BIOEFFICACY AND RESIDUE DYNAMICS OF COMBINATION PRODUCT OF NOVALURON AND LAMBDA CYHALOTHRIN AGAINST INSECT PESTS OF TOMATO

Thesis

by

VINIT KUMAR
(H-2017-16-M)

submitted to

Dr YASHWANT SINGH PARMAR UNIVERSITY
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Dr. Sapna Katna
Scientist

Department of Entomology
Dr Yashwant Singh Parmar University
of Horticulture & Forestry (Nauni) Solan
(HP)-173 230 India

CERTIFICATE – I

This is to certify that the thesis titled **"Bioefficacy and residue dynamics of combination product of novaluron and lambda cyhalothrin against insect pests of tomato"** submitted in partial fulfilment of the requirements for the award of the degree of **MASTER OF SCIENCE (AGRICULTURE) ENTOMOLOGY** in the discipline of **PLANT PROTECTION** to Dr. Yashwant Singh Parmar University of Horticulture and Forestry, (Nauni) Solan (HP)-173 230 is a bonafide research work carried out by **Mr. VINIT KUMAR (H-2017-16-M)** son of Sh. Rajesh Kumar 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 investigation have been fully acknowledged.

Place: Nauni, Solan
Dated: / /

(Dr. Sapna Katna)
Chairman
Advisory Committee

CERTIFICATE – II

This is to certify that the thesis titled, **"Bioefficacy and residue dynamics of combination product of novaluron and lambda cyhalothrin against insect pests of tomato"** submitted by **Mr. Vinit Kumar (H-2017-16-M)** son of Shri Rajesh Kumar to Dr. Yashwant Singh Parmar University of Horticulture and Forestry, (Nauni) Solan (HP) – 173230, India in partial fulfilment of the requirements for the degree of **MASTER OF SCIENCE (AGRICULTURE) ENTOMOLOGY** in the discipline of **PLANT PROTECTION** has been approved by the Advisory Committee after an oral examination of the student in collaboration with an External Examiner.

Dr. Sapna Katna
Major Advisor

Dr. Pankaj Sood
External Examiner

Dr. S.R. Dhiman
Dean's Nominee

Advisory Committee

Dr. J.K. Dubey
(Principal Scientist)
Department of Entomology

Dr. H.R. Sharma
(Principal Scientist)
Department of Vegetable Science

Dr. Divender Gupta
(Professor and Head)
Department of Entomology

Dean
College of Horticulture

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Place Date / /

Name
Vinit Kumar

CONTENTS

LIST OF ABBERIVATION

%	:	Per cent
ha^{-1}	:	Per hectare
@	:	at the rate
<	:	Less than
>	:	More than
°C	:	Degree Celsius
CS	:	Capsule Suspension
µg	:	Microgram (10^{-6} g)
µm	:	Micrometer
µV	:	Microvolt
µL	:	Microlitre
a.i.	:	Active ingredient
ADI	:	Acceptable Daily Intake
b	:	Slope of regression equation
e.g.	:	For example
EC	:	Emulsifiable concentrate
EPA	:	Environmental Protection Agency
ECD	:	Electron capture detector
et al.	:	etalia (Co - workers)
Fig.	:	Figure
G/g	:	Gram
GC	:	Gas Chromatograph
h	:	Hour (s)
HPLC	:	High Performance Liquid Chromatography
H.P.	:	Himachal Pradesh
ha	:	Hectare
i.e.	:	id est (that is)
ID	:	Internal Diameter
kg	:	Kilogram
L	:	Litre
LOD	:	Limit of Determination
m	:	Meter
LD_{50}	:	Lethal Dose
LC_{50}	:	Lethal Concentration
LOQ	:	Limit of Quantification
m^2	:	Square meter
mg	:	Milligram

max	:	Maximum
min	:	Minimum
min	:	Minute
ml	:	Millilitre
mm	:	Millimeter
MRL	:	Maximum Residue Limit
N	:	North direction
nm	:	Nano meter
ND	:	Non detectable
ng	:	Nanogram
ND	:	Not Detected
Pa	:	Pascal
ppm	:	Parts per million
PRL	:	Pesticide Residue Laboratory
PSA	:	Primary Secondary Amines
R^2	:	Correlation coefficient
RBD	:	Randomized Block Design
RL_{50}	:	Residue half life
rpm	:	Rotations per minute
SD	:	Standard deviation
SC	:	Suspension concentrate
SL	:	Soluble concentrate
SL	:	Soluble liquid
SPE	:	Solid phase extraction
$t_{1/2}$	:	Residue half-life
X dose	:	Single Dose
USEPA	:	United States Environmental Protection Agency
v/v	:	Volume by Volume
viz.,	:	Namely
W	:	Watt
w/v	:	Portion by weight to volume
w/w	:	Portion by weight
X	:	Times or independent variable
Y	:	Dependent variable
ZC	:	Mix formulation of SC and CS

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Chapter-1

INTRODUCTION

Tomato *(Solanum lycopersicum* L.) is one of the most important and widely grown vegetable crop over the world. It is a self-pollinated crop belonging to family Solanaceae having chromosome number 2n =24 (Rick, 1969). It is economically attractive and the area under cultivation is increasing daily (Pattnaik *et al*., 2012). It ranks next to potato in world acreage and first among the processed vegetables (Chaudhary, 1996). It is used directly as raw vegetable and also in the form of various processed products like ketchup, puree, juice and whole canned fruits etc. (Bhagta, 2017).

Tomato contributes as a good source of nutrition to the consumer and also a very good source of income for small and marginal farmers (Singh *et al.,* 2010). Tomato holds a significant position based on nutritional view point as it contains essential nutrients including vitamin A, C and E providing approximately 20 mg of vitamin C per 100 grams (Wilcox *et al.,* 2003). Besides these nutrients it also contains *β*-carotene, lycopene pigments, water and niacin, which are essential for metabolism (Olaniyi *et al.,* 2010). Due to the presence of lycopene, flavonoids and antioxidant properties, tomato is universally treated as ‘Protective food’ (Sepat *et al*., 2013). Tomato and its products are also used as a preventive strategy against major lifestyle diseases, such as cancer and cardiovascular diseases (Canene-Adams *et al*., 2005).

This crop is native to Central and South America (Vavilov, 1951) and perhaps introduced in India by the Portuguese, though there is no definite record of its introduction. Worldwide production of tomatoes reached 183.9 million tonnes in 2016 over an area of 7.6 million hectares (FAO, 2018). China is the major producer of tomato followed by India, USA, Turkey and Egypt (FAO, 2017). India ranks second in area as well as in production of tomato with about 7.97 lakh ha area and production of 20.7 million tonnes (NHB, 2017). Madhya Pradesh is the leading state followed by Andhra Pradesh and Maharashtra in terms of area under tomato crop in India, however, in Himachal Pradesh, the annual production is 4.89 lakh tonnes from an area of 11080 ha (NHB, 2017).

Tomato is warm season annual plant that grows with the average optimum temperature range of 25°C to 29°C (Ejaz *et al.,* 2011). It is generally grown as winter crop (October-April) in plains of India, but in Himachal Pradesh it is cultivated as an off-season crop during

April to October. However, like other vegetables its successful and economic cultivation is consistently threatened by many production constraints. Tomato is more prone to insect pests and diseases mainly due to the tenderness and softness as compared to other crops. The tomato yield in India is considerably lower because of several factors of which the damage caused by insect pests is most important. It is devastated by an array of pests like jassids, aphids, tobacco caterpillar, flea beetles, leaf minors, spider mites, and fruit borers. However, the major economic damage is caused by the fruit borer (Sajjad *et al*., 2011).

Tomato fruit borer, *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae) causing yield losses up to 31.53 per cent (Singh *et al.*, 2017) is the most destructive insect pest resulting in considerable losses in quantity as well as quality of tomato fruits (Singh and Chahal, 1978; Tewari and Moorthy, 1984; Reddy and Zehram, 2004). It reduces the market value of fruit up to 50 to 80 per cent.

The tomato pin worm, *Tuta absoluta* Meyrick, (Lepidoptera : Gelechiidae) is a serious pest of both outdoor and greenhouse tomatoes. The insect deposits eggs usually on the underside of leaves, stems and to a lesser extent on fruits. After hatching, young larvae penetrate into tomato fruits, leaves on which they feed and develop creating mines and galleries. On leaves, larvae feed only on mesophyll leaving the epidermis intact (OEPP/EPPO, 2005). Tomato plants can be attacked at any developmental stage, from seedlings to mature stage.

To control the fruit borer and pin worrn, different pesticides are being used in large quantities by farmers except in few cases where the crop is grown as per Good Agricultural Practices (GAP) for export purposes.

The synthetic pesticides are the important tool in Integrated Pest Management, but their indiscriminate use causes severe ecological consequences like destruction of natural enemy fauna, effect on non-target organisms, secondary pest outbreaks. Further change in food habits and consumption of tomato as salad, makes food safety issues more pertinent. Hence, GAP has to be recommended so as to reduce the pesticide load in food and environment.

Considering the economic importance of pest and fruit, the present study is conducted to study the bioefficacy and residue dynamics of combination product of novaluron and lambda cyhalothrin on tomato so as to recommend the safe waiting periods based on the Maximum Residue Limits (MRLs) calculated and recommending risk mitigation protocols for food safety. The following objectives are formulated for the proposed study.

Objectives-

1. To study the bioefficacy of combination product of novaluron and lambda cyhalothrin against insect pests of tomato
2. To study the residue dynamics of combination product of novaluron and lambda cyhalothrin in tomato

Chapter-2

REVIEW OF LITERATURE

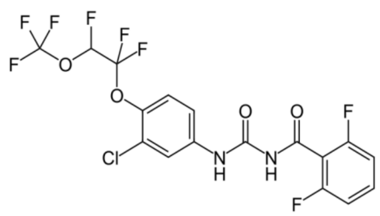
This chapter contains the relevant literature pertaining to the present study titled **“Bioefficacy and residue dynamics of combination product of novaluron and lambda cyhalothrin against insect pests of tomato”.** The available literature is reviewed under different headings as follows:

1. General information of novaluron
2. Bioefficacy of novaluron
3. Residue estimation of novaluron
4. Persistence of novaluron on crops
5. Persistence of novaluron in soil
6. General information of lambda cyhalothrin
7. Bioefficacy of lambda cyhalothrin
8. Residue estimation of lambda cyhalothrin
9. Persistence of lambda cyhalothrin on crops
10. Persistence of lambda cyhalothrin in soil

1. General information of novaluron

Novaluron belongs to a class of insecticides called insect growth regulators that slowly kill the insects over a few days by disrupting the normal growth and development of immature insects. Some compounds of this group are broad spectrum insecticides with an insect hormonal mimicking mode of action. These insect growth regulators affect chitin synthesis of immature insects disrupting their normal growth and development. Novaluron has low mammalian acute toxicity and has low risk to the environment and non target organisms (US EPA, 2002).

1.1 Chemical designation



Common name	Novaluron
Trade name	Rimon, Novaluron [ISO], Benzamide
IUPAC name	*N*-[[3-chloro-4-[1,1,2-trifluoro-2-(trifluoromethoxy)ethoxy]phenyl] carbamoyl]-2,6-difluorobenzamide
Molecular formula	$C_{17}H_9ClF_8N_2O_4$
Molecular weight	492.7 g/mol
Fomulation	Emulsified concentrate
Application type	Animal dip, spray
Solubility	Soluble in water 0.9531 mg/l at 25°C and in many organic solvents like acetone, dichloromethane, methanol, diethyl, ethyl acetate, hexane, toluene (Tomlin, 1997).

1.2 Toxicology

The acute toxicity through oral, dermal, and inhalation administrations were studied using rats. LD50 was determined as >5000 mg/kg body weight for oral dose acute toxicity to male and female rats, >2000 mg/mg body weight for dermal treatment acute toxicity to male and female rats, and >5150 mg/kg for inhalation acute toxicity to male and female rats (FAO 2004).

1.3 Uses

Novaluron is an insecticide which inhibits chitin synthesis, affecting the moulting stages of insect development. It acts by ingestion and contact and causes abnormal endocuticular deposition and abortive moulting. It is used in agriculture/horticulture on a wide range of crops including cotton, soya, maize, pome fruit, citrus, potato, tomato and vegetables against a wide range of pests. Novaluron is under evaluation by WHO Pesticide Evaluation Scheme as a mosquito larvicide (FAO 2004).

2. Bioefficacy of novaluron

Bioefficacy of emamectin benzoate 5 SG @ 8.9 and 11 g a.i. ha^{-1}, novaluron 10 EC @ 50, 75, and 100 g a.i. ha^{-1}, spinosad 45 EC @ 60 g a.i. ha^{-1} and profenofos 50 EC @ 750 g a.I. ha^{-1}, with conventional recommended insecticide, endosulfan 35 EC @ 350 g a.i.ha^{-1} were compared by Patil *et al*. (2007) against *Helicoverpa armigera* in chick pea and observed that minimum larval incidence of 1.68 larvae/m row length was recorded in novaluron 10 EC @

100 g/ha at 3DAS. In another study, seven different treatments along with untreated check were tested to assess the efficacy of new insecticides against *H. armigera* by Mahendra *et al*. (2011). Among them spinosad (0.006%) and indoxacarb (0.007%) were proved to be effective, which were followed by emamectin benzoate (0.001%), flubendiamide (0.004%) and novaluron (0.0075%) in reducing the larval population as compared to endosulfan (0.07%) and untreated check. Foliar application of novaluron at different doses @ 18.75, 37.5 and 75 g a.i. ha^{-1} was evaluated against *H. armigera* (Saini *et al.,* 2013). It was found that after 10 days of spray, novaluron at different doses was significantly superior to the standard check, quinalphos (525 g a.i. ha^{-1}) with respect to pod damage and grain yield. It was concluded that novaluron even at the lowest dose (18.75 g a.i. ha^{-1}) proved comparable/superior to the standard check, quinalphos and was significantly superior at higher dose (37.5 g a.i. ha^{-1}) in reducing larval population and pod damage, and thus increasing grain yield of chickpea. A combination product of novaluron 5.25 % + indoxacarb 4.5 % SC was also tested by Ghosal *et al.* (2015a) against pod borer of pigeon pea. They reported that the combination of novaluron + indoxacarb gave superlative effect over the sole insecticide novaluron & indoxacarb & standard check lambda cyhalothrin. Among the three selected dose of novaluron + indoxacarb (750, 825, 875 ml ha^{-1}) the said chemical @ 875 ml ha^{-1} was recorded as best in managing *H. armigera* population up to harvesting period (mean 0.03% infested pod of both years), while, @ 825 ml ha^{-1} also recorded remarkable effect on the target pest. Lal and Jat (2016) conducted experimental trials on the compatibility of urea 2 % with insecticides of different group viz., monocrotophos 36SL @ 500 ml ha^{-1}, cypermethrin 25EC @ 125 ml ha^{-1}, quinalphos 25EC @ 1000 ml ha^{-1} and novaluron 10EC @ 375 ml ha^{-1} against larval population of *H. armigera* in the field conditions on chickpea. These insecticides were found compatible with urea. The minimum larval population of *H. armigera* was observed in novaluron 10 EC at 3, 7 and 10 days after spraying. Monocrotophos, quinalphos and cypermethrin could not provide consistent results against *H. armigera*. Minimum per cent pod damage (7.3 %), maximum grain yield (14.6 q ha^{-1}) and monetary returns (6265 ha^{-1}) was realized from novaluron 10EC @ 375 ml ha^{-1} plus 2 per cent urea as compared to other treatments.

