.50	4.5
0.062	1.79	30	53.3	51.7	5.05	5.0
0.125	2.09	30	63.3	62.1	5.31	5.5
0.250	2.39	30	86.7	86.2	6.08	6.0
Control	-	30	3.3	0.0	-	-

Regression equation = 1.454X-2.592

LC₅₀ = 0.061 ppm Fiducial limits = 0.045 and 0.081 ppm

LC₉₀ = 0.460 ppm Fiducial limits = 0.270 and 1.197 ppm

$\chi^2_{cal}(p=0.05) = 0.414$ $\chi^2_{tab}(p=0.05) = 7.814$

Table 4.4 Concentration mortality response of cypermethrin to neonate larvae of *P. brassicae*

Conc. (ppm)	Log Conc. (ppm x 10 ⁵)	Number of insects treated	Observed mortality (%)	Corrected mortality (%)	Empirical probit	Expected probit (Y)
0.000062	0.79	30	16.7	13.8	3.92	3.9
0.000125	1.09	30	30	27.6	4.42	4.3
0.000250	1.39	30	46.7	44.8	4.87	4.8
0.000500	1.69	30	60	58.6	5.23	5.3
0.001000	2.00	30	80	79.3	5.81	5.7
Control	-	30	3.3	0.0	-	-

Regression equation = 1.450X-3.575

LC₅₀ = 0.00029 ppm Fiducial limits = 0.00022 and 0.00040 ppm

LC₉₀ = 0.00224 ppm Fiducial limits = 0.00127 and 0.00618 ppm

$\chi^2_{cal}(p=0.05) = 0.207$ $\chi^2_{tab}(p=0.05) = 7.814$

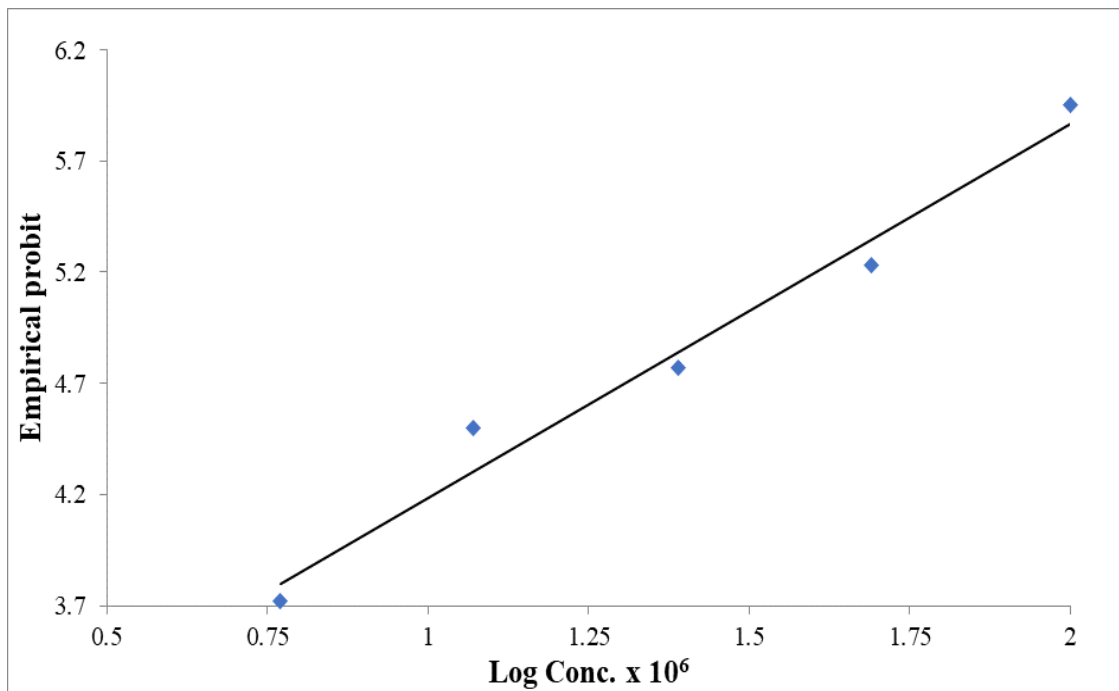


Fig 4.1 Concentration mortality response of cyantraniliprole to neonate larvae of *P. brassicae*

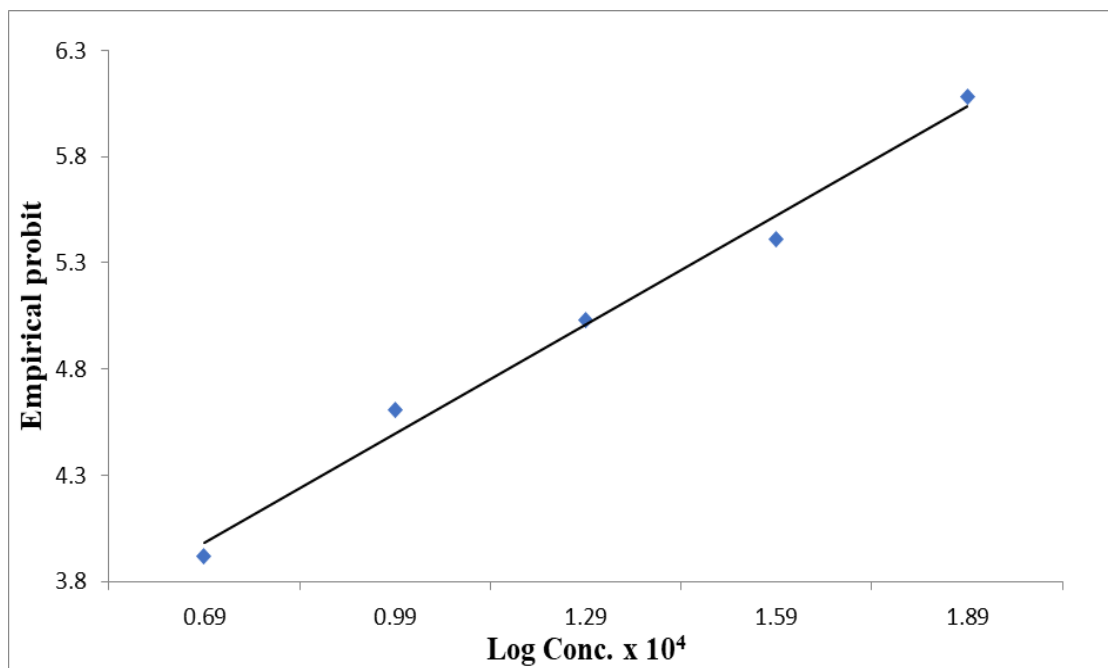


Fig 4.2 Concentration mortality response of emamectin benzoate to neonate larvae of *P. brassicae*

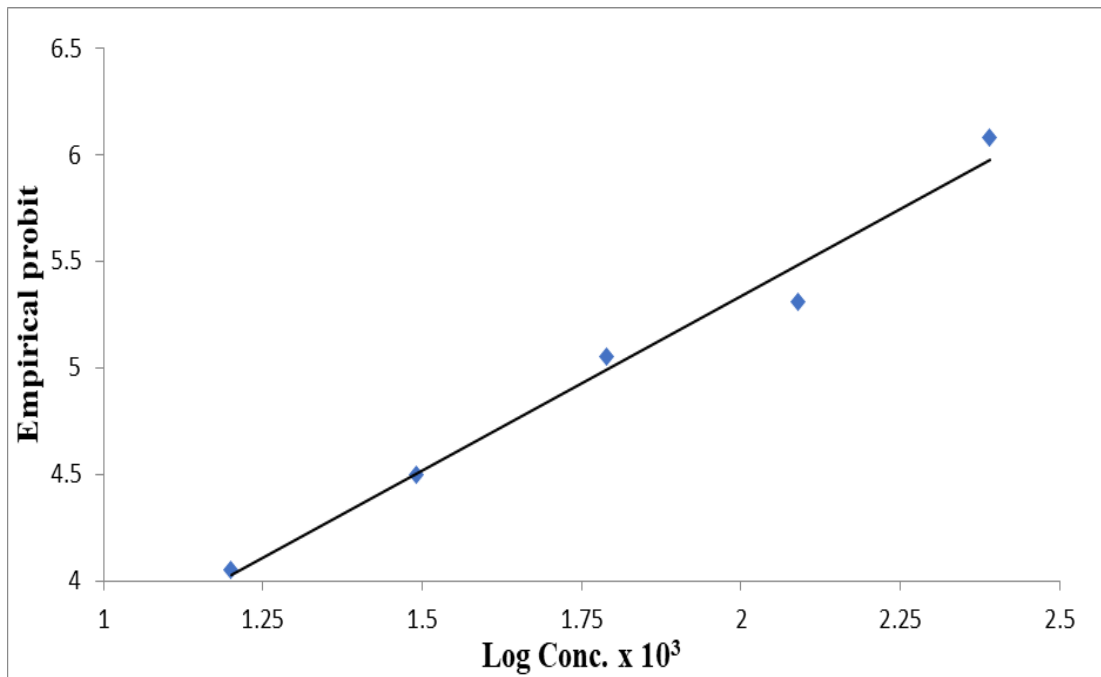


Fig 4.3 Concentration mortality response of spinosad to neonate larvae of *P. brassicae*

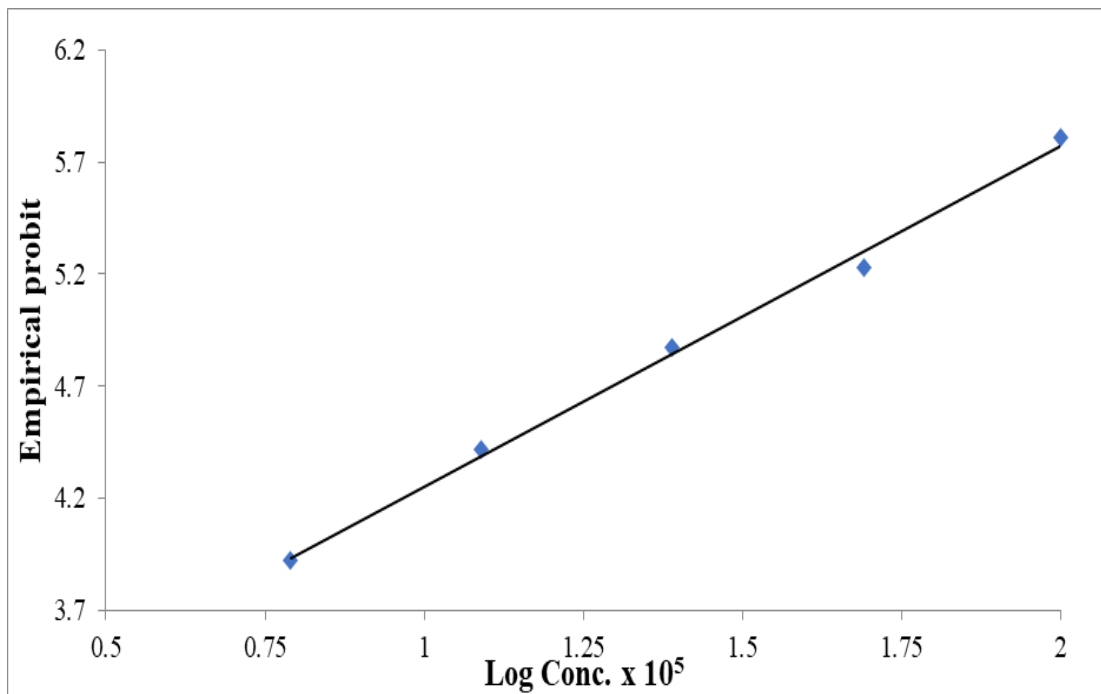


Fig 4.4 Concentration mortality response of cypermethrin to neonate larvae of *P. brassicae*

In the present investigations, lowest LC₅₀ value of 0.00003 ppm was observed in cyantraniliprole followed by cypermethrin (0.00029 ppm), emamectin benzoate (0.002 ppm) and spinosad (0.061 ppm). Kumar et al. (2010) reported higher LC₅₀ value of cypermethrin (0.0329 ppm) against 1-2 day old larvae of *P. brassicae*. The LC₅₀ value of spinosad against 1st instar larvae of *Spodoptera exigua* was observed as 0.096 ppm by Moadeli et al. (2014). Su et al. (2017) reported the LC₅₀ value of chlorantraniliprole against early (1st and 2nd) instar larvae of *P. rapae* as 0.95 ppm. Sukirno et al. (2017) reported the LC₅₀ values of spinosad against 1st instar larvae of *S. littoralis* and *S. exigua* as 2.19 and 1.40 ppm, respectively. The higher LC₅₀ values of these insecticides may be due to frequent use of insecticides for the management of the pest in respective regions. Shashni (2020) reported the LC₅₀ value of spinosad against neonate larvae of *P. brassicae* as 0.034 ppm which is lower than the present findings. The increased LC₅₀ value probably indicates the development of resistance to spinosad in *P. brassicae*. Similarly higher LC₅₀ values of emamectin benzoate (0.0874 ppm) and chlorantraniliprole (0.934 ppm) against 1st instar larvae of *A. ipsilon* were reported by Ismail (2021). The variations in the LC₅₀ values could be probably due to different agroclimatic regions and pesticide use pattern for the management of these pests.