Kumar *et al.* (2003) evaluated bioefficacy of different insecticides viz. malathion (2 ml/l), carbaryl (2 ml/l), *Bacillus thuringlensis* (2 g/l), azadirachtin (3 ml/l), endosulfan (2 ml/l), diflubenzuron (4 g/l) and fenvalerate (0.5 ml/l) as alone and in different combinations with novaluron against diamondback moth larvae under laboratory conditions. Five days after treatment, novaluron alone @ 0.75 ml/l provided 90 per cent mortality of diamondback moth larvae while all the combinations at full doses provided highest (100 %) mortality; whereas novaluron + *B.t.k* (0.375 ml + 1 g/l) also gave 100 per cent mortality which is a half doses combination. However, all the remaining combinations at half doses provided more than 70 per cent mortality of larvae. The findings of Srinivasan (2008) revealed that spraying of spinosad (0.045%) and indoxacarb (0.015%) attributed to higher yield and lesser larval incidence when compared to other treatments i.e. moncrotophos, lambda cyhalothrin, triazophos, profenophos, indoxacarb, bifenthrin, spinosad, endosulfan, novaluron, *Bt* and NSKE against spotted pod borer in short duration pigeonpea. Among biorationals, novaluron (0.01%) provided good protectton and registered significantly lesser incidence of spotted pod borer *M. vitrata* larvae and higher yield. Sharma and Jakhar (2010) studied the bioefficacy of insecticides emamectin benzoate (0.002 %), endosulfan (0.05 %), novaluron (0.01 %), lambda cyhalothrin (0.004 %), and spinosad (0.0024 %) against brinjal shoot and fruit borer, *Leucinodes orbonalis*. The results showed that in terms of shoot infestation, emamectin benzoate (0.002 %), endosulfan (0.05 %), novaluron (0.01 %) and lambda cyhalothrin (0.004 %) were found superior. The total number of drooping shoots was minimum (4.17) in emamectin benzoate followed by endosulfan (6.83) and novaluron (7.00), as compared to spinosad (9.17), deltamethrin (11.67) and *Bacillus thuringiensis* (13.17).

The bioefficacy of some newer insecticides against seed borer, *Trymalitis margarias* (Meyrick) was tested during three consecutive season of 2012-13, 2013-14 and 2014-15 on sapota by Bisane *et al*. (2017). The result revealed that profenofos 50 EC 0.075 % minimized fruit damage up to 4.03 per cent while novaluron 10 EC 0.005 % recorded 4.83 per cent fruit infestation put forward superiority over standard check acephate 75 SP 0.1125 % (9.86 %) and other pesticides. However on economics basis, the higher B: C ratio was recorded in novaluron 0.005 % (3.28), profenofos 0.075 % (3.14) and indoxacarb 0.0036% (2.46).

The results of study conducted by Cutler *et al*. (2007) suggest that novaluron could be a valuable tool in future for Colorado potato beetle *Leptinotarsa decemlineata* management programs. They conducted field trials in 2003 and 2004 to assess the effectiveness of novaluron (Rimon 10 EC), a benzoylphenyl urea chitin synthesis inhibitor, for management of Colorado potato beetle on potato. Foliar applications of novaluron did not significantly reduce numbers of *L. decemlineata* adults, egg masses, or first instar larvae, but second-fourth instars were greatly suppressed. At 50 g a.i ha^{-1}, novaluron provided excellent, prolonged protection, whether applied twice a season, or once in alternation with imidacloprid. In another study conducted by Alyokhin *et al*. (2008) on reduced viability of Colorado potato beetle, *Leptinotarsa decemlineata*, eggs exposed to novaluron, revealed a negative effect of novaluron on the number of progeny produced by the Colorado potato beetle. Direct toxicity did not explain all of the reduction in egg hatch observed, suggesting that novaluron probably acted on reproductive adults as well as on eggs after they were deposited.

Srimohanapriya *et al.* (2010) studied bioefficacy of insecticides against the rice leaf mite under laboratory and field conditions. Among the acaricides, methyl demeton 25 EC at 1000 ml/ha and novaluron 10 EC at 1000 ml/ha were the best treatments in reducing the population of mite and were equally effective at 1, 3, 7 and 14 days after treatment, followed by milbemectin under field conditions.

The seven novel chemical and biological insecticides [flubendiamide 39.35 SC, chlorantraniliprole 18.5 SC, emamectin benzoate 5 SG, novaluron 10 EC, indoxacarb 15.8 EC and two *Bacillus thuringiensis* var. *kurstaki* (Btk) formulations] were evaluated for their safety to *Trichogramma chilonis* by Duraimurugan and Lakshminarayana (2018). Based on the criteria suggested by IOBC (International Organisation for Biological Control) to assess the toxicity of insecticides to natural enemies, *Bt k* formulations, flubendiamide, chlorantraniliprole, indoxacarb and novaluron had lesser effect on *Trichogramma chilonis* and were categorized as harmless (<30 % mortality).

3. Residue estimation of novaluron

Dissipation and residual levels of novaluron in tomato under open field conditions were investigated by Malhat *et al*. (2014) using high performance liquid chromatography equipped with diode array detector (HPLC-DAD) with quick, easy, cheap, effective, rugged (QuEChERS) method. The method was validated using blank samples spiked at three levels and results showed that recoveries ranged from 93 to 99 per cent. Similarly, Shi *et al*. (2016) investigated chronic and acute risk assessments of the novaluron and bifenthrin based on the dissipation and residual level in cabbage determined by gas chromatography coupled with an electron capture detector (GC-ECD). At different spiked levels, mean recoveries were between 81 and 108 per cent with relative standard deviations (RSDs) from 1.1 to 6.8 per cent. The limit of quantification (LOQ) was 0.01 mg kg^{-1}, and good linearity with correlation coefficient (>0.9997) were obtained.

An analytical method combining off-line flow through extraction of a soil micro-sample and direct on column large volume injection (LVI up to 1.00 ml) of a methanol–water soil extract onto a conventional C18 RP HPLC column enabled fast (within 3.5 minutes) trace microanalysis of the relatively new chiral pesticides epoxiconazole (E) and novaluron (N), respectively. Linear calibration curves were evaluated from UV detection (230 nm) data in the range from 0.1 to 5 mg/kg in three most abundant Slovak agricultural soils. LOD (confidence band) at the levels 0.08 - 0.11 mg/kg and LOQ 0.4 - 0.6 mg/kg and LOD (S/N = 3) at the levels 0.007 - 0.018 mg/kg and LOQ (S/N = 10) 0.024 - 0.060 mg/kg, respectively, of dry soil were achieved. Recovery of pesticides in the overall LVI method including flow through 130 - 200 mg soil microsample extraction was: for epoxiconazole from 74 to 85 per cent and from 56 to 90 per cent for novaluron with reproducibility within ± 6 per cent RSD (Rybar *et al.*, 2007).

4. Persistence of novaluron on crop

The novaluron was rapidly dissipated in chilli and brinjal following first order reaction kinetics at all rates of application evaluated by Das *et al*. (2007) with half lives of 1.80 - 1.95 days (for chilli) and 1.80 -2.08 days (for brinjal) and the residue was found to be below detectable limit on 10th day samples. The study revealed that novaluron will not pose any problem of residual toxicity. Similarly, Malhat *et al*. (2014) found that novaluron residues tend to dissipate in tomato following first-order rate kinetics with half-life of 2.08 days. Data demonstrated that the use of novaluron at recommended doses would not pose any hazards to consumer. Recovery of novaluron done by Saini *et al.* (2013) in plant and soil samples of chickpea ranged from 82.22 to 90.64 per cent.

Ahlawat *et al*. (2017) conducted field experiment to study degradation of novaluron in chickpea at different doses (37.5, 75 and 150 g a.i. per ha) at pod formation stage. The average initial deposits of novaluron in green pods were found to be 0.642, 1.705, 2.251 mg per kg at the doses of 37.5, 75 and 150 g a.i. per ha, respectively. In case of green pods 91 per cent dissipation was observed within 5 days after application. Harvest time grains were found to be BDL (Below determination level). Similarly, Bisane *et al*. (2017) carried out residue study of profenofos and novaluron on sapota. The residue was not detected at harvest of fruits 10 days after spraying of profenofos 0.075 % and novaluron 0.005 %.

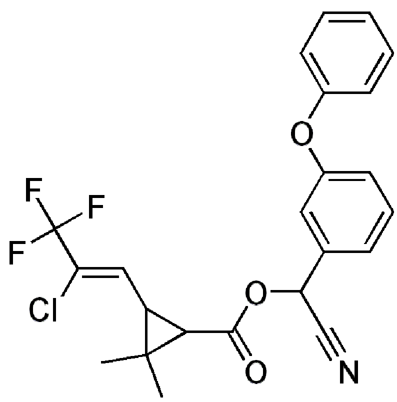
5. Persistence of novaluron in soil

In a study conducted by Fine *et al.* (2017) the residues of novaluron were found below the limit of detection in the apple field soil. The half life of novaluron in non sterilized soils evaluated by Das *et al.* (2008) ranged from 17.0 - 17.8 days (alluvial soil) 11.4-12.7 days (costal saline soil), while the values in sterilized soil were 53.7- 59.0 days (alluvial soil) and 28.9- 28.8 days (costal saline soil), respectively. Ahlawat *et al*. (2017) studied degradation of novaluron in chickpea cropped soil at different doses (37.5, 75 and 150 g a.i. per ha) at pod formation stage. Results of experiment revealed that the average initial deposits of novaluron in soil were found to be 0.144, 0.186, 0.223 mg per kg @ 37.5, 75 and 150 g a.i. per ha, respectively. In case of soil 77 per cent dissipation was observed within 5 days after application. At harvest time soil was found to be BDL.

6. General information of lambda cyhalothrin

Lambda cyhalothrin is a mixture of isomers of cyhalothrin. Cyhalothrin is an organic compound that is used as a pesticide. it is a pyrethroid that consists of a racemic mixture of two or more of the four active isomers of cyhalothrin (Moretto, 1991). According to He *et al*. (2008), lambda cyhalothrin is a class of synthetic insecticides that mimic the structure and insecticidal properties of the naturally occurring pyrethrin pesticide which comes from the flowers of chrysanthemums. Pyrethroids such as cyhalothrin are often preferred as an active ingredient in insecticides because they remain effective for longer periods of time than pyrethrin. It is a colourless solid, although samples can appear beige, with a mild odour. It has low water solubility and is non volatile. It has similar biochemical effects to that of natural pyrethrin.

6.1 Chemical designation



Common name	Lambda cyhalothrin
Trade name	Karate, Warrior, Passat, lambda-cyhalothrin, Cyhalothrin-k
IUPAC name	Cyano-(3-phenoxyphenyl)methyl] (1R,3R)-3-[(Z)-2-chloro-3,3,3- trifluoroprop-1-enyl]-2,2-dimethylcyclopropane-1-Carboxylate
Molecular formula	$C_{23}H_{19}ClF_3NO_3$
Molecular weight	449.9 g/mol
Fomulation	Capsule suspension (CS), EC, WP
Application type	Animal dip, spray
Solubility	Soluble in water 0.004 ppb at 20^{o}C and in many organic solvents acetone, dichloromethane, methanol, diethyl, ethyl acetate, hexane, toluene (Tomlin, 1994).

6.2 Toxicology

The acute oral LD_{50} for male rats is 166 mg/kg, for female rats is 114 mg/kg, for rabbits >1000 mg/kg (Tomlin, 1994). It is very toxic particularly to various species of fish and other aquatic organisms (He *et al*., 2008). Acute human exposure leads to irritation, burning, vomiting, headache, anesthesia (Sharda, 2013).

6.3 Uses

It is used for the control of several insects at home and agricultural field like aphids, beetles, butterfly larvae, cockroaches, mosquitoes, ticks and flies (Mergel, 2010). It is used in control of animal ectoparasites, especially *Boophilus microplus* or *Haematobia irritans* on cattle and *Bovicola ovis*, *Melophagus ovinus* on sheep (Tomlin, 1994).

7. Bioefficacy of lambda cyhalothrin

Ulaganathan and Gupta (2004) reported that six rounds of spray with newer insecticides acetamiprid 20 SP at 40 g a.i.ha^{-1}, imidacloprid 20 SL at 100 g a.i. ha^{-1}, betacyfluthrin 2.5 SL at 18 g a.i. ha^{-1}, spinosad 2.5 EC at 50 g a.i. ha^{-1}, bifenthrin 10 EC at 60 g a.i. ha^{-1} and lambda cyhalothrin 5 EC at 15 g a.i. ha^{-1} were promising in reducing the bollworms incidence and increasing the cotton yield. Similarly, Ghure *et al*. (2008) evaluated the bioefficacy of lambda cyhalothrin 5 EC, spinosad 45 SC, indoxacarb 14.5 SC, profenofos

50 EC, Dipel 8 L (*Bt* formulation) along with endosulfan 35 EC and NSKE 5% against bollworms of cotton. Lambda cyhalothrin 5 EC at 100 g a.i. ha^{-1} was found to be the most effective against cotton bollworms and was on par with indoxacarb 14.5 SC @ 75 g a.i. ha^{-1}, spinosad 45 SC @ 75 g a.i. ha^{-1} and profenofos 50 EC @ 1000 g a.i. ha^{-1}.

Duraimurugan *et al*. (2007) evaluated the efficacy of emamectin 5 SG against the cotton bollworm, *H. armigera* on cotton and indicated that formulation of emamectin @ 11 g a.i. ha^{-1} was effective than other insecticides like profenofos 50 EC @ 750 g a.i. ha^{-1}, lambda cyhalothrin 5 EC @ 15 g a.i. ha^{-1}, indoxacarb 14.5 SC @ 100 g a.i. ha^{-1} and spinosad 45 SC @ 60 g a.i. ha^{-1} in reducing the larval population and increasing the yield. In another study, Dhaka *et al*. (2011) evaluated the efficacy of different insecticides i.e. lambda cyhalothrin 5 EC at 500 ml ha^{-1}, carbosulfan 25 EC at 1000 ml ha^{-1}, indoxacarb 14.5 SC at 500 ml ha^{-1}, bifenthrin 20 EC at 500 ml ha^{-1}, novaluron 10 EC at 750 ml ha^{-1}, flubendiamide 39.35 EC at 75 ml ha^{-1}, spinosad 45 SC at 500 ml ha^{-1} and endosulfan 35 EC at 1250 ml ha^{-1} biopesticide viz., *Bt* at 1.5 kg ha^{-1} and botanical viz., neemarin 1500 ppm at 2500 ml ha^{-1}, on vegetable pea variety arkel against pod borer, *Etiella zinckenella* (Treitschke) and found that flubendiamide was the best with lowest pod and seed infestation of 11.37 and 12.98 per cent, respectively and maximum yield of 95.84 q ha^{-1} followed by indoxacarb, spinosad, novaluron, carbosulfan, bifenthrin, lambda cyhalothrin, endosulfan, neemarin and *Bt*, which gave 93.56, 91.63, 89.74, 83.22, 81.52, 79.42, 75.97, 72.78 and 68.99 q ha^{-1} yield, respectively.