Table 4.5 Relative toxicity of different insecticides to neonate larvae of *P. brassicae*

Insecticide	LC ₅₀ value (ppm)	Relative toxicity	LC ₉₀ value (ppm)	Relative toxicity
Cyantraniliprole	0.00003	2033.33	0.00019	2421.05
Cypermethrin	0.00029	210.34	0.00224	205.36
Emamectin benzoate	0.00200	30.5	0.0111	41.44
Spinosad	0.06100	1.0	0.460	1.0

It is evident from the data presented in table 4.5 that based on LC₅₀ values to neonate larvae, cyantraniliprole was 2033.33 times more toxic than spinosad, while cypermethrin was 210.34 times more toxic than spinosad. Emamectin benzoate was 30.5 times more toxic than spinosad. Almost similar trend of relative toxicity was observed on the basis of LC₉₀ values.

4.2 Intrinsic toxicity of novel insecticides against 3rd instar larvae of *P. brassicae*

Toxicity of three novel insecticides namely spinosad, emamectin benzoate and cyantraniliprole was tested against 3rd instar larvae of *P. brassicae* and the mortalities were compared with that of cypermethrin. The LC₅₀ and LC₉₀ values were calculated and the experimental findings are presented in the table 4.6 to 4.9, and depicted in the figure 4.5 to 4.8.

4.2.1 Cyantraniliprole

Cyantraniliprole when tested against 3rd instar larvae of *P. brassicae* at the concentrations of 0.0015 to 0.0250 ppm gave the mortality of 13.3 to 86.7 per cent (Table 4.8, Fig. 4.7). The LC₅₀ and LC₉₀ values were worked out to be 0.007 ppm and 0.038 ppm, respectively. The fiducial limits of LC₅₀ were 0.005 and 0.009 ppm. The fiducial limits of LC₉₀ were 0.024 and 0.080 ppm. The data were homogenous ($\chi^2 = 0.828$) at 5 per cent level of significance and 3 degrees of freedom (df). The slope was calculated to be 1.675.

4.2.2 Emamectin benzoate

The corrected mortalities of neonate larvae of *P. brassicae* for emamectin benzoate are presented in table 4.6 and the regression line is given in fig. 4.5. Emamectin benzoate when applied at the concentrations of 0.0039 to 0.0625 ppm gave the mortality of 13.3 to 83.3 per cent. The LC₅₀ values were worked out to be 0.017 ppm with the fiducial limits of 0.013 and 0.022 ppm. The LC₉₀ value was calculated to be 0.097 ppm with the fiducial limits of 0.062 and 0.206 ppm. The data were homogenous ($\chi^2 = 0.064$) at 5 per cent level of significance and 3 degrees of freedom (df). The slope was calculated to be 1.689.

4.2.3 Spinosad

The corrected mortalities of neonate larvae of *P. brassicae* for spinosad are presented in table 4.6 and the regression line is given in fig. 4.5. Spinosad at 0.0625 to 1 ppm gave the mortality of 20.0 to 86.7 per cent. The LC₅₀ and LC₉₀ values were worked out to be 0.202 ppm and 1.587 ppm, respectively. The fiducial limits of LC₅₀ were 0.147 and 0.272 ppm. The fiducial limits of LC₉₀ were 0.950 and 3.976 ppm. The slope was calculated to be 1.463. The data were homogenous ($\chi^2 = 1.101$) at 5 per cent level of significance and 3 degrees of freedom (df).

4.2.4 Cypermethrin

Cypermethrin when applied at the concentrations ranging from 0.125 to 2 ppm gave the mortality of 20.0 to 83.3 per cent. The corrected mortalities of third instar larvae of *P. brassicae* for cypermethrin are presented in table 4.9 and the regression line is given in fig. 4.8. The LC₅₀ and LC₉₀ values were worked out to be 0.484 ppm and 3.719 ppm, respectively. The fiducial limits of LC₅₀ were 0.357 and 0.652 ppm. The fiducial limits of LC₉₀ were 2.171 and 9.716 ppm. The data were homogenous ($\chi^2 = 0.405$) at 5 per cent level of significance and 3 degrees of freedom (df). The slope was calculated to be 1.447.

Table 4.6 Concentration mortality response of cyantraniliprole to 3rd instar larvae of *P. brassicae*

Conc. (ppm)	Log Conc. (ppm x 10 ⁴)	Number of insects treated	Observed mortality (%)	Corrected mortality (%)	Empirical probit	Expected probit (Y)
0.0015	1.18	30	13.3	13.3	3.87	3.9
0.0031	1.49	30	33.3	33.3	4.56	4.4
0.0062	1.79	30	46.7	46.7	4.92	4.9
0.0125	2.09	30	63.3	63.3	5.33	5.4
0.0250	2.39	30	86.7	86.7	6.13	6
Control	-	30	0.0	0.0	-	-

Regression equation = 1.675X-3.043

LC₅₀ = 0.007 ppm

Fiducial limits = 0.005 and 0.009 ppm

LC₉₀ = 0.038 ppm

Fiducial limits = 0.024 and 0.080 ppm

$\chi^2_{cal}(p=0.05) = 0.828$

$\chi^2_{tab}(p=0.05) = 7.814$

Table 4.7 Concentration mortality response of emamectin benzoate to 3rd instar larvae of *P. brassicae*

Conc. (ppm)	Log (Conc. x 10 ³)	Number of insects treated	Observed mortality (%)	Corrected mortality (%)	Empirical probit	Expected probit (Y)
0.0039	0.59	30	13.3	13.3	3.87	3.9
0.0078	0.89	30	30.0	30.0	4.48	4.4
0.0156	1.19	30	46.7	46.7	4.92	4.9
0.0312	1.49	30	66.7	66.7	5.44	5.4
0.0625	1.79	30	83.3	83.3	5.92	5.9
Control	-	30	0.0	0.0	-	-

Regression equation = 1.689X-2.079

LC₅₀ = 0.017 ppm

Fiducial limits = 0.013 and 0.022 ppm

LC₉₀ = 0.097 ppm

Fiducial limits = 0.062 and 0.206 ppm

$\chi^2_{cal}(p=0.05) = 0.064$

$\chi^2_{tab}(p=0.05) = 7.814$

Table 4.8 Concentration mortality response of spinosad to 3rd instar larvae of *P. brassicae*

Conc. (ppm)	Log Conc. (ppm x 10 ²)	Number of insects treated	Observed mortality (%)	Corrected mortality (%)	Empirical probit	Expected probit (Y)
0.06	0.77	30	20.0	20.0	4.16	4.2
0.12	1.07	30	43.3	43.3	4.82	4.5
0.25	1.39	30	53.3	53.3	5.08	5.1
0.50	1.69	30	66.7	66.7	5.44	5.5
1.00	2.00	30	86.7	86.7	6.13	6.0
Control	-	30	0.0	0.0	-	-

Regression equation = 1.434X-1.875

LC₅₀ = 0.202 ppm

Fiducial limits = 0.147 and 0.272 ppm

LC₉₀ = 1.587 ppm

Fiducial limits = 0.950 and 3.976 ppm

$\chi^2_{cal}(p=0.05) = 1.101$

$\chi^2_{tab}(p=0.05) = 7.814$

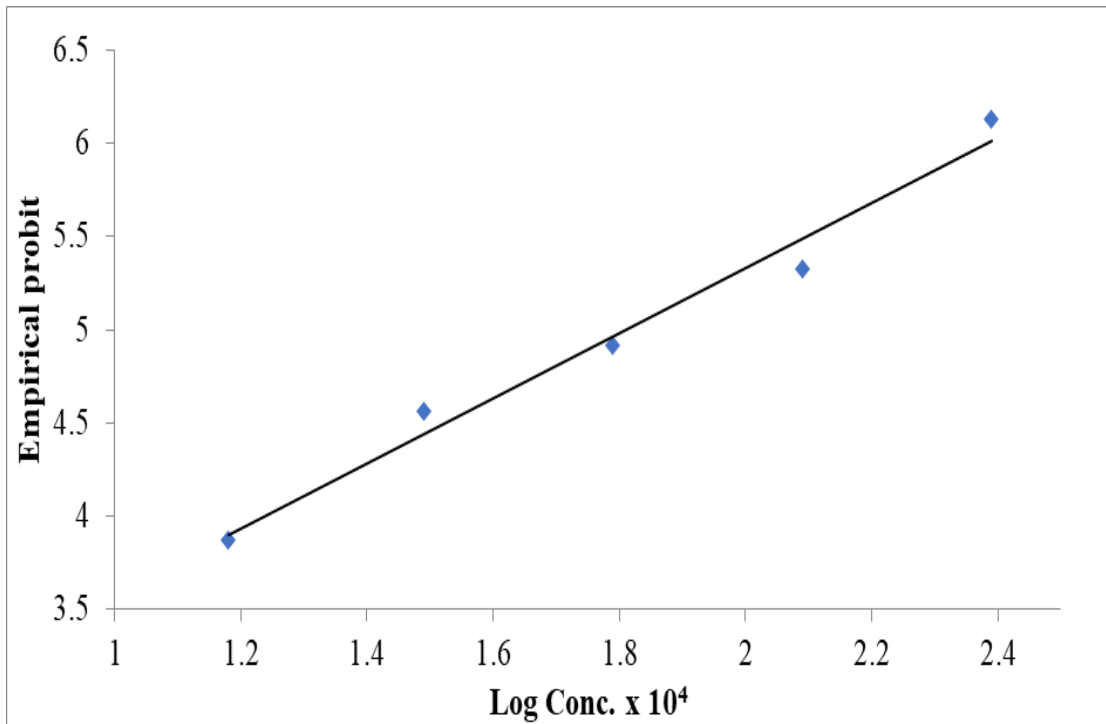


Fig 4.5 Concentration mortality response of cyantraniliprole to 3rd instar larvae of *P. brassicae*