Hammond (1996) conducted field experiment on residual activity of lambda cyhalothrin against bean leaf beetle in soyabean and reported that all weekly applications of lambda cyhalothrin provided significantly greater control of bean leaf beetles in terms of pod feeding until plant maturity in late September. When a large 2nd generation of bean leaf beetles is anticipated, a single application of lambda cyhalothrin provided long term control if applied at the beginning of the emergence of the 2nd generation beetles. Similarly another comparative study conducted by Oladimeji and Kannike (2010) on efficacy of neem (*Azadirachta indica* A. Juss), basil (*Ocimum basilicum* L.) leaf extracts and lambda cyhalothrin at three levels of concentration against flea beetle, *Podagrica spp*. (Jac.) in okra wherein the leaf extracts were applied at 5, 10 and 20 ml per litre while lambda cyhalothrin was applied at 2.5, 3.75 and 5.0 ml per litre and distilled water only served as control. The treated okra plants performed significantly better ($P < 0.05$) than the control. Percentage reduction in leaf damage ranged between 21 - 43 per cent in *O. basilicum*, 50 - 54 per cent in

Azadirachta indica and 72 - 81 per cent in lambda cyhalothrin. There was significant linear negative relationship between dry pod yield and surface area of leaf damaged by the insect (0.001 < P > 0.01).

8. Residue estimation of lambda cyhalothrin

The residues of lambda cyhalothrin (15 and 30 g a.i./ha) were extracted by Singh *et al.* (2007) from okra fruit samples macerated with acetone in a Warring blender. The extract was concentrated and partitioned with hexane (50, 25, 25 ml) by diluting with aqueous solution of sodium chloride. The organic phases were collected and concentrated. The hexane extract was cleaned by passing through a glass column packed with florisil and neutral alumina (5 g each) sandwiched between two layers of anhydrous sodium sulphate. The residues of lambda cyhalothrin were analyzed on a Chemito 1000 GLC which was equipped with Ni_{63} and a glass column packed with 3 per cent OV-101 on 80-100 mesh Chromosorb (WHP). In another study, Pal and Shah (2008) applied 2.0 ml of 5 EC lambda cyhalothrin on 4 kg grains and extracted its residues from wheat using the mixture of acetone:water (65:35) with vertical blender cum homogenizer at high speed. The extract was filtered, concentrated and set for liquid-liquid partitioning using hexane : methylene chloride (1:1). The organic phase was collected and concentrated. The quantitative estimation of concentrated sample was carried out using a GLC GC-1000 (Chemito) and Trace GC- ultra (Thermo Finnigan), both equipped with ECD Ni_{63} and micro- bore capillary column. For GC-1000 BP-1 SGE fused silica capillary (25 m x 0.22 mm x 0.25 mm) column was used.

The dissipation dynamics and residue amounts of lambda-cyhalothrin, thiamethoxam and clothianidin in apple were investigated by using rapid resolution liquid chromatography triple quadrupole mass spectrometer (RRLC-MS/MS) and gas chromatography mass spectrometry (GC-MS). The developed method performed satisfactory recoveries of 88 – 105% and the limit of quantitation (LOQ) was 0.01 mg kg^{-1} (Fan *et al.*, 2019).

Malhat *et al*. (2016) developed a validated analytical method for the detection and quantification of lambda cyhalothrin in tomato using gas chromatography electron capture detector (GC-ECD). The residues were successfully extracted from tomato samples using the QuEChERS method with slight modification.

9. Persistence of lambda cyhalothrin on crop:

Mathirajan *et al.* (2000) counducted a field trial to study the dissipation pattern of lambda cyhalothrin 5EC in chillies at three application rates i.e. 7.5, 15 and 30 g a.i./ha and observed deposition of initial residues ranging from 1.44 to 2.20 ppm when sprayed at 7.5 g a.i./ha. The medium (15 g a.i./ha) and highest (30 g a.i./ha) doses left initial residues ranging from 2.84 to 5.83, and from 5.48 to 7.15 ppm, respectively. The half-lives of residues were in the ranges 0.77-0.94, 0.81-0.97 and 0.82-0.99 for 7.5, 15 and 30 g a.i./ha, respectively. The waiting periods on fruits were 2.51 to 3.41 days at the lower dose and 4.19 to 4.89 days at the highest dose. Similarly, Sharma and Awasthi (2002) conducted field trial to study persistence of lambda cyhalothrin residues on cauliflower and found that the initial deposits of lambda cyhalothrin were ranging from 0.81 to 1.59 mg/ kg which dissipated quickly to reach below detectable limit by 10-15 days and half life of lambda cyhalothrin in cauliflower ranged between 2.0 to 2.4 days. In another study, Singh and Singh (2003) found that the application of lambda cyhalothrin at the rate of 25 and 50 g a.i./ ha on chickpea resulted in initial residues of 0.335 and 0.462 mg/ kg which was degraded with half life values of 4.9 and 5.0 days, respectively. Studies conducted by Jayakrishnan *et al*. (2005) revealed that lambda cyhalothrin when applied at the application rate of 15 g a.i./ha and 30 g a.i./ha on tomato fruits resulted in intial deposit of 0.38 mg/kg and 0.52 mg/kg which was degraded with half life values of 3.0 and 3.7 days, respectively.

Pesticide residues determination study conducted by Khan *et al*. (2011) on selected summer vegetables revealed that the residual level of cypermethrin was highest (16.2 mg/kg) in edible portion of bitter gourd, while lambda cyhalothrin and mancozeb residues were detected high (4.50 mg/kg, 6.26 mg/kg) in edible portion of bitter gourd and cucumber respectively. Cypermethrin residues were high (1.86 mg/kg) in okra leaves, while mancozeb and lambda cyhalothrin residual level was high (1.23 mg/kg, and 0.0002 mg/kg) in chili and tomato leaves. Similarly, Salghi *et al*. (2012) analyzed 8 pesticide residues in tomato samples and the residue levels for lambda cyhalothrin ranged from 0.001 to 0.010 mg/kg. In another study, Fan *et al*. (2013) examined the residues of four insecticides namely metalaxyl, fluazifop-P-butyl, chlorpyrifos, and lambda-cyhalothrin on six leafy vegetables. The limits of quantification (LOQ) of metalaxyl, fluazifop-P-butyl, chlorpyrifos, and lambda cyhalothrin were in the range of 0.001-0.01 mg kg^{-1} for all samples, and the average recoveries of all pesticides ranged from 67.6 to 119.1% at spiked levels of 0.01- 0.1 mg kg^{-1}. In supervised field trials, the half-lives of lambda cyhalothrin were in the range of 1.77-6.24 days. Safe waiting period reported by Bouri *et al*. (2012) for bifenthrin in green bean was 4 days in the winter and 3.5 days in the spring, whereas for lambda cyhalothrin it was 8 days in winter and 7.5 days in the spring.

Lofty *et al*. (2013) analysed the dissipation pattern of lambda cyhalothrin and malathion in zucchini. The insecticide incorporated into the plants decreased rapidly with a half-life time around 0.77 day for malathion and 4 days for lambda cyhalothrin. For lambda cyhalothrin, the preharvest interval is 5 days. Initial deposits of lambda cyhalothrin in fruits of pomegranate were 0.120 and 0.170 mg/kg which was degraded with half life values of 2.59 and 3.11 days after the application rate of 12.5 and 25 g a.i./ha, respectively (Kadam *et al.,* 2015). Similarly, Reddy *et al*. (2017) found that the application of lambda cyhalothrin @ 15.63 g a.i./ha on chilli resulted in initial residues of 0.78 mg/kg with a waiting period of 11.16 days.

Vemuri *et al*. (2017) evaluated the dissipation pattern of lambda cyhalothrin in open field and polyhouse conditions in capsicum fruits at the application rate of 15 g a.i./ha, and found that the initial deposit of 0.16 mg/kg and 0.37 mg/kg dissipated to 0.06 mg/kg and 0.05 mg/kg in 5 and 7 days of application.

10. Persistence of lambda cyhalothrin in soil

Hill and Inaba (1991) conducted a research and found that the more ideal soil temperature conditions within the crop canopy increased the microbial degradation of lambda cyhalothrin. One year after application, only 3.2% of the initial residues were recovered in the fallow area. A combination product of thiamethoxam (12.6%) and lambda cyhalothrin (9.4 %) (Alika 247 ZC) @ 33 g a.i. /ha and 66 g a.i. /ha, applied on paddy yielded no residues in the soil samples after harvest (Barik *et al*., 2010). The half life of 6.69 days in soil as well on tea was recorded by Chen *et al.* (2010) with the application of lambda cyhalothrin 2.5 ME.

Chauhan (2011) reported that the residues of lambda cyhalothrin when applied @ 15 and 30 g a.i./ha on tomato crop, dissipated completely in soil on 3^{rd} and 5^{th} day, respectively. Similarly, Chauhan *et al*. (2012) applied lambda cyhalothrin @ 15 g a.i./ha and 30g a.i./ ha at fruiting stage of tomato where residues reached below detectable level of 0.005 mg/kg on 3^{rd} and 7^{th} day at single and double dose, respectively in tomato cropped soil sample. The persistence and dissipation of fipronil (75 g a.i./ha.) and lambda cyhalothrin (30 g a.i./ ha.) on chili as well as on soil evaluated by Reddy and Reddy (2013) revealed no residues in soil after 15 days of spray.

Chapter-3

MATERIALS AND METHODS

The present investigation entitled **"Bioefficacy and residue dynamics of combination product of novaluron and lambda cyhalothrin against insect pests of tomato"** was carried out in the Department of Entomology, Dr. Y. S. Parmar University of Horticulture and Forestry, Nauni, Solan (H.P.) during the year 2018. The supervised field trials were conducted at the experimental farm of the department following all good agricultural practices as per standard package of practices of the university (Anonymous, 2014). The bioefficacy of insecticides on insect pests of crop were observed and the residues of respective insecticides were estimated in the Pesticide Residue Laboratory (PRL) of the department. The experimental details related to study sites, materials used, methodology adopted for the studies and observations recorded are presented in the chapter under different heads.

3.1 Location

The experimental site is located at Nauni, district Solan, Himachal Pradesh, at an elevation of about 1200 meters above mean sea level, lying between 30°51'23" N latitude and 77°10'7" E longitude. Agro-climatically, the location falls under zone-II mid-hills. The climate is sub-temperate, having annual rainfall between 1100-1300 mm, most of which occurs during monsoon (June - August).

3.2 Crop raising

The seeds of tomato *Solanum lycopersicum*, variety NS 108 were purchased from the local market. The seeds were sown in well prepared raised nursery bed. The nursery bed was regularly irrigated till the time of transplanting. One month old seedlings were transplanted on 21^{st} April, 2018 in the plots/beds of size 3 × 2 m at a distance of 90 × 30 cm as per standard packages of practices (Anonymous, 2014) to maintain optimum plant population in the field. Before transplanting, field was ploughed and sufficient quantity of well decomposed FYM and fertilizers were added into the soil. The experiment was laid out in Randomized Block Design (RBD) with 7 treatments including untreated control replicated thrice (Plate 3c).

3.3 Climatic conditions:

Table 3.1 Climatic parameters during experimental period of tomato crop

Average Maximum temperature (^{o}C)	26.80
Average Minimum temperature (^{o}C)	20.10
Average Relative Humidity (%)	80.00
Rainfall (mm)	332.00

Source: Meteorological Observatory, Department of Environmental Science, Dr. Y.S. Parmar University of Horticulture and Forestry, Nauni, Solan (H.P.) 173 230

3.4 Treatments

Insecticides presented in Table 3.2 were studied for their bioefficacy and residue study.

Table 3.2 Detail of different insecticides used

Sr. No.	Treatments	Insecticides		Dosage (g a.i./ha)	Source Company
		Common Name	Trade Name		
1.	T_0	Untreated Control	-	-	-
2.	T_1	Novaluron + lambda cyhalothrin(9.45+1.9%) ZC	GPI 1316	47+10	UPL Pvt. Limited, Mumbai (India)
3.	T_2	Novaluron + lambda cyhalothrin(9.45+1.9%) ZC	GPI 1316	71+14	
4.	T_3	Novaluron + lambda cyhalothrin(9.45+1.9%) ZC	GPI 1316	142+28	
5.	T_4	Novaluron 10 EC	Rimon	75	Indofil Agricultural Chemicals
6.	T_5	Lambda cyhalothrin 4.9% CS	Passat	14.7	Shanro Agritech Private Limited
7.	T_6	Chlorantraniliprole 18.5% SC	Coragen	28	DuPont India Private Limited

All the treatments (T_0 to T_6) were studied for bioefficacy against tomato pests whereas treatments T_0, T_2 and T_3 were evaluated for persistence studies. The insecticides were sprayed on tomato crop at the time of pest appearance with each insecticide dose sprayed twice at an interval of 10 days. The first spray was done on 2nd July, 2018 and second on 12th July, 2018. Control plots were sprayed with water only. Insecticides were sprayed with a knapsack sprayer fitted with a triple action nozzle. Tomato plants were thoroughly

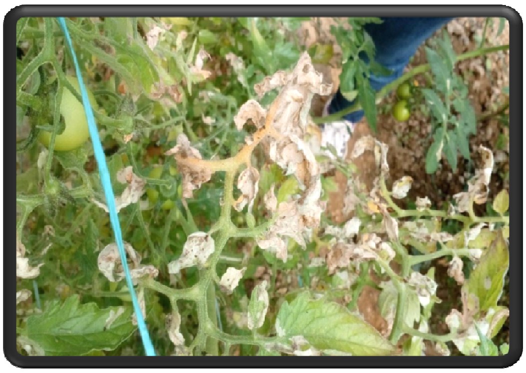


Plate 3a.*Tuta absoluta* infesting tomato fruits and leaves

Plate 3b. *Helicoverpa armigera* infesting tomato fruit

Plate 3c. View of experimental field trial

covered with spray fluid to run-off stage. Spray was done during a clear day, when there was minimum wind and all necessary precautions were taken to avoid the chances of drifting of spray fluid to adjacent plots. Care was taken that lower concentration was sprayed first, followed by higher concentration. The sprayer and measuring cylinder were washed thoroughly after each spray in order to avoid the carryover of insecticide from one treatment to another.

3.5 Bioefficacy studies

The observations on pest incidence and their number were recorded one day before spraying as pre-count. Post treatment count was taken at first, third, fifth, seventh and tenth days after each spraying for fruit borer (*Helicoverpa armigera)* and pin worm (*Tuta absoluta*). The field population data were subjected to statistical analysis.

3.5.1 Fruit borer (*Helicoverpa armigera)***:** For recording the pest population counts, ten plants were selected randomly in each plot. The data on mortality was recorded, based on the dead larvae of 3rd, 4th and 5th instars. All the counts were taken during morning hours. The data on fruit damage were taken on tenth day from each treatment after each spray by counting number of healthy and damaged fruits (Plate 3b).

3.5.2 Pin worm (*Tuta absoluta*)**:** The population of pin worm was recorded on ten randomly selected plants per plot. The population counts were recorded by randomly selecting two leaves from top, middle and bottom in each of the ten selected plants in every plot and mean number of pin worm larvae were calculated (Plate 3a).

3.5.3 Statistical analysis:

3.5.3.1 Fruit borer: The per cent reduction of larval population in all the treatments over control was calculated by using Henderson-Tilton's formula (Henderson and Tilton, 1955) as under:

$$\text{Per cent reduction in population} = 100\left(1 - \frac{Ta}{Tb} \times \frac{Cb}{Ca}\right)$$

Where, Ta = Number of insects after treatment

Tb = Number of insects before treatment

Ca = Number of insects in untreated check after treatment

Cb = Number of insects in untreated check before treatment.

Post treatment counts were made after 1, 3, 5, 7, and 10 days after insecticidal application. The per cent reduction in population was calculated at the different days interval. The data obtained were assigned arc sine transformations, analysed statistically by applying RBD as suggested by Gomez and Gomez (1984).

Total and damaged fruits were also recorded at 10 days after each spray. The damage percentage was calculated by using the following formula (Guru and Patil, 2018).

$$\text{Per cent fruit damage} = \frac{\text{Number of fruits damaged}}{\text{Total number of fruits}} \text{X } 100$$

3.5.3.2 Pin worm: The larval count was recorded from 10 randomly selected plants per replication. Percent larval reduction in all the treatments over control was calculated using Henderson-Tilton's formula as mentioned in the section 3.5.3.1. The data pertaining to pin worm incidence was analysed with arc sine transformations by applying RBD as suggested by Gomez and Gomez (1984). The fruit damage was calculated by using the above formula mentioned in the section 3.5.3.1 by Guru and Patil (2018). The overall effects of the treatments by combining these observations were also assessed by analyzing the data through ANOVA.

3.6 Phyto-toxicity Study: Phyto-toxicity was observed on tomato plants due to the application of treatment T_2 (X dose) and T_3 (2X dose) for yellowing, stunting, necrosis, chlorosis, vein clearing, epinasty and hyponasty at an interval of 1, 3, 5, 7 and 10 days as per the rating scale (0-10) as mentioned in Table 3.3.

Table 3.3: Phyto-toxicity Rating Scale (PRS):

Crop response/ Crop injury	Rating
0-00	0
1-10%	1
11-20%	2
21-30%	3
31-40%	4
41-50%	5
51-60%	6
61-70%	7
71-80%	8
81-90%	9
91-100%	10

3.7 Effect of insecticidal treatments on coccinellid beetles

The presence of coccinellids beetles was observed on ten plants in each replication in all the treatments at an interval of 1, 3, 5, 7 and 10 days after each spray.

3.8 Residue study

3.8.1 Laboratory materials

The following chemicals (analytical grade), glassware, instruments and gases were used to carry out the study:

3.8.1.1 Chemicals

1. Acetone (C_3H_6O), Genetix Biotech Asia Pvt. Ltd. Nazafgarh Road, NewDelhi
2. Acetonitrile (CH_3CN), Genetix Biotech Asia Pvt. Ltd. Nazafgarh Road, NewDelhi
3. Magnesium Sulphate ($MgSO_4$), Merck Specialities Pvt. Ltd. Worli, Mumbai
4. n-hexane (C_6H_{14}), HPLC Grade, Genetix Biotech Asia Pvt. Ltd. NazafgarhRoad, New Delhi
5. Potassium dichromate ($K_2Cr_2O_7$), Merck Specialities Pvt. Ltd. Worli, Mumbai
6. Primary Secondary Amines (PSA), Agilent Technology, USA
7. Reference standards, UPL Pvt. Limited, Mumbai (India)
8. Sodium Chloride (NaCl), Merck Specialities Pvt. Ltd. Worli, Mumbai
9. Sodium Sulphate (Na_2SO_4), Merck Specialties Pvt. Ltd. Worli, Mumbai
10. Sulphuric acid (H_2SO_4), Merck Specialties Pvt. Ltd. Worli, Mumbai
11. Teepol, Merck Specialities Pvt. Ltd. Worli, Mumbai

3.8.1.2 Glassware and plastic wares

1. Beakers of 50 and 100 ml capacity, Borosil Glassworks Ltd., Delhi
2. Capillary glass column, DB – 5, Agilent Technologies, USA
3. Graduated test tubes with stoppers of 5 ml capacity, Borosil Glassworks Ltd. Delhi
4. Injection vials (1.5 ml), Agilent Technologies, USA
5. Ivory PTFE / red silicone rubber septa, Agilent Technologies, USA
6. Plastic stands for holding tubes, Tarson Products Pvt. Ltd., Kolkata
7. Polypropylene centrifuge tubes (50 and 15 ml), Tarson Products Pvt. Ltd. Kolkata
8. Tips of 1 and 5 ml capacity, Tarson Products Pvt. Ltd., Kolkata
9. Turbo tubes of 30 ml capacity, Borosil Glassworks Ltd., Delhi

3.8.1.3 Instruments

1. Auto Pipettes (1 and 5 ml): Tarson Products Pvt. Ltd., Subhash Road, Kolkata
2. Centrifuge: Eppendorf India Ltd., Chandigarh
3. Electronic balance: Mettler Toledo India Pvt. Ltd., Mumbai
4. Gas chromatograph (GC) with ECD: Shimadzu corporation, Japan
5. Low volume high speed homogenizer: Heidolph, Germany
6. High volume homogenizer: Heidolph, Germany
7. Phillips mixer grinder: Phillips India Ltd., Nalagarh, Baddi, HP.
8. Refrigerator: Godrej India Ltd., Mumbai
9. Rotospin mixer: Tarson Products Pvt. Ltd., Subhash Road, Kolkata
10. Spinex test tube shaker: Tarson Products Pvt. Ltd., Subhash Road, Kolkata
11. Turboevaporator: Turbo Vap® LV, Caliper Life Service
12. Zero air Generator, PCI Analytics Pvt. Ltd. Mumbai
13. Sonicator: PCI India Pvt. Ltd. Biwandi, Mumbai

3.8.1.4 Gases

Nitrogen, Hydrogen, Helium and Zero air 99.99% purity: M/s Linde India Ltd., Mumbai.

3.9 Cleaning of Glasswares

Cleaning of glassware was done according to EPA (1980) procedure to eliminate any kind of contamination. The glassware was soaked in hot water followed by rinsing with organic solvent in order to remove the contamination. The deep penetrant and oxidizing agent, chromic acid 20 per cent [potassium dichromate (20 g) in sulphuric acid (100 ml) w/v] was used to remove traces of organic contaminants. The glassware was dipped in chromic acid for 4-5 hours and thereafter the same was washed under tap water to remove the chromic acid. The glassware was further washed with soap solution and tap water, rinsed with distilled water and finally with acetone to flush out the traces of organic contaminants.

3.10 Working standard solution

Certified reference material (CRMs) of insecticide under study were supplied by UPL, Pvt. Ltd Mumbai to prepare the respective stock solutions of 400 ppm each by applying the formula:

$$\text{Concentration of the stock solution } (\mu g) = \frac{Wt\,(g) \times 10^6 \times \text{Purity } (\%)}{V\,(mL)} \times 100$$

Where, Wt = Weight of the CRM (g)

V = Volume of the CRM to be prepared (mL)

From each stock solution, respective working solutions of 40 ppm, 10 ppm and 1 ppm were prepared by serial dilutions. To calculate the volume of a definite solution required to prepare solutions of other concentration, the following equation is used:

$$C_1V_1=C_2V_2$$

Where, C_1= Concentration of unknown solution

V_1= Volume of unknown solution

C_2= Concentration of known solution

V_2= Volume of known solution

Similarly, prepared lower concentrations *viz.*, 1.00, 0.50, 0.10, 0.05, 0.01 mg kg^{-1} etc. with n-hexane for analysis in GC.

3.11 Insecticide residue study

The residues of novaluron and lambda cyhalothrin were studied in / on tomato fruits and field soil as a component of combination product of novaluron + lambda cyhalothrin (GPI 1316) at X (71+14 g a.i./ha) and 2X (142+28 g a.i./ha) dose.

3.11.1 Sampling

3.11.1.1 Fruits

Tomato fruit samples (1 kg) were collected from each replication of second and third treatment at an interval of 0 (2 hours after spray), 1, 3, 5, 7, 10, 15 and 20 days, after second spray and 1kg fruit samples were also collected from untreated control which was sprayed with water only. The samples were packed in polyethylene bags, labelled well and brought to laboratory for residue analysis.

3.11.1.2 Soil

Soil samples (1 kg) from the X and 2X dose sprayed field were drawn from each replication after 20 days of last spray for analysis. During sampling, soil samples were collected from soil depth up to 15 cm randomly from treatment and control plots. The samples were spread over plastic sheets and allowed to shade dry at room temperature in the laboratory. The air dried samples were desegregated manually using a pestle and a marble mortar, passed through a No. 20 mm brass soil sieve and mixed thoroughly to achieve homogeneity.

3.11.2 Principle of QuEChERS technique

Tomato fruits and soil samples were analysed by QuEChERS technique. QuEChERS is an acronym for quick, easy, cheap, effective, rugged and safe technique for residue analysis. QuEChERS was developed and followed by selective analysis using a gas chromatography-mass spectrometry method for the quantification of pesticides in fruits and vegetable samples (Sharma, 2013). In this technique, samples were extracted with acetonitrile and in the extract sodium chloride was added in order to reduce the amount of polar interferences. The extract was cleaned up with dispersive solid-phase by using magnesium sulphate anhydrous and primary secondary amine (PSA). Magnesium sulphate anhydrous was used to remove water from organic phase. PSA was used to remove sugars, fatty acids, organic acids, lipids and some pigments.

3.11.3 Tomato fruits analysis

Tomato fruits (1kg) were chopped into small pieces, homogenized in high volume homogenizer and analyzed by QuEChERS technique (Sharma, 2013).

3.11.3.1 Extraction:

Out of homogenized sample, 15 g representative sample was taken with 30 ml acetonitrile in 50 ml polypropylene centrifuge tube and homogenized at 15000 rpm for 3 minutes by using low volume high speed homogenizer. Anhydrous sodium chloride (3 g) was added into the tube, shaken at 50 rpm for 5 minutes with Rotospin shaker and then centrifuged at 3000 rpm for 3 minutes. The top organic layer of 18 ml was transferred to another 50 ml polypropylene centrifuge tube containing 9 g anhydrous sodium sulphate and was shaken for 5 minutes at 50 rpm.

3.11.3.2 Dispersive solid phase cleanup:

Anhydrous magnesium sulphate (1150 mg) and PSA (400 mg) were taken in 15 ml polypropylene centrifuge tube. The tube was capped and shaken for one minute on Spinix test tube shaker. Approximately 11ml fraction from 18 ml extract was added into the centrifuge tube, shaken for one minute at 50 rpm in Rotospin shaker and then centrifuged at 3000 rpm for 5 minutes. The 6 ml fraction was transferred to the 30 ml turbo glass tube and evaporated in turbo evaporator to dryness at 45° C in the presence of zero air. The extract was dissolved in 3 ml n-hexane for the analysis of novaluron and lambda cyhalothrin and injected 1 µl into gas chromatograph.

3.11.4 Soil Analysis

3.11.4.1 Extraction

Soil samples were analyzed by QuEChERS technique, modified for analysis of soil (Asensio-Ramos *et al*., 2010). A representative 10 g sieved ground dry soil sample was taken in a 50 ml polypropylene centrifuge tube, to which 20 ml acetonitrile was added and allowed for shaking up to 1 minute using a Rotospin shaker.

3.11.4.2 Dispersive solid phase cleanup

Add 4 g of anhydrous magnesium sulphate and 1 g of sodium chloride and centrifuged at 3300 rpm for 3 minutes. After centrifugation, 10 ml of supernatant was taken in another centrifuge tube of 15 ml containing 1.5 g of magnesium sulphate and 0.250 g of PSA, thereafter allowed for 3 minutes shaking. After shaking, the tube was sonicated for 3 minutes and then centrifuged for 10 minute at 4400 rpm. From this tube, 4 ml aliquot of the supernatant was taken in a turbo tube and evaporated to dryness in presence of air current at 45° C. The dried residues of cropped soil were dissolved in 2 ml of n-hexane for injection (1µl) in to GC-ECD.

3.11.5 Determination of novaluron and lambda cyhalothrin residues:

Parameters of GC-ECD

Novaluron and lambda cyhalothrin residues were estimated on gas chromatograph SHIMADZU GC 2010 equipped with ECD and capillary column (DB-1, 30m long, 0.25mm ID. and 0.25µm film thickness). Temperature of injection port and detector was kept at 250^{o} C and 300^{o} C, respectively. Oven temperature was initially kept at 100^{o} C for 5 minutes and then raised to 220^{o} C at the rate of 40^{o} C/ minute with a hold time of 5 minutes thereafter column temperature was finally raised to 280^{o} C at the rate of 4^{o} C/ minute and hold for 7 minutes. The gas flow (nitrogen) was 1.0 ml/ min and retention time of novaluron and lambda cyhalothrin was 7.530 and 23.537 min., respectively. The limit of quantification for novaluron was 0.01 mg/ kg and for lambda cyhalothrin was 0.1 mg/ kg.

3.12 Recovery studies

The recovery was determined by spiking the untreated tomato fruits and soil samples at 0.01, 0.05, 0.10, 0.50 and 1.00 mg kg^{-1} levels with different test insecticides. Fortified samples were processed as per the procedure described for analysis of sample and linearity was tested. The per cent recovery was calculated as follows:

$$\text{Per cent Recovery} = \frac{\text{Amount recovered (mg/kg)}}{\text{Amount added (mg/kg)}} \times 100$$

3.13 Dissipation studies

3.13.1 Calculation of Residue Half Life (RL_{50}) Values

The RL_{50} values were calculated as per Hoskins (1961) formula, which is as follows:

$$t_{1/2} = \frac{\log 2}{k_1} = \frac{0.301}{k_1} = \frac{0.301}{b}$$

Where,

$t_{1/2}$	=	half-life value (RL_{50}) in days
k_1	=	slope of regression equation of the log residues determined in mg/kg or ppm (y) on the time elapsed in days (x)

3.13.2 Calculation for safe waiting period

$$T_{si} = \frac{\log k_2 - \log tol}{k_1} = \frac{\log k_2 - \log tol}{b}$$

Where,

T_{si}	=	time taken in days by the insecticide to reach tolerance limit
$\log k_2$	=	log of initial deposit
log tol	=	log of proposed tolerance limit
$k_1 = b$	=	slope of regression equation

3.13.3 Residues dissipation rate

The per cent dissipation of the residue over the initial deposit was calculated for various sampling intervals as per the following mathematical formula:

$$\text{Per cent dissipation (\%)} = 100 - \frac{\text{Residue (mg/kg)}}{\text{Initial deposit (mg/kg)}} \times 100$$

Chapter-4

RESULTS AND DISCUSSION

The present investigation was carried out to assess the "**Bioefficacy and residue dynamics of a combination product of novaluron and lambda cyhalothrin against insect pests of tomato"**. Experimental results of the present study are described in the following text under different heads.