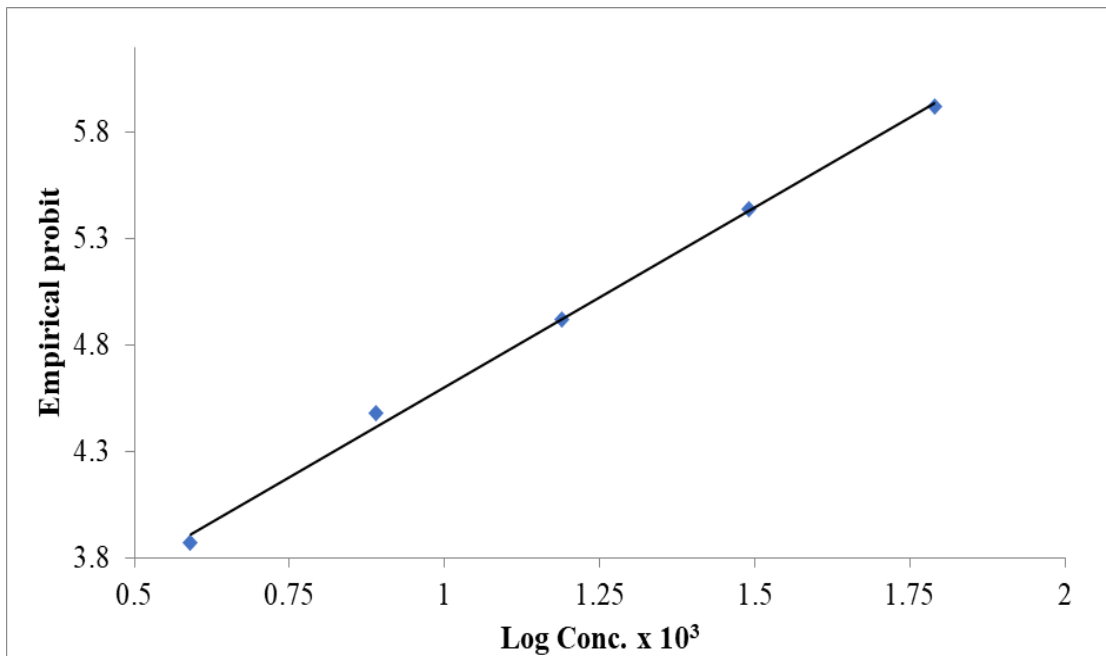


Fig 4.6 Concentration mortality response of emamectin benzoate to 3rd instar larvae of *P. brassicae*

Table 4.9 Concentration mortality response of cypermethrin to 3rd instar larvae of *P. brassicae*

Conc. (ppm)	Log Conc. (ppm x 10 ²)	Number of insects treated	Observed mortality (%)	Corrected mortality (%)	Empirical probit	Expected probit (Y)
0.125	1.09	30	20.0	20.0	4.16	4.1
0.250	1.39	30	33.3	33.3	4.56	4.5
0.500	1.69	30	53.3	53.3	5.08	5.0
1.000	2.00	30	63.3	63.3	5.33	5.4
2.000	2.30	30	83.3	83.3	5.92	5.8
Control	-	30	0.0	0.0	-	-

Regression equation = $1.447X - 2.438$

LC₅₀ = 0.484 ppm

Fiducial limits = 0.357 and 0.652 ppm

LC₉₀ = 3.719 ppm

Fiducial limits = 2.171 and 9.716 ppm

$\chi^2_{cal}(p=0.05) = 0.405$

$\chi^2_{tab}(p=0.05) = 7.814$

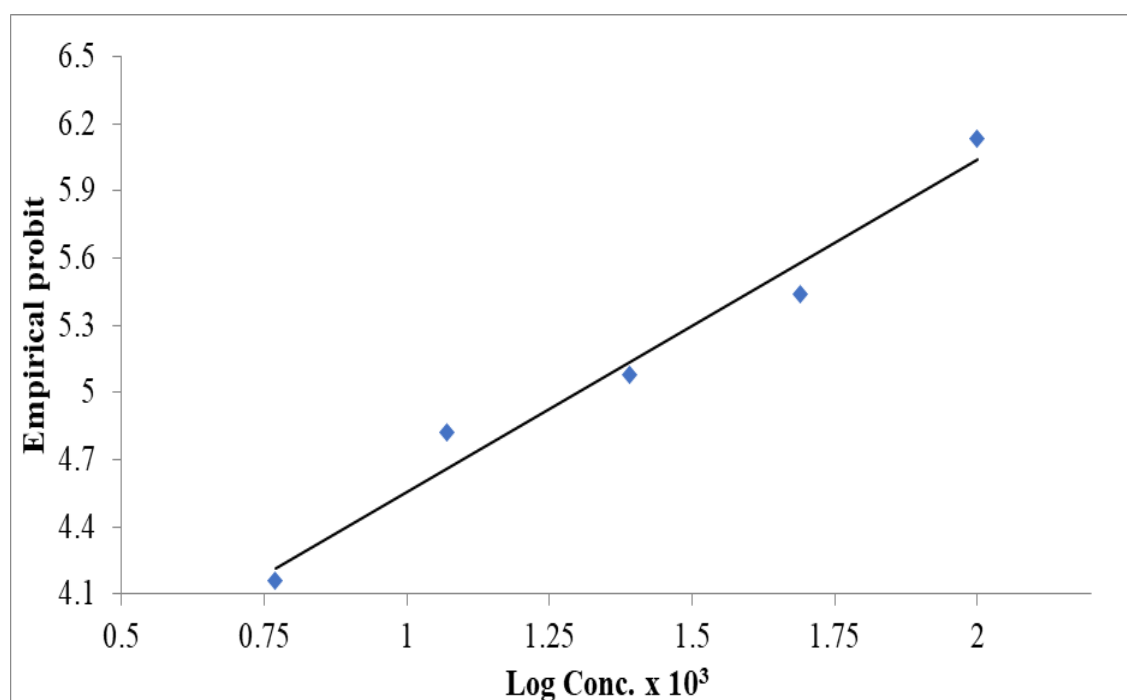


Fig 4.7 Concentration mortality response of spinosad to 3rd instar larvae of *P. brassicae*

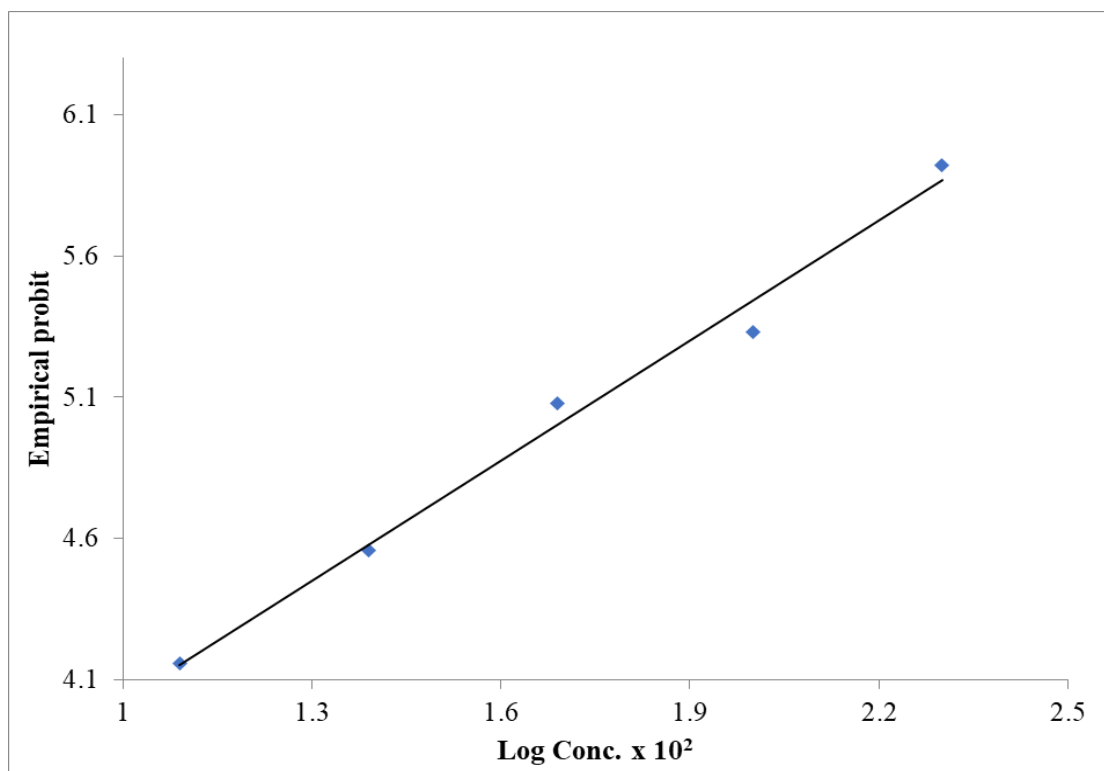


Fig 4.8 Concentration mortality response of cypermethrin to 3rd instar larvae of *P. brassicae*

The results of the present investigation indicated that LC₅₀ values of cypermethrin, cyantraniliprole, emamectin benzoate and spinosad against the 3rd instar larvae of *P. brassicae* were 0.484 ppm, 0.007, 0.017, and 0.202 ppm, respectively. The findings of Dhawan et al. (2010) on the toxicity of spinosad and chlorantraniliprole against the 3rd instar larvae of *P. brassicae* showed the LC₅₀ values as 0.017 and 0.002 ppm, respectively. The difference in toxicity may be due to different agroecological regions. Kumar et al. (2010) reported the LC₅₀ of cypermethrin against 3rd instar larvae of *P. brassicae* as 0.124 ppm, while it was 0.484 ppm in the present studies, thus indicating the frequent use of this insecticide in cabbage. The LC₅₀ values of chlorantraniliprole, cyantraniliprole, emamectin benzoate and spinosad against the 3rd instar larvae of *S. litura* were 2.26, 0.63, 0.50 and 8.41 ppm, respectively (Natarikar and Balikai 2015). The present findings get support from the results of Saleh et al. (2015) who reported the LC₅₀ value of emamectin benzoate as 0.019 and 0.039 ppm against the 4th instar larvae of lab and field strain of *S. littoralis*, respectively. Su et al. (2017) observed that the LC₅₀ value of chlorantraniliprole tested against late instar larvae (3rd and 4th) was 4.32 ppm. Similarly, Sharma et al. (2019) reported the LC₅₀ values of emamectin benzoate,

chlorantraniliprole and spinosad as 0.00007, 0.002 and 0.006 ppm, respectively. The present findings on spinosad toxicity are in close proximity to those of Khoramdel et al. (2021) who worked out the LC₅₀ value of 0.298 ppm for spinosad to 3rd instar larvae of *P. brassicae*. Again, the difference in the LC₅₀ values of all the insecticides could be attributed to usage pattern of pesticides in these regions.

Table 4.10 Relative toxicity of different insecticides to 3rd instar larvae of *P. brassicae*

Insecticide	LC ₅₀ value (ppm)	Relative toxicity	LC ₉₀ value (ppm)	Relative toxicity
Cyantraniliprole	0.007	69.14	0.038	97.87
Cypermethrin	0.484	1.0	3.719	1.0
Emamectin benzoate	0.017	28.47	0.097	38.34
Spinosad	0.202	2.40	1.587	2.34

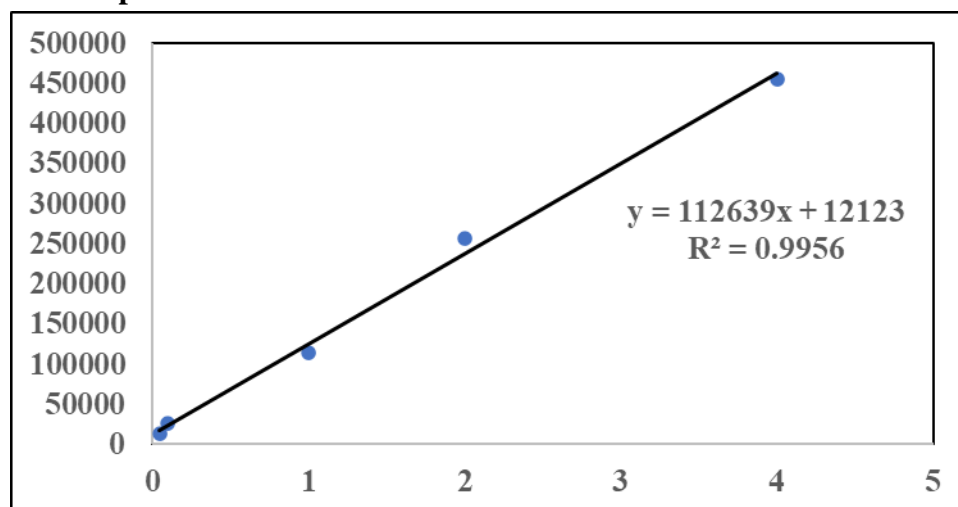
Based on LC₅₀ values, it is clear that cyantraniliprole, emamectin benzoate, spinosad are 69.14, 28.47, 2.40 times toxic than cypermethrin to third instar larvae of *P. brassicae* (Table 4.10). Similar trend in toxicity was observed on the basis of LC₉₀ values. Dhawan et al. (2010) reported that chlorantraniliprole was 8.5 times more toxic than spinosad to 3rd instar larvae of *P. brassicae* which is different from the present findings which show that cyantraniliprole is 28.85 times more toxic than spinosad. This could be due to only recent introduction of the new chemistry insecticides in the study area. Sharma et al. (2019) observed that chlorantraniliprole and emamectin benzoate were 3 and 85.71 times more toxic than spinosad against 3rd instar larvae of *P. brassicae*. Kannan et al. (2011) also observed that emamectin benzoate was more toxic than spinosad to 3rd instar larvae of *A. ipsilon*.

4.3 Residues of insecticides on cabbage heads

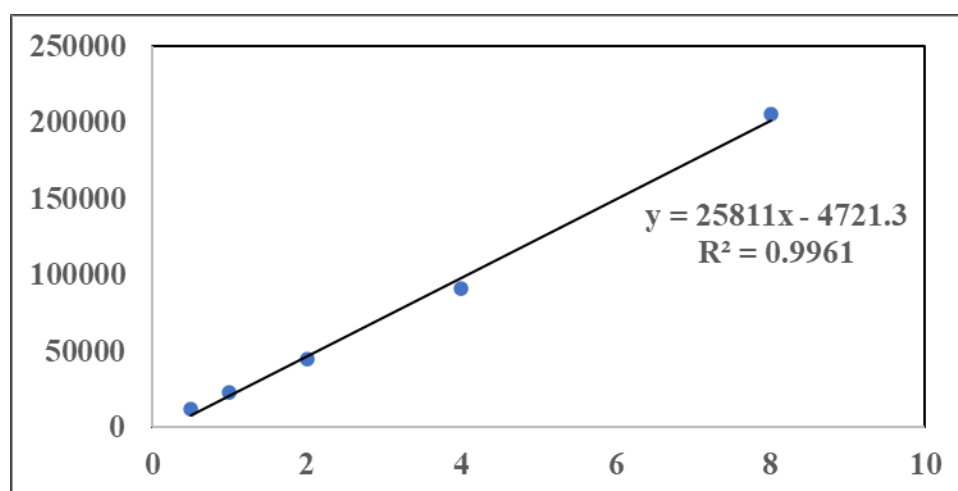
4.3.1 Standard curves of insecticides

Standard solutions of the three insecticides *viz.*, cyantraniliprole, emamectin benzoate and spinosad were prepared with acetonitrile (HPLC grade). The chromatograms of standard insecticides are presented in figure 4.10. The peak areas obtained in HPLC are plotted in the graph. The peak area of standards and standard curves of the insecticides are shown in the table 4.11 and figure 4.9, respectively. The calibration curves of all the three insecticides showed a linear relationship.

a) Cyantraniliprole



b) Emamectin benzoate



c) Spinosad

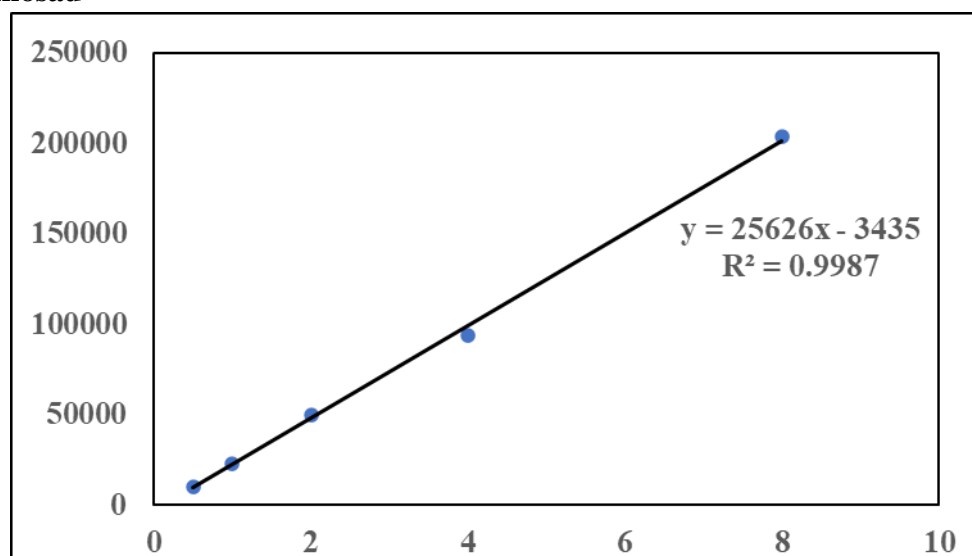


Fig. 4.9 Calibration curves of insecticides

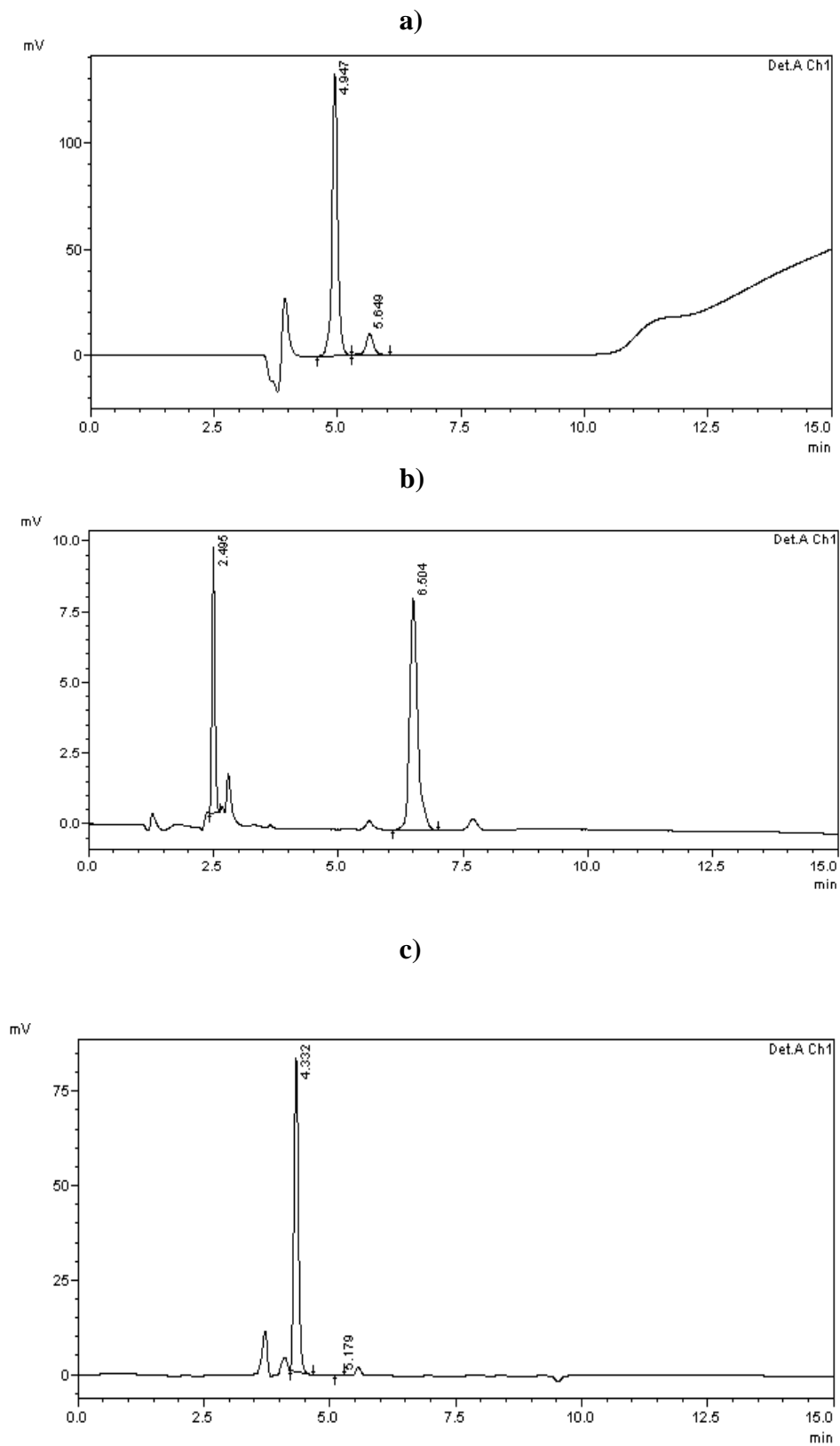


Fig. 4.10 Chromatograms of insecticide standards (HPLC)
a) Cyantraniliprole, b) Emamectin benzoate, and c) Spinosad

Table 4.11 Peak area of insecticide standards at different concentrations

Insecticides	Concentration (ppm)	Peak Area (mV)
Cyantraniliprole	0.05	13136
	0.1	25767
	1	114235
	2	257677
	4	455174
Emamectin benzoate	0.5	12408
	1	22687
	2	44773
	4	90995
	8	205594
Spinosad	0.5	10171
	1	22783
	2	49464
	4	94080
	8	203537

4.3.2 Recovery of insecticides from cabbage heads

The results of recoveries of cyantraniliprole, emamectin benzoate and spinosad from the spiked samples of cabbage heads are given in table 4.12. Cabbage samples spiked with cyantraniliprole at 0.1, 1 and 2 ppm level of fortification gave recoveries of 82.4, 88.67 and 88.65 per cent, respectively. The mean recovery of cyantraniliprole was found out to be 86.57 per cent which is in accordance with the results of Baek Soo-jin (2018), who reported the average recovery of chlorantraniliprole to be 70.3 and 88.0% when the samples were spiked at 0.1 and 0.01 level of fortification. However, Kar et al. (2017) obtained higher recoveries of 88.5, 89.4, 94.3 and 89.3% at 0.1, 0.25, 0.5 and 1 mg/kg levels of fortification. Kumar et al. (2021) also reported recoveries of 97.5, 97.3, 91.0 and 89.8% at 0.5, 0.1, 0.05 and 0.01 mg/kg levels of fortification. The higher recoveries may be due to variations in the method of extraction and analysis.