4.1 Bioefficacy studies

4.2 Insecticide persistence study

4.3 Statistical constants of insecticide(s)

4.1 Bioefficacy studies:

Bioefficacy of combination product of novaluron and lambda cyhalothrin (GPI 1316) was evaluated against fruit borer (*Helicoverpa armigera*) and pin worm (*Tuta absoluta*) on tomato during the year 2018.

4.1.1 Bioefficacy of novaluron + lambda cyhalothrin (GPI 1316) against fruit borer (*Helicoverpa armigera*)

4.1.1.1 Reduction of larval population after first spray:

Observations recorded at one day before application revealed that population of fruit borer was uniformly distributed in the field ranging from 1.30 to 1.63 larvae per plant (Table 4.1).

Data presented in Table 4.1 revealed that there was a significant reduction in the population of larvae after the application of different insecticidal treatments. After one day of first spray highest per cent larval reduction (77.72) was found in treatment novaluron + lambda cyhalothrin @ 142+28 g a.i./ha (T_3) and it proved to be significantly superior over all other treatments. It was followed by novaluron + lambda cyhalothrin @ 71+14 g a.i./ha (T_2) and novaluron + lambda cyhalothrin @ 47+10 g a.i./ha (T_1) with 74.85 and 73.66 per cent reduction, respectively. Among all the treatments evaluated lambda cyhalothrin @ 14.7 g a.i./ha (T_5) was noticed with lowest per cent larval reduction (60.54). Similarly, at 10th day after spray (DAS) maximum per cent larval reduction was observed in treatment T_3 (90.33%) followed by treatment T_2 (86.00%) whereas minimum per cent larval reduction (65.15%) was

Table 4.1 Bioefficacy of novaluron + lambda cyhalothrin (GPI 1316) against fruit borer (*Helicoverpa armigera*) on tomato after first spray during 2018

Treatments	Insecticides	Dosage g a.i./ha	Pre-count (No. of larvae/ plant)	Per cent reduction					
				1DAS	3DAS	5DAS	7DAS	10 DAS	Mean
T_1	Novaluron + lambda cyhalothrin (9.45+1.9%) ZC	47+10	1.46	73.66 (59.17)	75.55 (60.36)	76.29 (60.86)	78.44 (62.33)	80.36 (63.74)	76.86[c] (61.29)
T_2	Novaluron + lambda cyhalothrin (9.45+1.9%) ZC	71+14 (X)	1.50	74.85 (59.96)	77.24 (61.50)	80.66 (63.92)	84.33 (66.67)	86.00 (68.07)	80.61[b] (64.02)
T_3	Novaluron + lambda cyhalothrin (9.45+1.9%) ZC	142+28 (2X)	1.30	77.72 (61.83)	80.15 (63.54)	84.21 (66.62)	86.69 (68.63)	90.33 (72.05)	83.82[a] (66.54)
T_4	Novaluron 10 EC	75	1.56	67.57 (55.30)	69.83 (56.62)	70.86 (57.31)	73.83 (59.23)	76.00 (60.66)	71.62[d] (57.83)
T_5	Lambda cyhalothrin 4.9% CS	14.7	1.50	60.54 (51.07)	62.99 (52.51)	64.00 (53.11)	66.60 (54.68)	68.41 (55.78)	64.51[f] (53.43)
T_6	Chlorantraniliprole 18.5% SC	28	1.63	68.01 (55.54)	69.45 (56.43)	70.81 (57.28)	68.64 (55.97)	65.15 (53.80)	68.41[e] (55.81)
	Mean			70.39 (57.14)	72.53 (58.50	74.47 (59.85)	76.42 (61.25)	77.71 (62.35)	

Figures in parentheses are arc sin transformed values, DAS- Days after spray

$CD_{0.05}$ (Days):1.37

$CD_{0.05}$ (Treatments): 1.50

$CD_{0.05}$ (Days × Treatments): 3.36

noticed in treatment T_5. When treatments were compared at different days it was found that per cent reduction in larval population get increased up to 10^{th} DAS in all the treatments (T_1, T_2, T_3, T_4 and T_5) except in treatment T_6 (chlorantraniliprole @ 28 g a.i./ha) whose per cent reduction in larval population get increased up to 5^{th} DAS then decreased at 7^{th} and 10^{th} DAS. Overall comparison of different treatments revealed that significantly maximum larval reduction of 83.82 per cent was recorded with novaluron + lambda cyhalothrin @ 142+28 g a.i./ha (T_3). Novaluron + lambda cyhalothrin @ 71+14 g a.i./ha (T_2) was proved to be next effective treatment after causing 80.61 per cent larval reduction followed by novaluron + lambda cyhalothrin @ 47+10 g a.i./ha (T_1), novaluron @ 75 g a.i./ha (T_4) and chlorantraniliprole @ 28 g a.i./ha (T_6) with 76.86, 71.62 and 68.41 per cent reduction, respectively. However, lambda cyhalothrin @ 14.7 g a.i./ha (T_5) was reported with minimum larval reduction of 64.51 per cent.

Table 4.2: Bioefficacy of novaluron + lambda cyhalothrin (GPI 1316) against fruit borer (*Helicoverpa armigera*) on tomato after second spray during 2018

Treatments	Insecticides	Dosage g a.i./ha	Per cent reduction					
			1DAS	3DAS	5DAS	7DAS	10DAS	Mean
T_1	Novaluron + lambda cyhalothrin (9.45+1.9%) ZC	47+10	82.66 (65.59)	84.55 (66.83)	85.29 (67.53)	87.44 (69.24)	89.36 (70.29)	85.86 [c] (68.11)
T_2	Novaluron + lambda cyhalothrin (9.45+1.9%) ZC	71+14 (X)	83.85 (66.42)	86.24 (68.21)	89.66 (71.38)	93.33 (75.19)	95.00 (77.59)	89.61 [b] (71.76)
T_3	Novaluron + lambda cyhalothrin (9.45+1.9%) ZC	142+28 (2X)	86.72 (68.62)	89.15 (70.90)	93.21 (75.05)	95.69 (78.47)	97.33 (81.03)	92.42 [a] (74.81)
T_4	Novaluron 10 EC	75	76.57 (61.12)	78.83 (62.61)	79.86 (63.31)	82.83 (65.56)	85.00 (67.28)	80.62 [d] (63.98)
T_5	Lambda cyhalothrin 4.9% CS	14.7	69.54 (56.48)	71.99 (58.02)	73.00 (58.67)	75.60 (60.38)	77.41 (61.61)	73.51 [f] (59.03)
T_6	Chlorantraniliprole 18.5% SC	28	77.01 (61.34)	78.45 (62.34)	79.81 (63.32)	77.64 (61.90)	74.15 (59.43)	77.41 [e] (61.67)
	Mean		79.39 (63.26)	81.53 (64.83)	83.47 (66.54)	85.42 (68.46)	86.37	

Figures in parentheses are arc sin transformed values, DAS- Days after spray

C.D $_{0.05}$ (Days): 1.7

C.D $_{0.05}$ (Treatments): 1.86

C.D $_{0.05}$ (Days × Treatments): 4.16

4.1.1.2 Reduction of larval population after second spray

After second spray, similar trend was observed in the reduction of larval population due to different treatments. Novaluron + lambda cyhalothrin (9.45+1.9%) ZC @ 142+28 g a.i./ha (T_3) caused the highest per cent reduction (86.72) of larval population on 1 DAS followed by novaluron + lambda cyhalothrin @ 71+14 g a.i./ha (T_2) with 83.85 per cent larval reduction. However, lowest per cent larval reduction (69.54) was noticed in lambda cyhalothrin treatment @ 14.7 g a.i./ha (T_5). On an average, novaluron + lambda cyhalothrin @ 142+28 g a.i./ha (T_3) was reported the best treatment with maximum 92.42 per cent reduction. Next effective treatment was novaluron + lambda cyhalothrin @ 71+14 g a.i./ha (T_2) with 89.61 per cent reduction followed by novaluron + lambda cyhalothrin @ 47+10 g a.i./ha (T_1) showing 85.86 per cent reduction. Novaluron 10 EC @ 75 g a.i./ha (T_4) and chlorantraniliprole 18.5% SC @ 28 g a.i./ha (T_6) showing 80.62 and 77.41 per cent larval reduction, respectively were next in terms of efficacy. Whereas minimum (73.51) per cent reduction was noticed in lambda cyhalothrin @ 14.7g a.i./ha (T_5).

4.1.1.3 Fruit Damage

4.1.1.3.1 After first spray

The data on fruit damage at the time of harvesting after 10^{th} day of each spray (Table 4.3) revealed that all the treatments proved superior with less fruit damage compared to control plot. Minimum fruit damage of 6.19 per cent was observed in novaluron + lambda cyhalothrin @ 142+28 g a.i./ha (T_3) treatment followed by novaluron + lambda cyhalothrin @ 71+14 g a.i./ha (T_2) treated plots (7.76% fruit damage). The plots sprayed with novaluron + lambda cyhalothrin @ 47+10 g a.i./ha, novaluron 10 EC @ 75 g a.i./ha and chlorantraniliprole 18.5% SC @ 28 g a.i./ha recorded 11.05, 15.67 and 21.33 per cent of fruit damage, respectively. Maximum fruit damage of 25.68 per cent was seen in the treatment sprayed with lambda cyhalothrin 4.9% CS@ 14.7 g a.i./ha (T_5). Control plots (T_0) where crop was sprayed with water only, 34.05 per cent fruits were found infested with the larvae of *H. armigera*.

4.1.1.3.1 After second spray

Similar trend of investigating treatments effect was noticed after second spray on fruit damage due to fruit borer. Data presented in Table 4.3 revealed that all the insecticidal treatments significantly reduced the incidence of fruit borer. Minimum fruit damage (3.61%) was recorded in T_3 (novaluron + lambda cyhalothrin @ 142+28 g a.i./ha) which was statistically at par with T_2 (novaluron + lambda cyhalothrin @ 71+14 g a.i./ha) with 5.49 per cent fruit damage followed by T_1 (novaluron + lambda cyhalothrin @ 47+10 g a.i./ha), T_4 (novaluron 10 EC @ 75 g a.i./ha) and T_6 (chlorantraniliprole 18.5% SC @ 28 g a.i./ha) showing 8.69, 13.67 and 20.06 per cent fruit damage, respectively. Maximum per cent fruit damage (25.68%) was found in the fruits harvested from the plots treated with lambda cyhalothrin @ 14.7 g a.i./ha (T_5), whereas 36.98 per cent fruit damage was noticed in the control plots (T_0).

Table 4.3 Bioefficacy of novaluron + lambda cyhalothrin (GPI 1316) against fruit borer (*Helicoverpa armigera*) on tomato during 2018

Treatments	Insecticides	Dosage g a.i./ha	Per cent fruit damage	
			10^{th} day after first spray	10^{th} day after second spray
T_0	Untreated control	-	34.05 (35.67)^{g}	36.98 (37.42)^{g}
T_1	Novaluron + lambda cyhalothrin (9.45+1.9%) ZC	47+10	11.05 (19.40)^{bc}	8.69 (17.09)^{bc}
T_2	Novaluron + lambda cyhalothrin (9.45+1.9%) ZC	71+14 (X)	7.76 (16.02)^{ab}	5.49 (13.51)^{ab}
T_3	Novaluron + lambda cyhalothrin (9.45+1.9%) ZC	142+28 (2X)	6.19 (14.36)^{a}	3.61 (10.91)^{a}
T_4	Novaluron 10 EC	75	15.67 (23.28) d	13.67 (21.48)^{cd}
T_5	Lambda cyhalothrin 4.9% CS	14.7	25.68 (30.40)^{ef}	25.68 (30.41)^{ef}
T_6	Chlorantraniliprole 18.5% SC	28	21.33 (27.48)^{e}	20.06 (26.57)^{e}
	$CD_{0.05}$		3.78	4.53

Figures in parentheses are arc sin transformed values

The bioefficacy of novaluron and lambda cyhalothrin against fruit borer was studied separately and in combination with other insecticides by several workers but no study has so far been conducted on the combination product of novaluron and lambda cyhalothrin in any part of the world. So, the similar studies conducted to evaluate the bioefficacy of novaluron and lambda cyhalothrin were discussed here to support the present study. The results of present study showed 83.82 – 92.42, 71.62 – 80.62 and 64.51 –73.51 per cent reduction in larval population of fruit borer after two foliar applications of novaluron + lambda cyhalothrin @ 142 + 28 g a.i./ ha, novaluron @ 75 g a.i./ha and lambda cyhalothrin @ 14.7 g a.i./ha, respectively which was in closer proximity with the findings of Ghosal *et al.* (2015a) who reported 95.64, 79.82 and 79.49 per cent larval reduction over control in fruit borer population after three application of novaluron 5.25% + indoxacarb 4.5% SC @ 875 ml/ ha, novaluron @ 750 ml/ha and lambda cyhalothrin 5% EC @ 400 ml/ha, respectively in first season (2011). In second season (2012), reduction in larval population was 96.12%, 80.71% and 80.38% in plots treated with novaluron 5.25% + indoxacarb 4.5% SC @ 875 / ha, novaluron @ 750 ml/ha and lambda cyhalothrin 5% EC @ 400 ml/ha, respectively.

The results of present investigation are in conformity with the observations of Kumar *et al.* (2003) who reported novaluron alone @ 0.75 ml/l providing 90 per cent mortality of diamond back moth larvae while all the combinations at full doses provided highest (100%) mortality, whereas novaluron + *Bt.k* (0.375 ml + 1 g/l) which is a half doses combination also gave 100 per cent mortality. Saini *et al*. (2013) reported that novaluron @ 18.75, 37.5 and 75 g a.i./ha was found significantly superior against *H. armigera* as compared to the standard check, quinalphos (525 g a.i./ha) with respect to pod damage and grain yield in chick pea. Novaluron 10 EC @ 200 g a.i./ha reduced population of spotted pod borer, *Maruca vitrata* up to 70 per cent with less than 17 per cent pod damage (Mahalakshmi *et al.*, 2013).

The present experimental findings are also supported by the results of Yogeeswarudu and Venkata (2014) who reported 87.12 - 94.38 and 87.12 - 90.83 per cent reduction over control in larval population of gram pod borer *H. armigera* in chickpea after two applications of novaluron 10 EC @ 1.5 ml/ l and lambda cyhalothrin 5EC 1ml/ l, respectively.