The recovery percentage of emamectin benzoate was found to be 84, 86.67 and 84.61 per cent from 0.5, 1 and 2 ppm level of spiking, respectively. The average recovery of emamectin benzoate was found to be 85.09 per cent. The experimental findings are consistent with the recovery obtained by Liu et al. (2012) who reported the mean recoveries of emamectin benzoate to be 86.4, 84 and 87.4% at 0.01, 0.1 and

1 ppm levels of fortification, respectively from spiked cabbage samples. Wang et al. (2012) also obtained recoveries of 85.7, 91.6 and 97% at 0.001, 0.01 and 0.1 mg/kg levels of fortification, respectively. Singh et al. (2013) reported the recoveries of emamectin benzoate at 0.5, 0.25 and 0.05 mg/kg levels of fortification as more than 92%. Dong et al. (2015) found the recoveries of 91.1, 99.0, and 98% at 0.5, 0.1, and 0.01 mg/kg level of fortification. The higher recovery percentage in their studies were perhaps due to different extraction, clean up and analytical methods.

The results of the recovery of the three insecticides indicated that the per cent recovery of spinosad was 88, 86.33 and 85.56 per cent at 0.5, 1 and 2 ppm of fortification, respectively. The average recovery of spinosad was found to be 86.7 per cent which is similar with the recoveries reported by Kashyap et al. (2015) and Mandal et al. (2009) who reported the mean recoveries of spinosad to be 80.6 to 90.1 per cent and 83.3 to 86.6 per cent, respectively from fortified cabbage heads. Sharma et al. (2007) reported the average recoveries of 80-83% at 0.05 and 2 mg/kg levels of fortification. The present findings are in close proximity to those of Singh and Battu (2012) where average recovery of spinosad ranged from 80 to 88.7% when the samples were spiked at 0.01, 0.05, 0.1 and 0.2 mg/kg level of fortification. Shashni (2020) also reported the mean recoveries of 84-87% at 0.05, 0.1 and 0.2 ppm levels of fortification level.

Table 4.12 Recovery of insecticides in fortified samples of cabbage heads

Insecticide	Spiked level (mg/kg)	Amount Recovered (%) *Mean \pm SD	RSD_r
Cyantraniliprole	0.1	82.40 \pm 1.20	1.45
	1	88.67 \pm 1.52	1.71
	2	88.65 \pm 1.26	1.42
	Mean	86.57 \pm 3.54	
Emamectin benzoate	0.5	84.00 \pm 2.00	2.38
	1	86.67 \pm 2.25	2.59
	2	84.61 \pm 2.38	2.81
	Mean	85.09 \pm 2.73	
Spinosad	0.5	88.00 \pm 2.00	2.27
	1	86.33 \pm 1.78	2.06
	2	85.56 \pm 2.35	2.74
	Mean	86.64 \pm 2.50	

Mean of 3 replicates

SD = Standard Deviation

RSD_r = Relative Standard Deviation (Repeatability)

4.33 Limit of Detection (LOD) and Limit of Quantification (LOQ)

The limit of detection (LOD) of cyantraniliprole was 0.003 mg/kg, whereas for emamectin benzoate and spinosad, LODs were calculated to be 0.005 mg/kg each. The limits of quantification for cyantraniliprole, emamectin benzoate, and spinosad were calculated as 0.01, 0.02 and 0.02 mg/kg, respectively (Table 4.13).

Table 4.13 Limit of Detection (LOD) and Limit of Quantification (LOQ) of insecticides

Insecticide	Disturbance area	Noise level	LOD (mg/kg)	LOQ (mg/kg)
Cyantraniliprole	1207	0.0010	0.003	0.01
Emamectin Benzoate	431	0.0018	0.005	0.02
Spinosad	406	0.0017	0.005	0.02

Baek Soo-jin (2018) reported that LOQ of chlorantraniliprole as 0.01 mg/kg in cabbage whereas Sun et al. (2012) reported that the LOQ value of cyantraniliprole in pakchoi cabbage as 0.01 mg/kg which is similar to the value obtained in the present study. Preethi et al. (2019) reported the LOD and LOQ of chlorantraniliprole as 0.015 and 0.05 mg/kg, respectively. Liu et al. (2012) also obtained the LOQ value of emamectin benzoate in cabbage samples as 0.01 mg/kg. The LOD and LOQ of emamectin benzoate in cabbage heads was worked to be 0.017 and 0.05 mg/kg, respectively (Singh et al. 2013) which is in close conformity to the present findings. The present values of LOQ and LOD of spinosad are lower as compared to 0.05 and 0.017 mg/kg as reported by Chahil et al. (2014). The findings of the present investigation are similar to the results of Mandal et al. (2009) and Kashyap et al. (2015) who also reported the LOQ of spinosad as 0.02 mg/kg on cauliflower and tomato samples, respectively. However, Singh and Battu (2012) calculated the LOD and LOQ value of spinosad as 0.01 and 0.03 mg/kg on cabbage. While analysing the residues of spinosad on cauliflower, Shashni (2020) reported the LOD and LOQ of spinosad as 0.003 and 0.011 mg/kg, respectively.

4.3.4 Insecticide residues

The data on insecticide residues after two sprays are presented in the table 4.14, 4.15 and figure 4.11 and their dissipation curves are shown in the figure 4.12.

a) Cyantraniliprole

The average initial deposits of cyantraniliprole on cabbage when applied at a dosage of 60 and 120 g a.i./ha were 0.4515 ± 0.015 and 1.1487 ± 0.174 mg/kg, respectively. In the treatment with the dosage of 60 g a.i./ha, the residues dissipated following the order of 0.3417 ± 0.018 , 0.1497 ± 0.011 and 0.0332 ± 0.009 mg/kg on 1st, 3rd and 5th day. They were not quantifiable on the 7th day. The per cent dissipation of initial deposit was 24.32, 66.84 and 92.64% after 1, 3 and 5 days of spraying. Whereas in the case of double dosage, the residues followed the order of 0.9102 ± 0.015 , 0.7207 ± 0.023 , 0.5246 ± 0.106 , 0.3972 ± 0.025 , 0.1983 ± 0.020 mg/kg on 1st, 3rd, 5th, 7th and 10th day. The residues were not quantifiable on the 15th day. The residues reduced by 20.76, 37.25, 54.33, 65.42, and 82.74 % by the 1st, 3rd, 5th, 7th and 10th day. The results of this experiment are in accordance with the findings of Kumar et al. (2021), who reported the initial deposits of cyantraniliprole to be 0.403 and 1.195 mg/kg on cabbage when applied at a dosage of 60 and 120 g a.i./ha, respectively. The present findings are also in agreement to those of Sun et al. (2012) who reported the mean initial deposit of cyantraniliprole ranged from 0.02-1.27 mg/kg on Pakchoi at 3-7 days after spraying at a dosage of 60-90 g a.i./ha. Kar et al. (2012, 2017) reported that the initial deposits of chlorantraniliprole ranged from 0.12 to 0.22 mg/kg when applied at the doses of 9.25 and 18.5 g a.i./ha.

b) Emamectin benzoate

The mean initial deposits of emamectin benzoate on cabbage when applied at a dosage of 10 and 20 g a.i./ha were 0.2991 ± 0.009 and 0.3663 ± 0.008 mg/kg, respectively. In the treatment with the dosage of 10 g a.i./ha, the residues dissipated to 0.1735 ± 0.005 and 0.0306 ± 0.007 mg/kg on 1st and 3rd day. These were not quantifiable on the 5th day. The per cent dissipation of emamectin benzoate initial deposit was 41.99 and 89.76% after 1 and 3 days of spraying. Whereas in the case of double dosage, the residues followed the order of 0.1761 ± 0.004 , 0.0832 ± 0.012 mg/kg on 1st, 3rd day and were not quantifiable on the 5th day *i.e.*, below the LOQ of 0.02 mg/kg. The residues reduced by 51.93% on 1st day and 77.28% on 3rd day. The findings of the current study are close to the mean initial deposit of emamectin benzoate on cauliflower as 0.22 and 0.30 mg/kg when applied at a dosage of 8.5 and 17 g a.i./ha, respectively (Chahil et al. 2014). Wang et al. (2012) reported the average

initial deposits of emamectin benzoate in cabbage as 0.001-0.052 mg/kg at a dose of 4.5 g a.i./ha after 7-14 days of spraying which corroborates the present findings. Rajasekaran et al. (2013) reported the mean initial deposits of emamectin benzoate in cauliflower as 0.064 and 0.116 mg/kg at a dose of 1.5 and 3.0 L/ha, respectively. The present findings are almost similar to those of Singh et al. (2013) who observed the initial deposit of emamectin benzoate in cabbage as 0.11 and 0.21 mg/kg when applied at a dose of 8.5 and 17 g a.i./ha.

c. Spinosad

Spinosad when applied at a concentration of 15 and 30 g a.i./ha resulted in the average initial deposits of 0.5151 ± 0.027 and 0.9713 ± 0.128 mg/kg, respectively. The dissipation of its residues followed the order of 0.3914 ± 0.027 , 0.2369 ± 0.016 and 0.1081 ± 0.009 mg/kg for 1st, 3rd and 5th day after spraying, respectively when applied @ 15 g a.i./ha. There was a reduction of 24.01, 54 and 79.01 per cent of initial deposit after 1st and 3rd day of spraying. The residues were not quantifiable on 5th day. While for the dosage of 30 g a.i./ha, the order of dissipation was 0.7668 ± 0.0861 , 0.6107 ± 0.027 , 0.4939 ± 0.017 and 0.2976 ± 0.009 mg/kg for 1st, 3rd, 5th and 7th day after spraying. The reduction of initial deposit after 1st, 3rd and 5th day was 21.05, 37.13, 49.15 and 69.36 % per cent, respectively. The findings of this experiment are in accordance with the results of Mandal et al. (2009) where initial deposits of spinosad on cabbage were 0.57 and 1.34 mg/kg at the dosage of 15 and 30 g a.i./ha, respectively. However, Singh and Battu (2012) reported mean initial deposits of spinosad on cabbage as 0.33 and 0.56 mg/kg at 15 and 30 g a.i./ha which are less as compared to those obtained in the present investigation. The present findings are corroborated with the earlier results of Kashyap et al. (2015) who observed the mean initial deposit of spinosad on cabbage as 0.45 and 0.93 mg/kg when applied @ 15 and 30 g a.i./ha, respectively. The findings of the present study are in close proximity to those of Shashni (2020) who determined the average initial deposit of spinosad (0.015%) as 0.467 mg/kg on cauliflower.

4.3.5 Half-life values of insecticides

The half-life values and safety intervals of the insecticides are given in table 4.16 and 4.17, respectively. The linear curves of the insecticides are shown in the fig 4.13 and 4.14.

The half-life values of cyantraniliprole when applied @ 60 g and 120 g a.i./ha were calculated as 1.3 and 4.2 days, with corresponding safety intervals of 2.9 and 3.3 days, respectively. Sun et al. (2012) also reported the half-life values of 2.9-3.6 days on cabbage at a dosage 60-90 g a.i./ha, thus corroborating the present findings. Kar et al. (2017) obtained the half-life values of chlorantraniliprole on cabbage as 1.80 and 1.46 days at a dose of 9.25 and 18.5 g a.i./ha. Preethi et al. (2019) reported the half-life values of chlorantraniliprole as 2.29-2.53 days on cabbage. The present findings are somewhat similar to those of Kumar et al. (2021) reporting the half-life values of cyantraniliprole as 3.5-4.8 days on cabbage at a dose of 60 and 120 g a.i./ha.

Table 4.14 Residues of insecticides at the recommended doses on cabbage during Rabi 2021-2022

Days after treatment	Mean residues (mg/kg)		
	Cyantraniliprole	Emamectin benzoate	Spinosad
0 (2 hrs after spray)	0.4515±0.015	0.2991±0.009	0.5151±0.027
1	0.3417±0.018 (24.32)	0.1735±0.005 (41.99)	0.3914±0.027 (24.01)
3	0.1497±0.011 (66.84)	0.0306±0.007 (89.76)	0.2369±0.016 (54.00)
5	0.0332±0.009 (92.64)	< LOQ	0.1081±0.009 (79.01)
7	< LOQ	< LOQ	< LOQ

Figures after ± sign denote the standard deviation

Figures in parentheses denote the per cent dissipation of insecticides

LOQ= Limit of Quantification

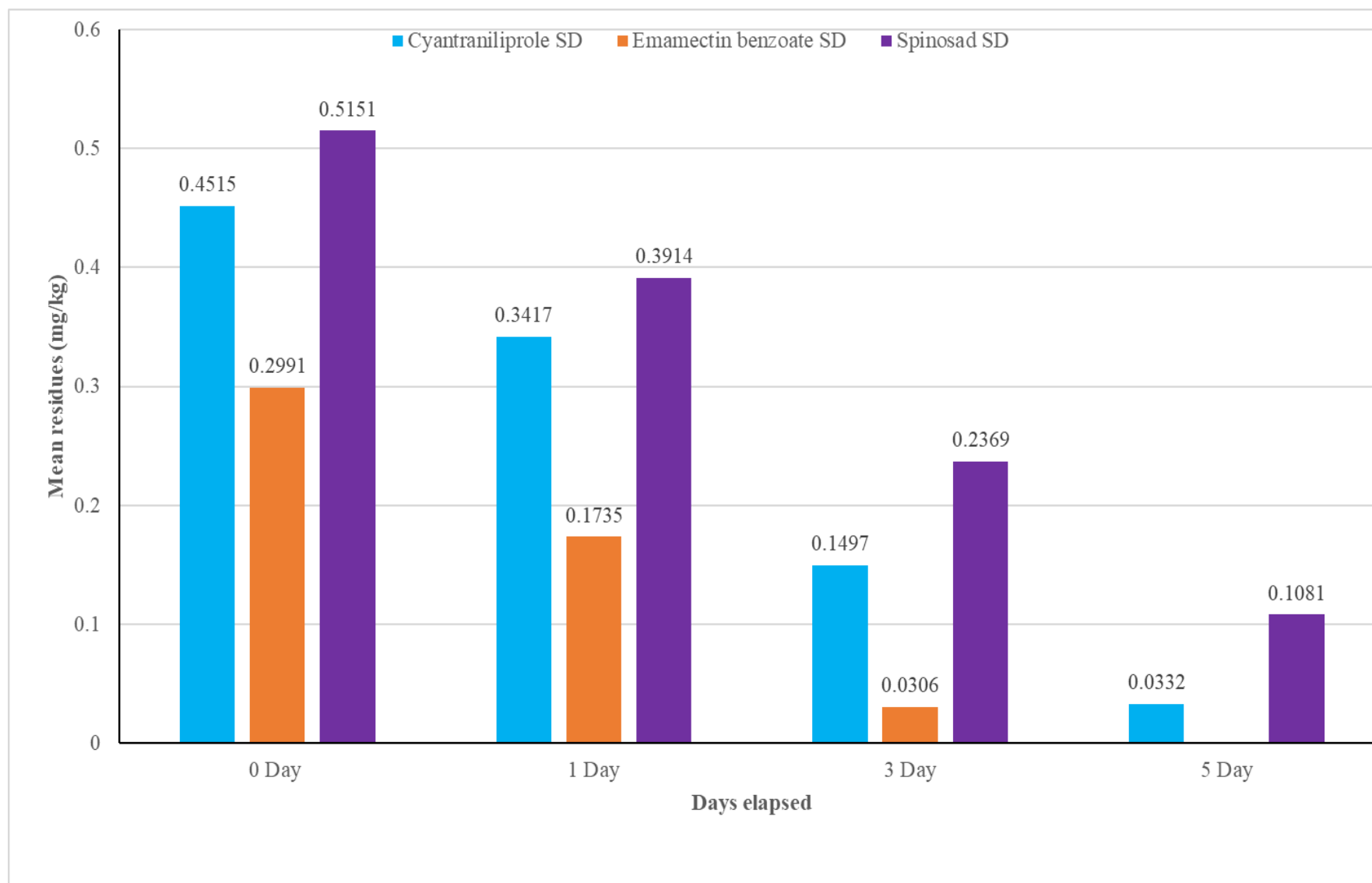


Fig. 4.11a Residues of insecticides at recommended dose

Table 4.15 Residues of insecticides at double the recommended doses on cabbage during Rabi 2021-2022

Days after treatment	Mean residues (mg/kg)		
	Cyantraniliprole	Emamectin benzoate	Spinosad
0 (2hrs after spray)	1.1487±0.174	0.3663±0.009	0.9713±0.128
1	0.9102±0.015 (20.76)	0.1761±0.004 (51.93)	0.7668±0.086 (21.05)
3	0.7207±0.023 (37.25)	0.0832±0.012 (77.28)	0.6107±0.027 (37.13)
5	0.5246±0.106 (54.33)	< LOQ	0.4939±0.017 (49.15)
7	0.3972±0.025 (65.42)	< LOQ	0.2976±0.009 (69.36)
10	0.1983±0.020 (82.74)	< LOQ	< LOQ
15	< LOQ	< LOQ	< LOQ

Figures followed after ± denote the standard deviation

Figures in parentheses denote the per cent dissipation of insecticide

LOQ= Limit of Quantification

The half-life values of emamectin benzoate when applied @ 10 and 20 g a.i./ha are 0.9 and 1.5 days, respectively. The safety intervals were worked out to be 3.48 and 6.09 days for the doses 10 and 20 g a.i./ha, respectively. The present findings get support from the results of Liu et al. (2012) who reported the half-life values of emamectin benzoate as 1 day and Singh et al. (2013) who worked out the half-life values as 0.88 and 1.25 days at a dose of 8.5 and 17 g a.i./ha. However, Chahil et al. (2014) worked out the half-life values of emamectin benzoate as 1.72 and 2.26 days at a dose of 8.5 and 17 g a.i./ha. Dong et al. (2015) reported the half-life values of emamectin benzoate on cabbage as 1.08-2.70 days which also support the present results.