Lal and Jat (2016) reported minimum (7.3 %) pod damage in chickpea following application of novaluron 10EC @ 375 ml ha^{-1} plus 2 per cent urea.

4.1.2 Bioefficacy of novaluron + lambda cyhalothrin (GPI 1316) against pin worm (*Tuta absoluta*)

4.1.2.1 Reduction of larval population after first spray

The larval population before one day of application was uniformly distributed in the field ranging from 2.06 - 2.26 larvae per plant (Table 4.4).

Results presented in Table 4.4 showed that there was a significant reduction in the population of larvae after the application of different insecticidal treatments. After one day of first spray, highest larval reduction of 69.10 per cent was noticed in treatment T_3 (novaluron + lambda cyhalothrin @ 142+28 g a.i./ha) and it proved to be significantly superior over all

Table 4.4 Bioefficacy of novaluron + lambda cyhalothrin (GPI 1316) against pin worm (*Tuta absoluta*) on tomato after first spray during 2018

Treatments	Insecticides	Dosage g a.i./ha	Pre-count (No. of larvae/ plant)	Per cent reduction					
				1DAS	3DAS	5DAS	7DAS	10DAS	Mean
T_1	Novaluron + lambda cyhalothrin (9.45+1.9%) ZC	47+10	2.10	60.88 (51.27)	63.69 (52.92)	66.73 (54.76)	69.22 (56.29)	71.8 (57.90)	66.46 (54.63)^{d}
T_2	Novaluron + lambda cyhalothrin (9.45+1.9%) ZC	71+14 (X)	2.20	65.72 (54.14)	67.11 (54.99)	70.29 (56.95)	72.67 (58.47)	75.10 (60.07)	70.18 (56.93)^{b}
T_3	Novaluron + lambda cyhalothrin (9.45+1.9%) ZC	142+28 (2X)	2.06	69.10 (56.22)	74.08 (59.39)	76.33 (60.96)	80.63 (63.91)	81.42 (64.47)	76.31 (60.99)^{a}
T_4	Novaluron 10 EC	75	2.26	64.49 (53.44)	66.51 (54.63)	69.40 (56.40)	71.94 (58.07)	72.14 (58.13)	68.89 (56.13)^{bc}
T_5	Lambda cyhalothrin 4.9% CS	14.7	2.16	55.52 (48.15)	54.70 (47.68)	57.31 (49.19)	59.62 (50.53)	57.40 (49.23)	56.91 (48.96)^{f}
T_6	Chlorantraniliprole 18.5% SC	28	2.23	56.47 (48.70)	69.10 (56.24)	75.52 (60.33)	68.16 (55.71)	62.58 (52.27)	66.37 (54.65)^{de}
	Mean			62.03 (51.99)	65.86 (54.31)	69.26 (56.43)	70.37 (57.16)	70.07 (57.01)	

Figures in parentheses are arc sin transformed values, DAS- Days after spray

$CD_{0.05}$ (Days): 1.35

$CD_{0.05}$(Treatments): 1.48

$CD_{0.05}$(Days × Treatments): 3.32

other treatments at all the days of observation. It was followed by T_2 (novaluron + lambda cyhalothrin @ 71+14 g a.i./ha) with 65.72 per cent larval reduction and T_4 (novaluron 10 EC @ 75 g a.i./ha) with 64.49 per cent larval reduction. Among all the treatments investigated, lambda cyhalothrin 4.9% CS @ 14.7 g a.i./ha (T_5) was noticed with lowest per cent larval reduction (55.52%). Overall comparison of the different treatments after 10 DAS revealed maximum per cent larval reduction (76.31) in treatment T_3 followed by treatment T_2 with 70.18 per cent larval reduction which is statistically at par with treatment T_4 showing 68.89 per cent larval reduction. Whereas least per cent larval reduction of 56.91 was observed in treatment T_5. Novaluron + lambda cyhalothrin @ 47+10g a.i./ha (T_1) and chlorantraniliprole 18.5% SC @ 28 g a.i./ha (T_6) were statistically at par with each other showing 66.46 and 66.37 per cent larval reduction, respectively.

4.1.2.2 Reduction of larval population after second spray

The data recorded after one DAS of second application showed similar trend (Table 4.5) as observed in first spray. Maximum larval population reduction (84.10 %) was found in T_3 (novaluron + lambda cyhalothrin @ 142+28 g a.i./ha) followed by T_2 (novaluron + lambda cyhalothrin @ 71+14 g a.i./ha) showing 80.72 per cent larval population reduction. The other promising treatments were T_1 (novaluron + lambda cyhalothrin @ 47+10 g a.i./ha) and T_4 (novaluron 10 EC @ 75 g a.i./ha) showing 75.88 and 75.49 per cent reduction followed by T_6 (Chlorantraniliprole @ 28 g a.i./ha) with 71.47 per cent larval population reduction. The minimum per cent larval reduction (70.52) was observed in treatment T_5 (lambda cyhalothrin @ 14.7 g a.i./ha). The data on over all efficacy revealed that the best treatment was T_3 followed by T_2 with 91.31 and 85.18 per cent larval reduction, respectively. Treatment T_1 and T_4 were statistically at par with each other showing 81.46 and 79.89 per cent larval reduction. Treatment T_6 was also statistically at par with T_4 with 78.17 per cent larval reduction. The least effective treatment of all the treatments was T_5 which recorded 73.91per cent reduction of larvae of *T. absoluta*.

4.1.2.3 Fruit damage

4.1.2.3.1 After first spray

All the insecticidal treatments recorded significantly lower per cent fruit damage over control. The order of per cent fruit damage in various treatment is novaluron + lambda cyhalothrin @ 142+28 g a.i./ha (T_3) < novaluron + lambda cyhalothrin @ 71+14 g a.i./ha (T_2) < novaluron + lambda cyhalothrin @ 47+10g a.i./ha (T_1) < novaluron @ 75 g a.i./ha (T4) <

chlorantraniliprole @ 28 g a.i./ha (T_6) < lambda cyhalothrin @ 28 g a.i./ha (T_5) showing 3.66, 4.66, 5.66, 9.00, 10.33 and 12.33 per cent fruit damage, respectively. Treatments T_3, T_2 and T_1 were found statistically at par with each other. Plots treated with novaluron, chlorantraniliprole and lambda cyhalothrin were also found statistically at par with each other. Maximum fruit damage was observed in untreated plots (14.33%).

Table 4.5 Bioefficacy of novaluron + lambda cyhalothrin (GPI 1316) against pin worm (*Tuta absoluta*) on tomato after second spray during 2018

Treatments	Insecticides	Dosage g a.i./ha	Per cent reduction					
			1DAS	3DAS	5DAS	7DAS	10DAS	Mean
T_1	Novaluron + lambda cyhalothrin (9.45+1.9%) ZC	47+10	75.88 (60.59)	78.69 (62.49)	81.73 (64.70)	84.22 (66.62)	86.80 (68.67)	81.46 (64.61) c
T_2	Novaluron + lambda cyhalothrin (9.45+1.9%) ZC	71+14 (X)	80.72 (63.94)	82.11 (64.99)	85.29 (67.44)	87.67 (69.50)	90.10 (71.77)	85.18 (67.53) b
T_3	Novaluron + lambda cyhalothrin (9.45+1.9%) ZC	142+28 (2X)	84.10 (66.52)	89.08 (70.76)	91.33 (73.44)	95.63 (78.65)	96.42 (79.49)	91.31 (73.77) a
T_4	Novaluron 10 EC	75	75.49 (60.39)	77.51 (61.69)	80.40 (63.72)	82.94 (65.72)	83.14 (65.77)	79.89 (63.46) cd
T_5	Lambda cyhalothrin 4.9% CS	14.7	70.52 (57.10)	72.70 (58.49)	74.31 (59.54)	76.62 (61.08)	75.40 (60.25)	73.91 (59.29) f
T_6	Chlorantraniliprole 18.5% SC	28	71.47 (57.69)	80.10 (63.57)	86.52 (68.46)	79.16 (63.04)	73.58 (59.05)	78.17 (62.36) de
	Mean		76.36 (61.04)	80.03 (63.67)	83.26 (66.22)	84.37 (67.43)	84.24 (67.50)	

Figures in parentheses are arc sin transformed values, DAS- Days after spray

CD $_{0.05}$ (Days): 1.81

CD $_{0.05}$(Treatments): 1.99

CD $_{0.05}$ (Days × Treatments): 4.45

4.1.2.3.2 After second spray

Similar trend of investigating treatments effect was noticed after second spray on fruit damage due to pin worm. Minimum fruit damage (2.33 %) was recorded in treatment T_3 (novaluron + lambda cyhalothrin @ 142 + 28 g a.i./ha) which was statistically at par with T_2 (2.66 %) and T_1 (4.33 %). Treatment T_4 (novaluron 10 EC @ 75 g a.i./ha), T_5 (lambda cyhalothrin 4.9% CS @ 14.7 g a.i./ha) and T_6 (chlorantraniliprole 18.5% SC @ 28 g a.i./ha) showing 8.33, 10.66 and 9.00 per cent fruit damage, respectively were statistically at par with each other. Among insecticidal treatments, maximum per cent fruit damage (10.33%) was found in the fruits harvested from the plots treated with lambda cyhalothrin (T_5), whereas 13.66 per cent fruit damage was noticed in the control plots (T_0).

Table 4.6 Bioefficacy of novaluron + lambda cyhalothrin (GPI 1316) against pin worm (*Tuta absoluta*) on tomato during 2018

Treatments	Insecticides	Dosage g a.i./ha	Per cent fruit damage	
			10^{th} day after first spray	10^{th} day after second spray
T_0	Untreated control	-	14.33^{g} (3.89)	13.66^{g} (3.81)
T_1	Novaluron + lambda cyhalothrin (9.45+1.9%) ZC	47+10	5.66^{bc} (2.57)	4.33^{bc} (2.27)
T_2	Novaluron + lambda cyhalothrin (9.45+1.9%) ZC	71+14 (X)	4.66^{ab} (2.33)	2.66^{ab} (1.90)
T_3	Novaluron + lambda cyhalothrin (9.45+1.9%) ZC	142+28 (2X)	3.66^{a} (2.13)	2.33^{a} (1.79)
T_4	Novaluron 10 EC	75	9.00^{d} (3.15)	8.33^{d} (3.04)
T_5	Lambda cyhalothrin 4.9% CS	14.7	12.33^{ef} (3.63)	10.66^{ef} (3.38)
T_6	Chlorantraniliprole 18.5% SC	28	10.33^{de} (3.35)	9.00^{de} (3.14)
	$CD_{0.05}$		3.02	3.24

Figures in parentheses are square root transformed values

The results of present investigation are supported by the work of Ghosal *et al.* (2015b) who reported that novaluron 5.25 % + emamectin 0.9 % @ 875 ml ha^{-1} and novaluron 10 EC @ 750 ml ha^{-1} reduced 92.91 and 72.91 per cent population of diamond back moth larvae over control, respectively and the maximum yield was obtained from the plots treated with novaluron 5.25% + emamectin 0.9% @ 875 ml ha^{-1} in first season. Similar results were observed in second season (2013) where novaluron 5.25 % + emamectin 0.9 % @ 875 ml ha^{-1} and novaluron 10 EC @ 750 ml ha^{-1} reduced 90.45 and 68.99 per cent population over control. Like previous year, maximum yield was obtained from the plots treated with novaluron 5.25 % + emamectin 0.9 % @ 875 ml ha^{-1}. Bisane *et al*. (2017) reported that the novaluron 10 EC 0.005 % minimized fruit damage due to sapota seed borer, *Trymalitis margaritas* up to 4.83 per cent.

4.1.3 Phyto-toxicity studies

The perusal of data presented in the Table 4.7 revealed that no phyto-toxicity symptoms like yellowing, stunting, necrosis, chlorosis, vein clearing, epinasty and hyponasty were observed on tomato plants after 1, 3, 5, 7 and 10 days of two applications of each dose of test insecticide i.e. novaluron + lambda cyhalothrin @ 71+14 g a.i./ha (X dose) and novaluron + lambda cyhalothrin @ 142+28 g a.i./ha (2X dose). Our results are in conformity with the observations of Ghosal *et al.* (2015b) who reported that there were no phyto-toxicity symptoms like leaf injury, wilting, epinasty and hyponasty, necrosis and vein clearing etc. in all the three doses of novaluron 5.25 % + emamectin 0.9 % @ 875, 1750 and 3500 ml ha^{-1} and stated the insecticide safer for cabbage. Kumar *et al.* (2014) tested six fungicides and six insecticides alone and in combination for phytotoxicity and observed no phytotoxicity symptoms when novaluron was applied individually at recommended dose @ 1 ml/l in groundnut. Price and Nagle (2006) also reported no phyto-toxicity symptoms on plants of strawberry when novaluron was applied alone and in combination with bifenthrin for sap beetles.

Table 4.7 Phyto-toxicity effects of novaluron + lambda cyhalothrin on tomato plants

Treatments	Insecticides	Dosage (g a.i./ha)	Phyto-toxicity symptoms*									
			After first spray					After second spray				
			1 DAS	3 DAS	5 DAS	7 DAS	10 DAS	1 DAS	3 DAS	5 DAS	7 DAS	10 DAS
T_2	Novaluron + lambda cyhalothrin (9.45+1.9%) ZC	71+14 (X)	0	0	0	0	0	0	0	0	0	0
T_3	Novaluron + lambda cyhalothrin (9.45+1.9%) ZC	142+28 (2X)	0	0	0	0	0	0	0	0	0	0

DAS= days after spraying, *Yellowing, stunting, necrosis, chlorosis, vein clearing, epinasty and hyponasty

4.1.4 Effect of insecticidal treatments on coccinellid beetles

During the period of experimentation, no population of coccinellid beetles was observed in any treatment.

4.2 Insecticide persistence study

4.2.1 Efficiency of analytical method

4.2.1.1 Linearity

4.2.1.1.1 Novaluron

To establish the linearity of novaluron, calibration curve was plotted by running the standards of various concentrations *viz.*, 0.01, 0.05, 0.10, 0.50 and 1.00 mg/kg in GC-ECD and working out its correlation coefficient (R^2). Figure 1 shows the linear response of novaluron in GC-ECD with correlation coefficient of 0.992.