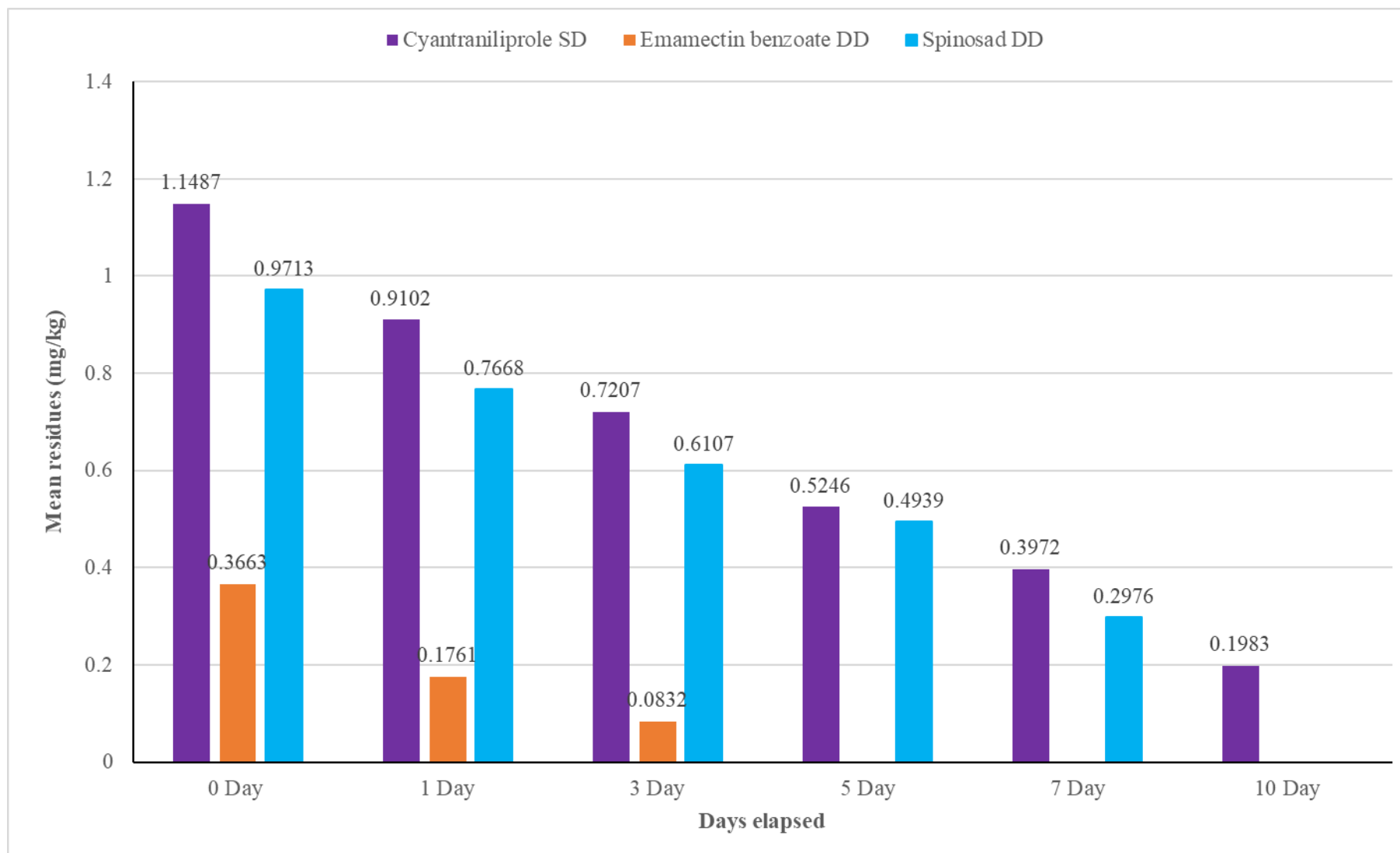


Fig. 4.11b Residues of insecticides at double the recommended dose

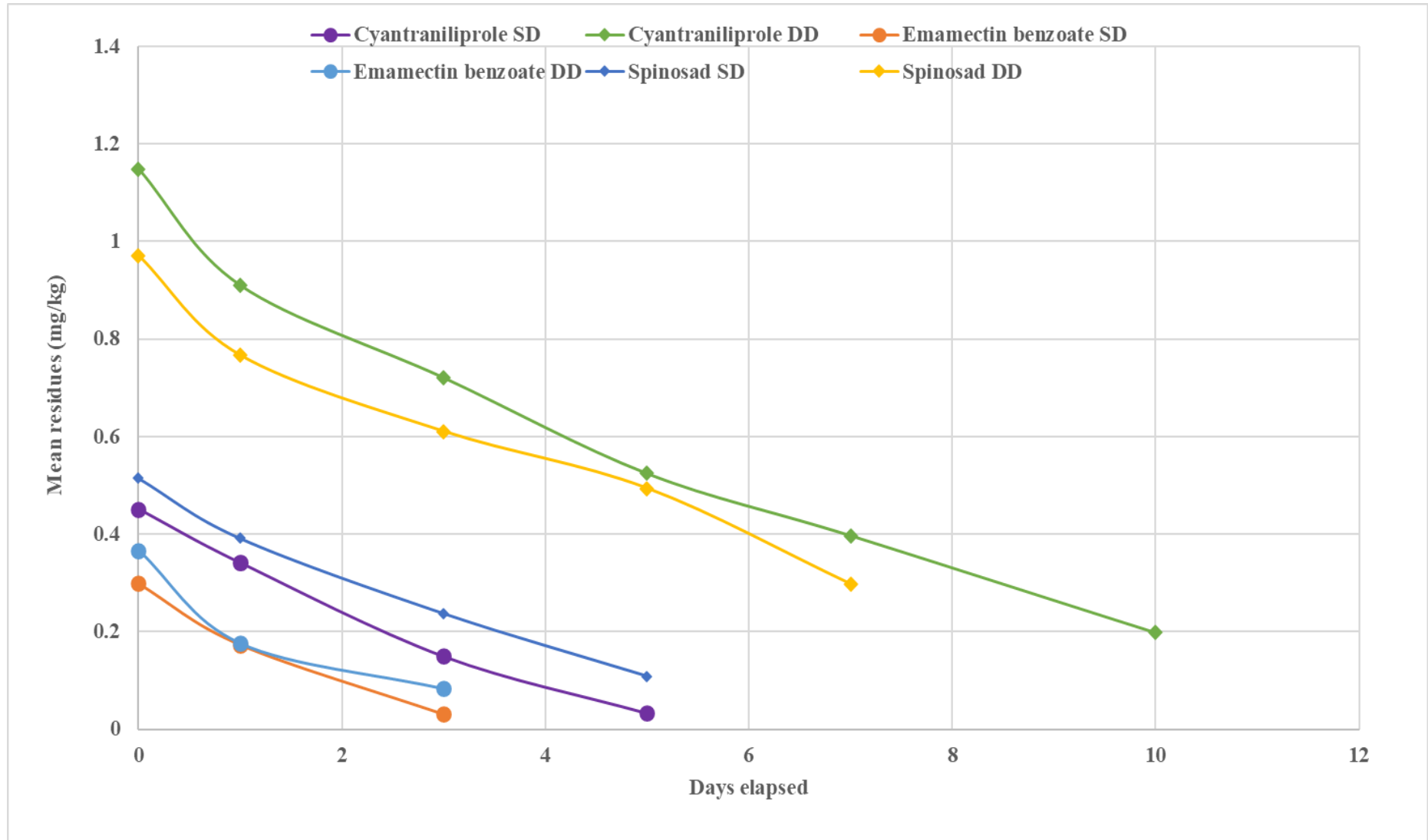


Fig 4.12 Dissipation curves of insecticides on cabbage heads

The half-life values of spinosad when applied @ 15 and 30 g a.i./ha was calculated as 2.3 and 4.5 days, respectively. The safety intervals were worked out to be 4.4 days and 4.6 days at the doses of 15g and 30 g a.i./ha. The present findings are in accordance with results reported by Sharma et al. (2007) who worked out the half-life of spinosad as 2.8 days when applied at a concentration of 17.5 g a.i./ha and 2.0 days when applied at 35.0 g a.i./ha. Mandal et al. (2009) reported lower half-life values of spinosad as 1.20 and 1.58 days at 15 and 30 g a.i./ha and suggested a safety interval of 6 days on cauliflower. Singh and Battu (2012) reported the half-life values of spinosad in cabbage as 1.4 and 1.5 days with a safe waiting period of 5.5 and 7.2 days at a dose of 15 and 30 g a.i./ha. The higher waiting period was calculated based upon the MRL value of spinosad as 0.02 mg/kg on cabbage during that period as compared to present MRL value of 2.0 mg/kg.

Table 4.16 Half-life values of insecticides

Insecticides	Dose (g a.i./ha)	Regression equation	Half-life (days)
Cyantraniliprole	60	$Y = -0.2255x - 0.2716$	1.3
	120	$Y = -0.0725x + 0.0631$	4.2
Emamectin benzoate	10	$Y = -0.3367X - 0.4841$	0.9
	20	$Y = -0.2072X - 0.4805$	1.5
Spinosad	15	$Y = -0.1335X - 0.2715$	2.3
	30	$Y = -0.0675x - 0.0189$	4.5

Table 4.17 Safety intervals of insecticides

Parameters	Cyantraniliprole		Emamectin benzoate		Spinosad	
	60	120	10	20	15	30
Tolerance limit (mg/kg)	2.0* [#]	2.0* [#]	0.02 [#]	0.02 [#]	2.0* [#]	2.0* [#]
Log tolerance limit	0.301	0.301	-1.698	-1.698	0.301	0.301
Initial deposit (mg/kg)	0.4515	1.1487	0.2991	0.3663	0.5151	0.9713
Log Initial deposit	-0.345	0.060	-0.524	-0.436	-0.288	-0.013
Slope	-0.226	-0.073	-0.337	-0.207	-0.134	-0.068
Safety interval (days)	2.9	3.3	3.48	6.09	4.4	4.6

*FSSAI, 2020 #FAO, 2020

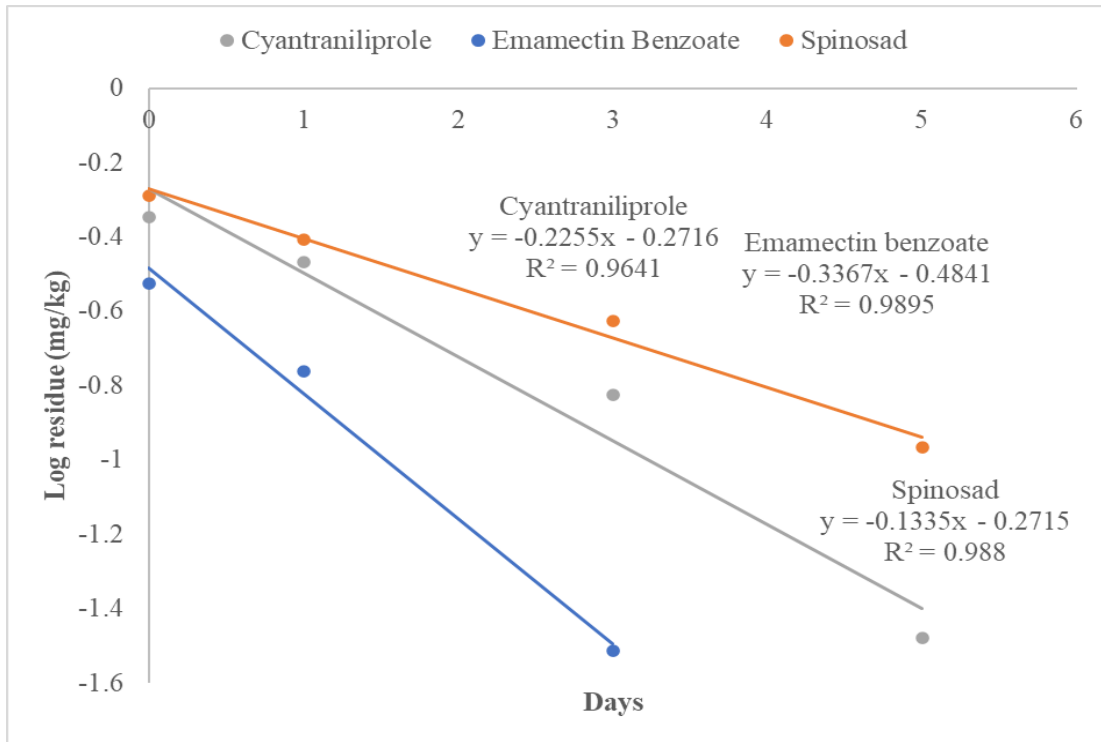


Fig 4.13a Regression lines of insecticides at the recommended doses on cabbage during *Rabi* 2021-22

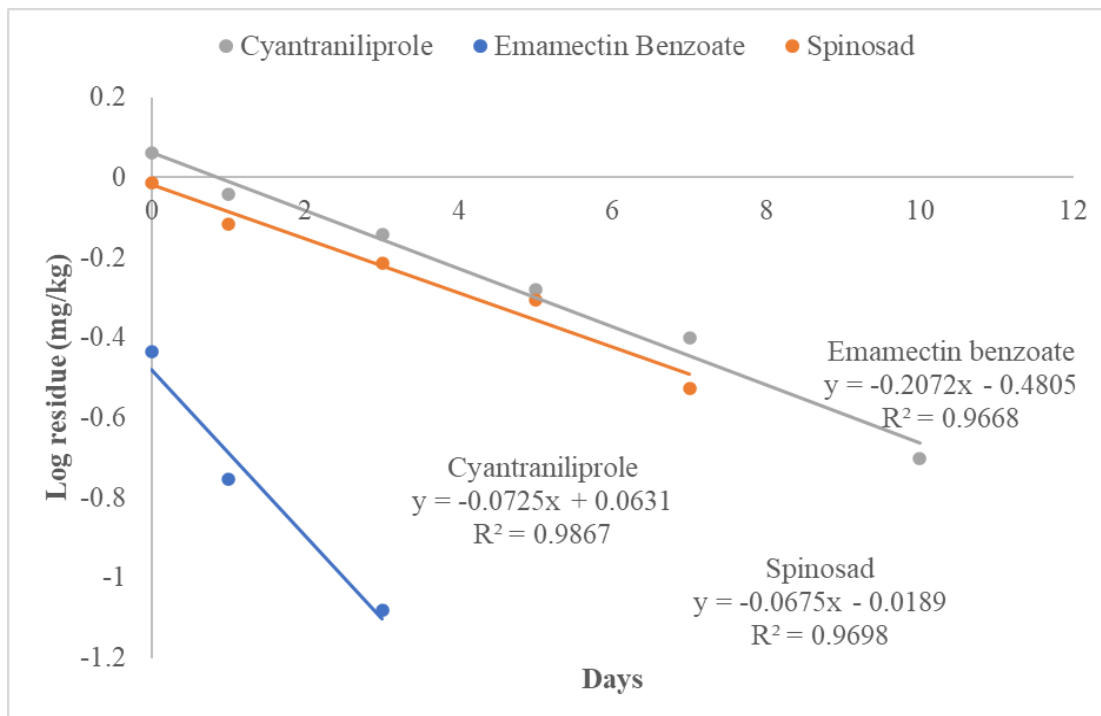


Fig 4.13b Regression lines of insecticides at double the recommended doses on cabbage during *Rabi* 2021-22

4.4 Effect of processing on insecticide residues in cabbage

Three processing methods *viz.*, removing the outer leaves, washing under tap water for 1 minute and boiling for about 5 minutes were followed. On the day of spray, washing the cabbage heads under tap water resulted in reduction of residues. The residues of cyantraniliprole, emamectin benzoate and spinosad were 0.2248, 0.0903 and 0.3064 ppm, respectively at their recommended doses (Table 4.18). The respective residues at double the recommended doses were 0.3453, 0.1271 and 0.5690 ppm upon washing the zero day samples (Table 4.19). Boiling and removing the outer leaves removed 100% residues followed by washing for all the insecticides. Washing removed 40.5 and 41.4% of initial deposit of spinosad when applied @ 15 g and 30 g a.i./ha, respectively. Spinosad residues were not quantifiable after 1st day when the samples were washed. Average initial deposit of emamectin benzoate was reduced by 69 and 65% for treatments with the dosage of 10 g and 20 g a.i./ha, respectively. Emamectin benzoate residues were below LOD after 1st day when the samples are washed. Cyantraniliprole residues were reduced by 50.21 and 97.98% in the treatment with the dosage of 60 g a.i./ha on zero day and 1st day, respectively with washing. Whereas washing the cabbages removed 69.9 and 98.33% of cyantraniliprole residues in the treatment with the dosage of 120 g a.i./ha on zero day and 1st day, respectively. Cyantraniliprole were not quantifiable after the 1st day. The current findings indicated that boiling and removal of outer leaves are the most effective processing methods for removing the residues of all the three insecticides which indeed is in coherence with the reports of Kumari (2008), Kar et al. (2012) and Preethi et al. (2019). According to Chung (2018), boiling is the most effective procedure for the removal of residues. Panhwar et al. (2014) reported that washing removed emamectin benzoate residues by 65% which is very similar to results of the present study. Spinosad residues were reduced by 64.4% by washing at the dosage of 30 g a.i./ha on the 1st day, thus is in accordance with the results of Vijayashree et al. (2014).