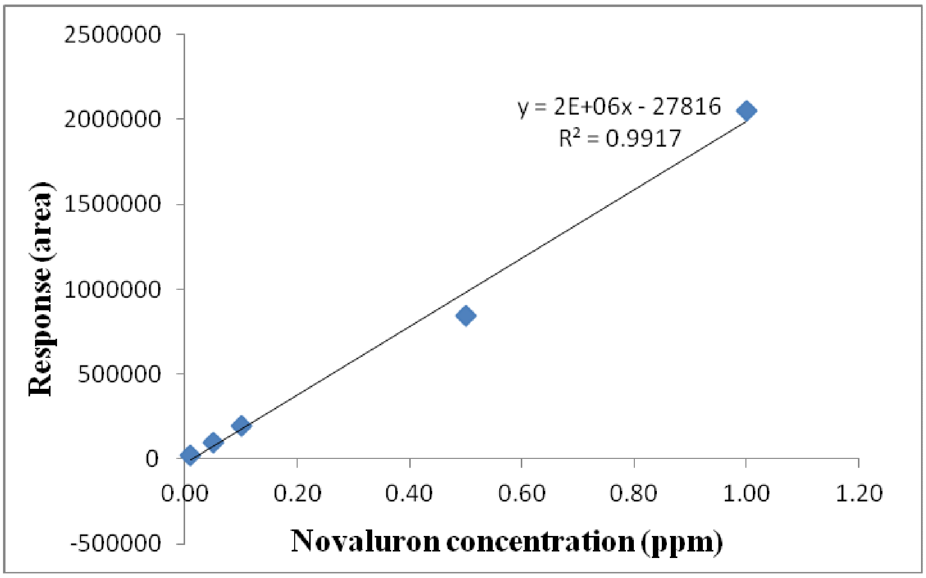


Fig .1 Linearity of novaluron

4.2.1.1.2 Lambda cyhalothrin

A calibration curve has been plotted by running various concentrations viz., 0.01, 0.05, 0.10, 0.50 and 1.00 mg/kg of lambda cyhalothrin in GC-ECD and the linearity of the method was determined by calculating its correlation coefficient (R^2). The linear response of lambda cyhalothrin with correlation coefficient of 0.997 was obtained as depicted in figure 2.

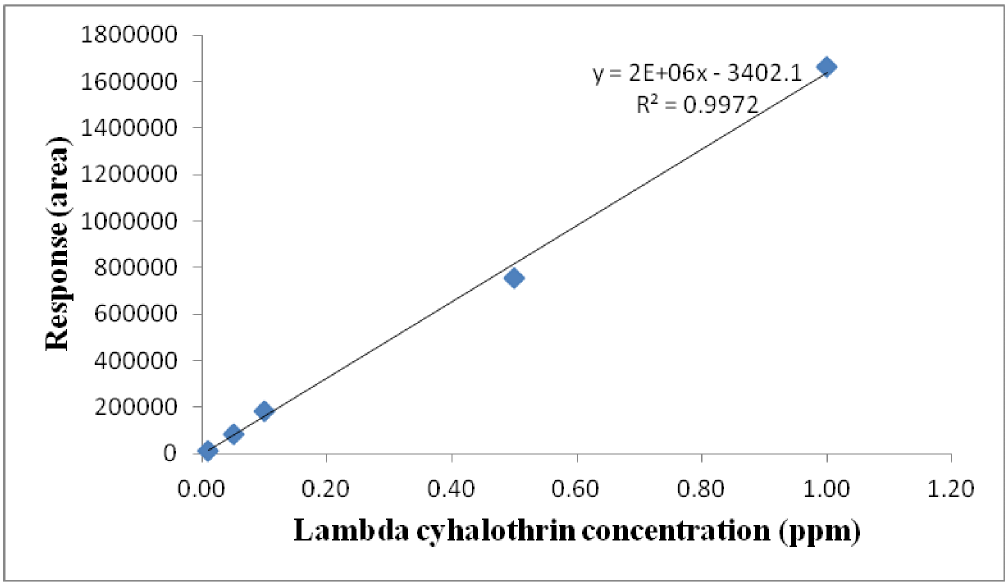


Fig. 2 Linearity of lambda cyhalothrin

4.2.1.2 Recovery studies

4.2.1.2.1 Fruits

4.2.1.2.1.1 Novaluron

As persual from the data (Table 4.8), recovery of novaluron from fortified tomato fruits at different fortification levels *viz*., 0.01, 0.05, 0.10, 0.50 and 1.00 mg/kg was 110.00, 94.00, 96.00 and 83.60 and 101.60 per cent, respectively. The average recovery was between 83.60 – 110.00 per cent. The results of present findings showed a close similarity to those obtained by Malhat *et al*. (2014) using blank samples of tomato spiked at three levels of novaluron and results showed that recoveries ranged from 93 to 99 per cent. Similarly, Shi *et al*. (2016) found that mean recoveries of novaluron and bifenthrin at different spiked levels were between 81 and 108 per cent at limit of quantification (LOQ) of 0.01 mg kg^{-1}. Recovery of novaluron in chickpea plant ranged from 82.22 to 90.64 per cent (Saini *et al.*, 2013). Das *et al*. (2007) reported the average recovery of novaluron in chilli and brinjal spiked at 0.10 - 1.00 ppm as 92.00 - 94.10 per cent, respectively.

Kumar *et al*. (2018) reported that recovery of novaluron ranged between 81 to 103 per cent in fresh chilli and from 82 to 110.2 per cent in dry chilli spiked at 1, 0.5, 0.25, 0.1, 0.05, 0.025, 0.01 and 0.005 mg /l fortification levels.

Table 4.8 Recovery of novaluron from fortified tomato fruits

Fortification levels (mg kg^{-1})	**Recovery (mg kg^{-1})**						**Average recovery (%)**
	R_1	R_2	R_3	R_4	R_5	Mean ± SD	
0.01	0.010	0.012	0.010	0.012	0.010	0.011 ± 0.001	110.00
0.05	0.041	0.045	0.048	0.051	0.050	0.047 ± 0.004	94.00
0.10	0.104	0.100	0.094	0.080	0.100	0.096 ±0.009	96.00
0.50	0.400	0.427	0.432	0.431	0.401	0.418 ± 0.016	83.60
1.00	1.007	1.056	0.968	1.061	0.986	1.016 ± 0.041	101.60

4.2.1.2.1.2 Lambda cyhalothrin

A scrutiny of Table 4.9 showed that the recovery of lambda cyhalothrin from tomato fruits at different fortification levels *viz*., 0.01, 0.05, 0.10, 0.50 and 1.00 mg/kg was 90.00, 106.00, 113.00, 92.40 and 102.00 per cent, respectively. The average recovery ranged between 90.00 – 113.00 per cent as supported by the result of Kelageri *et al.* (2017) who reported that recovery of lambda cyhalothrin was 91.44, 105.08 and 113.46 per cent in tomato when spiked at 0.05, 0.25 and 0.50 mg/kg fortification levels, respectively. In another study, Vemuri *et al.* (2017) observed that the recovery of lambda cyhalothrin from chilli samples at 0.05, 0.25 and 0.5 mg/kg fotification levels was 91.69, 95.88 and 109.66 per cent, respectively. The lambda cyhalothrin recovery was 84.2 and 88.0 per cent from fortified brinjal fruits at 0.5 and 1.0 mg/kg levels as observed by Gupta *et al.* (2015). Aly (2017) recovered 89.29 per cent λ-cyhalothrin from sweet pepper fortified at 1.00 mg/kg.

Table 4.9 Recovery of lambda cyhalothrin from fortified tomato fruits

Fortification levels (mg kg^{-1})	**Recovery (mg kg^{-1})**						**Average recovery (%)**
	R_1	R_2	R_3	R_4	R_5	Mean ± SD	
0.01	0.008	0.009	0.010	0.010	0.008	0.009 ± 0.001	90.00
0.05	0.048	0.050	0.055	0.056	0.056	0.053 ± 0.003	106.00
0.10	0.116	0.119	0.115	0.103	0.111	0.113 ± 0.006	113.00
0.50	0.448	0.469	0.486	0.455	0.450	0.462 ± 0.016	92.40
1.00	0.907	0.918	1.170	0.959	1.145	1.020 ± 0.127	102.00

Chromatographic response of different concentrations (0.01, 0.05, 0.10, 0.50 and 1.00 mg/kg) of novaluron and lambda cyhalothrin in tomato fruit matrix is presented in Figure 3.

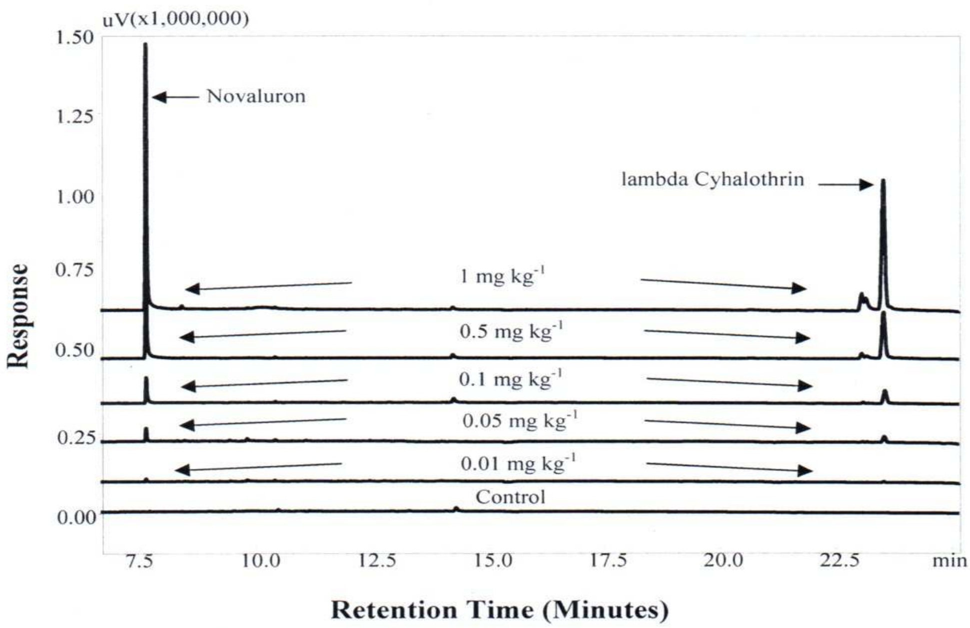


Fig. 3 Chromatographic response of different concentrations of novaluron and lambda cyhalothrin in tomato

4.2.1.2.2 Soil

4.2.1.2.2.1 Novaluron

The data presented in Table 4.9 showed that the recovery of novaluron from fortified tomato field soil varied from 98.00 - 102.00 per cent at various fortification levels (0.01, 0.05, 0.10, 0.50 and 1.00 mg/kg). Our results are in line with those documented by Ahlawat *et al*. (2017) who recovered 85.96 – 89.46 per cent of novaluron from control plot soil spiked at level of 0.01, 0.05 and 0.10 mg kg^{-1} in chickpea.

Table 4.10 Recovery of novaluron from fortified tomato crop field soil

Fortification levels (mg kg^{-1})	Recovery (mg kg^{-1})						Average recovery (%)
	R_1	R_2	R_3	R_4	R_5	Mean ± SD	
0.01	0.010	0.009	0.010	0.009	0.011	0.010 ± 0.000	100.00
0.05	0.053	0.052	0.045	0.048	0.048	0.049 ± 0.003	98.00
0.10	0.097	0.102	0.100	0.099	0.113	0.102 ± 0.006	102.00
0.50	0.542	0.534	0.472	0.485	0.466	0.500 ± 0.035	100.00
1.00	0.886	0.926	0.901	0.896	0.932	0.908 ± 0.019	90.80

4.2.1.2.2.2 Lambda cyhalothrin

The data presented in Table 4.11 indicated that the recovery of lambda cyhalothrin from fortified tomato field soil at various fortification levels *viz*., 0.01, 0.05, 0.10, 0.50 and 1.00 mg/kg was found to be 90.00, 96.00, 88.00, 90.80 and 83.00 per cent, respectively. Hence, the average recovery from tomato cropped soil varied from 83.00 – 90.80 per cent.

Table 4.11 Recovery of lambda cyhalothrin from fortified tomato crop field soil

Fortification levels (mg kg^{-1})	Recovery (mg kg^{-1})						Average recovery (%)
	R_1	R_2	R_3	R_4	R_5	Mean ± SD	
0.01	0.010	0.009	0.009	0.009	0.009	0.009 ± 0.000	90.00
0.05	0.044	0.044	0.051	0.050	0.049	0.048 ± 0.003	96.00
0.10	0.088	0.091	0.083	0.090	0.090	0.088 ± 0.003	88.00
0.50	0.474	0.462	0.440	0.435	0.461	0.454 ± 0.016	90.80
1.00	0.842	0.831	0.832	0.800	0.846	0.830 ± 0.018	83.00

Similar trend of recoveries has been reported by Beevi *et al.* (2014) who reported the recovery of lambda cyhalothrin from cardamom field soil between 99.70 and 104.80 per cent at fortification levels of 0.05 and 0.25 mg/kg, respectively. Mao *et al.* (2010) observed the average recovery of lambda cyhalothrin as 91.36 - 96.64 per cent from fortified tobacco field soil at different fortification levels between 0.05 - 0.25 mg/kg. Fan *et al.* (2019) reported the average recoveries of lambda cyhalothrin, thiamethoxam and clothianidin in apple between 88 -105 per cent at the limit of quantitation (LOQ) of 0.01 mg kg^{-1}.

4.2.2 Initial deposits and persistence of novaluron and lambda cyhalothrin in/on tomato fruits

Initial deposits of a pesticide depend upon a number of factors like concentration, formulation, weather conditions, substrate characteristics, type of sprayer used, distance between the nozzle and plant surface and properties of carrier etc. Besides these factors, plant type (erect or prostrate), shape of plant parts (broad, narrow or linear) and growth of plant parts (slow or fast) influence the deposits persistence on plant surface (Ebling, 1963) whereas the dissipation of insecticides depend upon crop factors like stage of crop, rind of fruit, internal material, weather factors like moisture, temperature, rain and surface characteristics to hold deposits and chemical factors like structural stability of chemical compound either as parent compound or metabolites, volatilization, solubility, formulation, site and method of application (Edwards, 1975). Keeping most of these factors constant the discussion will be around the deposits and dissipation due to the levels of insecticides.

4.2.2.1 Novaluron

4.2.2.1.1 Initial deposits

The average initial deposits (two hours after application) were 0.567 mg/kg and 1.241 mg/kg on tomato fruits due to the application of combination product of novaluron and lambda cyhalothrin on tomato (Table 4.12 & 4.13, Fig.4). The initial deposits of novaluron at standard dose (71 g a.i./ha) were 2.81 times lower than that obtained from double dose (142 g a.i./ha). The results of present findings showed a closer proximity to those obtained by Ahlawat *et al.* (2017) who reported the average initial deposits of 0.642, 1.705 and 2.251 mg/kg in green pods after the application of novaluron 10 EC @ 37.5, 75 and 150 g a.i. per ha in chick pea.

Similarly, Kumar *et al.* (2018) observed that the initial deposits of novaluron at 37.5 and 75 g a.i. ha^{-1} were 0.11 and 0.20 mg kg^{-1} in fresh chilli whereas in dry chilli peppers these were 0.31 and 0.59 mg kg^{-1}, respectively.