Table 4.18 Effect of processing on residues of insecticides when applied at recommended doses

Insecticide (Dose g a.i./ha)	Day of sampling	Residues			
		Before processing	Removal of outer leaves	After processing Washing	Boiling
Cyantraniliprole (60)	0	0.4515±0.015	BDL	0.2248±0.014 (50.21)	BDL
	1	0.2906±0.018	BDL	0.0091±0.010 (97.98)	BDL
	3	0.1497±0.011	BDL	BDL	BDL
	5	0.0332±0.009	BDL	BDL	BDL
Emamectin benzoate (10)	0	0.2991±0.009	BDL	0.0903±0.001 (69.00)	BDL
	1	0.1735±0.005	BDL	BDL	BDL
	3	0.0306±0.007	BDL	BDL	BDL
Spinosad (15)	0	0.5151±0.027	BDL	0.3064±0.106 (40.50)	BDL
	1	0.3914±0.027	BDL	BDL	BDL
	3	0.2369±0.016	BDL	BDL	BDL
	5	0.1081±0.009	BDL	BDL	BDL

Figures followed after ± denote the standard deviation

Figures in parentheses indicate the per cent reduction of residues

BDL=below detectable level

Table 4.19 Effect of processing on residues of insecticides when applied at double the recommended doses

Insecticide (Dose g a.i./ha)	Day of sampling	Residues			
		Before processing	Removal of outer leaves	After processing Washing	Boiling
Cyantraniliprole (120)	0	1.1487±0.174	BDL	0.3453±0.018 (69.90)	BDL
	1	0.8243±0.015	BDL	0.0191±0.009 (98.33)	BDL
	3	0.6445±0.023	BDL	BDL	BDL
	5	0.4135±0.106	BDL	BDL	BDL
	7	0.2916±0.025	BDL	BDL	BDL
	10	0.1019±0.020	BDL	BDL	BDL
Emamectin benzoate (20)	0	0.3663±0.008	BDL	0.1271±0.097 (65.00)	BDL
	1	0.1761±0.004	BDL	BDL	BDL
	3	0.0832±0.012	BDL	BDL	BDL
Spinosad (30)	0	0.9713±0.128	BDL	0.5690±0.016 (41.40)	BDL
	1	0.6299±0.086	BDL	0.3438±0.011 (64.60)	BDL
	3	0.4079±0.027	BDL	BDL	BDL
	5	0.2504±0.017	BDL	BDL	BDL
	7	0.0946±0.009	BDL	BDL	BDL

Figures followed after '±' denote the standard deviation

Figures in parentheses indicate the per cent reduction of residues

BDL= below detectable level

5. SUMMARY AND CONCLUSIONS

Studies on “**Toxicity of novel insecticides to *Pieris brassicae* (Linnaeus) and their residue dynamics on cabbage**” were conducted in the Department of Entomology, CSK HPKV, Palampur during *Rabi* 2021-2022.

Intrinsic toxicity studies were conducted in the toxicology laboratory of the Department of Entomology. Three novel insecticides *viz.*, cyantraniliprole, emamectin benzoate and spinosad along with cypermethrin (check) were used for the intrinsic toxicity studies and leaf dip method of bioassay was employed. Residue studies of these insecticides were also carried out as well as the effect of different processing methods on removal of residues was studied. The cabbage crop was sprayed with recommended and double the recommended doses of insecticides. Two sprays were given at 14 days interval and the samples were collected after second spray at different intervals for residue analysis by using HPLC. The findings are summarized below:

- ❖ The LC_{50} values of cyantraniliprole, emamectin benzoate, spinosad and cypermethrin tested against neonate larvae of *P. brassicae* were 0.00003, 0.00029, 0.002 and 0.061 ppm, respectively. Cyantraniliprole, cypermethrin and emamectin benzoate were 2033.33, 210.34 and 30.5 times more toxic than spinosad to the neonate larvae of *P. brassicae*.
- ❖ The LC_{50} values of cyantraniliprole, emamectin benzoate, spinosad and cypermethrin tested against 3rd instar larvae of *P. brassicae* were 0.007, 0.484, 0.017 and 0.202 ppm, respectively. Cyantraniliprole, emamectin benzoate and spinosad were 69.14, 28.47 and 2.40 times more toxic to 3rd instar larvae of *P. brassicae* when compared to cypermethrin.
- ❖ The mean recoveries of cyantraniliprole when fortified at 0.1, 1 and 2 mg/kg levels of fortification were 82.40, 88.67 and 88.65 per cent, respectively. While the recoveries of 84.00, 86.67 and 84.61 per cent were obtained when the cabbage samples were fortified with emamectin benzoate at 0.5, 1 and 2

mg/kg level of fortification, respectively. The per cent recovery of spinosad was 88, 86.33 and 85.56 per cent at 0.5, 1 and 2 ppm levels of fortification.

- ❖ The limits of detection (LOD) of cyantraniliprole, emamectin benzoate, spinosad were calculated as 0.003, 0.005 and 0.005 mg/kg respectively. Whereas the limit of quantification (LOQ) of cyantraniliprole, emamectin benzoate, spinosad were worked out to be 0.01, 0.02 and 0.02 mg/kg.
- ❖ The average initial deposits of cyantraniliprole when applied at a dosage of 60 g and 120 g a.i./ha were 0.4515 ± 0.015 and 1.1487 ± 0.174 mg/kg, respectively. The residues for the dose of 60 g a.i./ha reduced to 0.3417 ± 0.018 (24.32%), 0.1497 ± 0.011 (66.84%) and 0.0332 ± 0.009 (92.64%) mg/kg on 1st, 3rd and 5th day, respectively. Whereas in the case of double dosage, the residues decreased to 0.9102 ± 0.015 (20.76%), 0.7207 ± 0.023 (37.25%), 0.5246 ± 0.106 (54.33%), 0.3972 ± 0.025 (65.42%), 0.1983 ± 0.020 (82.74%) mg/kg on 1st, 3rd, 5th, 7th and 10th day. The residues were not quantifiable on the 15th day.
- ❖ The mean initial deposits of emamectin benzoate on cabbage when applied at a dosage of 10 g and 20 g a.i./ha is 0.2991 ± 0.009 and 0.3663 ± 0.008 mg/kg, respectively. The residues dissipated to 0.1735 ± 0.005 (41.99%) and 0.0306 ± 0.007 (89.76%) mg/kg on 1st and 3rd day for 10 g a.i./ha dosage. Whereas in the case of double dosage, the residues reduced to 0.1761 ± 0.004 (51.93%) mg/kg on 1st day and 0.0832 ± 0.012 (77.28%) mg/kg on 3rd day and were not quantifiable on the 5th day.
- ❖ The mean initial deposits of spinosad were 0.5151 ± 0.027 and 0.9713 ± 0.128 mg/kg at the doses of 15 g and 30 g a.i./ha, respectively. The residues dissipated to 0.3914 ± 0.027 (24.01%), 0.2369 ± 0.016 (54.0%) and 0.1081 ± 0.009 (79.01%) mg/kg for 1st, 3rd and 5th day after spraying when applied @ 15 g a.i./ha. While for the dosage of 30 g a.i./ha, the residues decreased to 0.7668 ± 0.0861 (21.05%), 0.6107 ± 0.027 (37.13%), 0.4939 ± 0.017 (49.15%) and 0.2976 ± 0.009 (69.36%) mg/kg for 1st, 3rd, 5th and 7th day after spraying, respectively.
- ❖ The half-life values of cyantraniliprole, emamectin benzoate and spinosad were 1.3 and 4.2 days, 0.9 and 1.5 days, 2.3 and 4.5 days, respectively

corresponding to their recommended and double the recommended doses. Accordingly, the safety intervals of cyantraniliprole, emamectin benzoate and spinosad were worked out to be 2.9 and 3.3, 3.5 and 6.1, and 4.4 and 4.6 days, respectively.

- ❖ Removal of outer leaves and boiling removed 100% of residues of cyantraniliprole, emamectin benzoate and spinosad even on the day of application of the insecticides.
- ❖ While washing removed 50.21 and 69.9% of cyantraniliprole residues on the day of application and 97.98 and 98.33% of cyantraniliprole residues after 1 day of application at the doses of 60 and 120 g a.i./ha, respectively.
- ❖ Washing removed emamectin benzoate residues by 69 and 65% on the day of application at the doses of 10 and 20 g a.i./ha, respectively.
- ❖ Washing the heads also reduced spinosad residues by 40.5 and 41.4% when applied at the doses of 15 and 30 g a.i./ha, respectively.

Conclusions

- ❖ On the basis of present investigations, it can be suggested that application of novel insecticides should be done at early stage of infestation by *Pieris brassicae* for effective control. Novel insecticides being used at low application rates can give better control of the pest at its early stages thereby reducing the number of applications. Further, removal of outer 2-3 leaves of cabbage before consumption can help in almost complete removal of residues of these novel insecticides.

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APPENDIX-I

Meteorological data recorded during 15th -19th standard meteorological week (SMW) of 9th April, 2022 to 13th May, 2022

Standard week	Maximum temperature (°C)	Minimum temperature (°C)	Maximum Relative humidity (%)	Minimum Relative humidity (%)	Rainfall (mm)	Sunshine hours
15	31.3	16.8	50.6	31.3	8.6	2.8
16	30.0	16.6	43.0	29.3	8.0	8.2
17	30.2	16.6	40.3	26.9	9.5	3.6
18	30.3	17.8	51.0	32.6	4.9	1.8
19	29.4	17.9	52.8	46.2	6.6	55.3

Source: All India Coordinated Research Project on Agrometeorology (AICRPAM) website
<http://www.cropweatheroutlook.in/>

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Qualification	Year	School/Board/University	Marks (%)	Division
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12 th	2016	Board of Intermediate Education, Andhra Pradesh	97.7	First
B.Sc. (Hons.) Agriculture	2020	Acharya N.G. Ranga Agricultural University, Guntur	81.3	First
M.Sc. (Ag.) Entomology	2022	CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur	79.5	First

Thesis Title in M.Sc.: Toxicity of novel insecticides to *Pieris brassicae* (Linnaeus) and their residue dynamics on cabbage

Fellow/Scholarship/Gold Medals/Any Other Distinction:

National Talent Scholarship during M.Sc.

Publications:

Jabez Raju B, Karthik S and Anil G. 2021. Programmed Cell Death: A Destructive and Constructive process in Insects. *Indian Entomologist* 2(2): 53-64

Jabez Raju B and Karthik S. 2021. Contributions of Dr. M.S. Mani to the Field of Entomology. *Indian Entomologist* 2(1): 116-118

Sai Reddy MS, Karthik S, Jabez Raju B and Yashaswini G. 2022. Multi-omics Approaches in Insect-Plant Interactions. **In:** Tanda AS (eds) *Molecular Advances in Insect Resistance of Field Crops*. Springer, Cham. P.335. https://doi.org/10.1007/978-3-030-92152-1_13