4.2.2.1.2 Persistence in/on tomato fruits

The initial deposits of novaluron at standard dose were 0.567 which dissipated to 0.337, 0.163, 0.098 and 0.037 mg/kg after 1, 3, 5, days 7 days of application, respectively. At double dose, the initial deposits of novaluron were 1.241 mg/kg which dissipated to 0.628, 0.368, 0.224, 0.111 and 0.027 mg/kg after 1, 3, 5, 7 and 10 days, respectively (Table 4.12 & 4.13, Fig. 4). Persistence data showed that the novaluron residues on tomato fruits were reduced from 0.337 to 0.037 mg/kg in 1 to 7 days at standard dose, thus recorded 41.49 – 93.58 per cent dissipation at standard dose and at double dose, residues were reduced from 0.628 - 0.027 mg/kg in 1 to 10 days showing 49.40 to 97.82 per cent dissipation on tomato fruits. Our results are in agreement withthose of Ahlawat *et al*. (2017) who observed that the mean initial deposits were reduced by more than 35 per cent in one day. These residues were further reduced by more than 90 per cent on 5^{th} day. The residues reached below determination limit of 0.01 mg kg^{-1} on 7^{th} day with the application of novaluron @ 37.5 g a.i. ha^{-1}; whereas at 75 and 150 g a.i. ha^{-1}, these residues reached below 0.01 mg kg^{-1} in 15 and 20 days, respectively, showing 100 per cent dissipation in chickpea green pods. Kumar *et al*. (2018) reported that residues of novaluron persisted up to 7 days in fresh chilli pepper when applied at 37.5 g a.i. ha^{-1} and 75 g a.i. ha^{-1} and residues were below the limit of quantification on the 10^{th} day for both doses, whereas residues of novaluron in dry chilli peppers reached below the limit of quantification on the 15^{th} day at both the dosages.

Table 4.12 Persistence of novaluron (@ 71 g a.i./ha) as a component of combination product novaluron and lambda cyhalothrin in/on tomato fruits

Interval (Days)	Residues (mg/kg)				Dissipation (%)
	R_1	R_2	R_3	Mean residues ± SD	
0	0.58	0.57	0.578	0.567 ±0.005	-
1	0.31	0.358	0.343	0.337 ± 0.025	41.49
3	0.168	0.159	0.161	0.163 ± 0.005	71.70
5	0.104	0.095	0.095	0.098 ± 0.005	82.99
7	0.038	0.033	0.041	0.037 ± 0.004	93.58
10	BDL	BDL	BDL	-	-
Control	ND	ND	ND	ND	-

BDL = Below Determination Limit; ND = Not Detected

Table 4.13 Persistence of novaluron (@ 142 g a.i./ha) as a component of combination product novaluron and lambda cyhalothrin in/on tomato fruits

Interval (Days)	Residues (mg/kg)				Dissipation (%)
	R_1	R_2	R_3	Mean residues ± SD	
0	1.258	1.223	1.242	1.241 ± 0.018	-
1	0.635	0.621	0.628	0.628 ±0.007	49.40
3	0.387	0.355	0.362	0.368 ± 0.017	70.35
5	0.214	0.228	0.23	0.224 ± 0.009	81.95
7	0.109	0.112	0.111	0.111 ± 0.002	91.06
10	0.027	0.03	0.025	0.027 ± 0.003	97.82
15	BDL	BDL	BDL	BDL	-
Control	ND	ND	ND	ND	

BDL = Below Determination Limit; ND = Not Detected

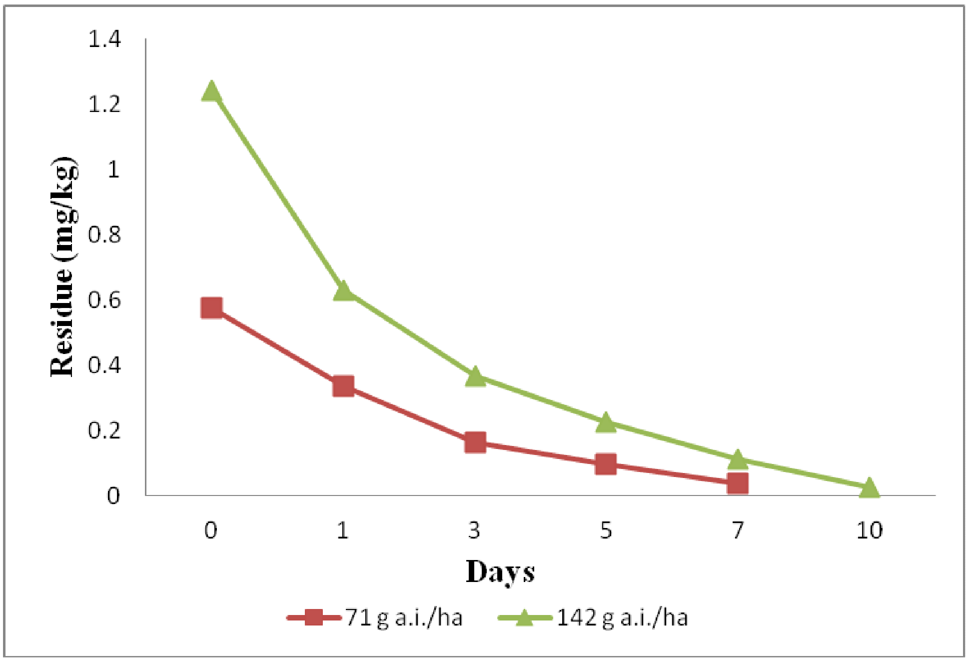


Fig 4 Dissipation pattern of novaluron (@ 71 and 142 g a.i./ha) as a component of combination product novaluron and lambda cyhalothrin in/on tomato fruits

4.2.2.2 Lambda cyhalothrin

4.2.2.2.1 Initial deposits

The average initial deposits of lambda cyhalothrin were 0.282 mg/kg and 0.531 mg/kg on tomato fruits due to the application of combination product of novaluron + lambda cyhalothrin on tomato fruits (Table 4.14 & 4.15, Fig.5). The initial deposits of lambda cyhalothrin at standard dose (14 g a.i./ha) were 1.88 times lower than that obtained from double dose (28 g a.i./ha). Results of study conducted by Jayakrishnan *et al*. (2005) revealed that lambda cyhalothrin when applied at the application rate of 15 g a.i./ha and 30 g a.i./ha on tomato fruits yielded initial deposit of 0.38 mg/kg and 0.52 mg/kg, while Singh and Singh (2003) found that the application of lambda cyhalothrin (@ 25 and 50 g a.i./ ha) on chickpea resulted in initial deposition of residues ranging from 0.335 and 0.462 mg/ kg which showed conformity with the present findings.

Mathirajan *et al.* (2000) conducted a field trial to know the dissipation pattern of lambda cyhalothrin 5EC in chillies at three application rates i.e. 7.5, 15 and 30 g a.i./ha and observed that the deposition of initial residues ranging from 1.44 to 2.20 ppm when sprayed at 7.5 g a.i./ha. The medium (15 g a.i./ha) and highest (30 g a.i./ha) doses left initial residues ranging from 2.84 to 5.83, and from 5.48 to 7.15 ppm, respectively. Sharma and Awasthi (2002) conducted field trial to study persistence of lambda cyhalothrin residues on cauliflower and found that the initial deposits of lambda cyhalothrin ranged from 0.81 to 1.59 mg/ kg. The initial deposits of lambda cyhalothrin residues on brinjal fruits was 0.138 mg/kg when sprayed at the rate of 15 g a.i./ha (Gupta *et al.*, 2015). The difference in the initial deposits of lambda cyhalothrin may be attributed to the variations in type of crop, doses applied and agroclimatic conditions.

4.2.2.2.2 Persistence in/on tomato fruits

At standard dose the initial deposits of 0.282 mg/kg dissipated to 0.150, 0.098 and 0.035 mg/kg after 1, 3 and 5 days of application, respectively. At double dose, the initial deposits of 0.531 mg/kg dissipated to 0.280, 0.168, 0.102 and 0.038 in 1, 3, 5 and 7 days, respectively. The per cent dissipation values of the initial deposit at standard dose were found to be 46.75, 65.09 and 87.46 at 1, 3 and 5 days intervals, respectively and at double dose, the per cent dissipation values were 47.37, 68.32, 80.80 and 92.85 at 1, 3, 5 and 7 days intervals, respectively (Table 4.14 & 4.15, Fig.5). The results of present findings showed a close proximity to those obtained by Vemuri *et al*. (2016). The residues of lambda cyhalothrin dissipated to 0.06 mg/kg and 0.05 mg/kg in 5 and 7 days of application at the rate of 15 g a.i./ha in open field and polyhouse conditions in capsicum fruits. Sharma and Awasthi (2002) studied persistence of lambda cyhalothrin residues on cauliflower which dissipated quickly to reach below detectable limit by 10 - 15 days.

Table 4.14 Persistence of lambda cyhalothrin (@ 14 g a.i./ha) as a component of combination product novaluron and lambda cyhalothrinin/on tomato fruits

Interval (Days)	Residues (mg/kg)				Dissipation (%)
	R_1	R_2	R_3	Mean residues ± SD	
0	0.281	0.279	0.285	0.282 ±0.003	-
1	0.162	0.145	0.143	0.150 ± 0.010	46.75
3	0.107	0.098	0.09	0.098 ± 0.009	65.09
5	0.038	0.031	0.037	0.035 ± 0.004	87.46
7	BDL	BDL	BDL	-	-
Control	ND	ND	ND	ND	-

BDL = Below Determination Limit; ND = Not Detected

Table 4.15 Persistence of lambda cyhalothrin (@ 28 g a.i./ha) as a component of combination product novaluron and lambda cyhalothrinin/on tomato fruits

Interval (Days)	Residues (mg/kg)				Dissipation (%)
	R_1	R_2	R_3	Mean residues ± SD	
0	0.529	0.543	0.522	0.531 ± 0.011	-
1	0.271	0.294	0.274	0.280 ±0.013	47.37
3	0.169	0.160	0.176	0.168 ± 0.008	68.32
5	0.102	0.100	0.104	0.102 ± 0.002	80.80
7	0.034	0.039	0.041	0.028 ± 0.004	92.85
10	BDL	BDL	BDL	BDL	-
Control	ND	ND	ND	ND	

BDL = Below Determination Limit; ND = Not Detected

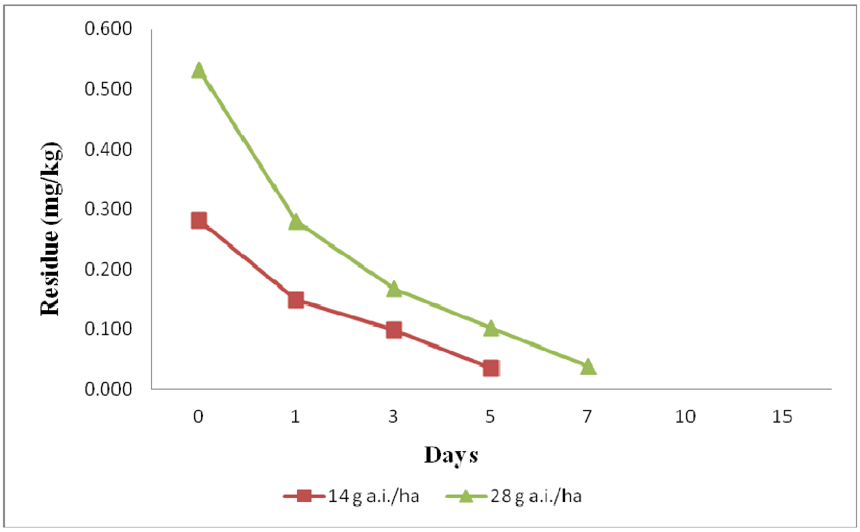


Fig 5 Dissipation pattern of lambda cyhalothrin (@ 14 and 28 g a.i./ha) as a component of combination product novaluron and lambda cyhalothrin in/on tomato fruits

4.2.3 Persistence of novaluron and lambda cyhalothrin in soil

The transport, persistence or degradation of pesticides in soil depends on the chemistry of the pesticide as well as physical, chemical and biological properties of the soil (Beevi *et al.*, 2014). The main processes which potentially affect the ultimate fate of pesticides in soil are adsorption/desorption processes, transformation processes (biological and chemical degradation), and transportation through soil, atmosphere, surface water, or ground water (Kumari *et al*., 2008).

4.2.3.1 Persistence of novaluron in tomato field soil

The data presented in Table 4.16 showed that in tomato field soil the residues of novaluron were found to be below determination level (<0.01 mg/kg) after 20 days of application of combination product of novaluron + lambda cyhalothrin at standard (71+ 14 g a.i./ha) and double dose (142+ 28 g a.i/ha). Our results are in line with those of Ahlawat *et al*. (2017) who observed that the average initial deposits in chickpea field soil at 37.5, 75 and 150 g a.i. ha^{-1} dosages were 0.144, 0.186 and 0.223 mg kg^{-1}, respectively. At 37.5 g a.i. ha^{-1} residues were reduced to BDL level on 7^{th} day and at higher application @ 75.0 and 150.0 g a.i. ha^{-1} residues persisted up to 7 and 10 days, respectively.

Table 4.16 Residues of novaluron in tomato field soil as a component of combination product novaluron and lambda cyhalothrin

Interval (Days)	Novaluron residues (mg/kg)							
	@ 71g a.i./ha				@ 142g a.i./ha			
	R_1	R_2	R_3	Mean residues ±SD	R_1	R_2	R_3	Mean residues ± SD
20	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
Control	ND	ND	ND	ND	ND	ND	ND	ND

BDL = Below Determination Limit; ND = Not Detected

4.2.3.2 Persistence of lambda cyhalothrin in tomato field soil

The residues of lambda cyhalothrin were found to be below determination limit (<0.01 mg/kg) after 20 days due to the application of combination product of novaluron + lambda cyhalothrin at standard (71 + 14 g a.i./ha) and double dose (142 + 28 g a.i/ha) on tomato field soil (Table 4.17). Chauhan *et al*. (2012) applied lambda cyhalothrin @ 15 g a.i./ha and 30 g a.i./ ha at fruiting stage of tomato and observed residues reaching below detectable level of 0.005 mg/kg on 3^{rd} and 7^{th} day at single and double dose in tomato cropped soil sample, respectively. Studies on the persistence and dissipation of fipronil (75 g a.i./ha.) and lambda cyhalothrin (30 g a.i./ ha.) on chili as well as in soil by Reddy and Reddy (2013) reported no residues in soil after 15 days of spray. Chauhan (2011) observed that residues of lambda cyhalothrin when applied @ 15 g a.i/ha and 30 g a.i/ha on tomato crop, dissipated completely in soil on 3rdand 5th day in case of single dose and double dose, respectively. All these findings supported our results.

Table 4.17 Residues of lambda cyhalothrin in tomato field soil as a component of combination product novaluron and lambda cyhalothrin

Interval (Days)	Lambda cyhalothrin residues (mg/kg)							
	@ 14 g a.i./ha				@ 28 g a.i./ha			
	R_1	R_2	R_3	Mean residues ±SD	R_1	R_2	R_3	Mean residues ±SD
20	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
Control	ND	ND	ND	ND	ND	ND	ND	ND

BDL = Below Determination Limit; ND = Not Detected

4.3 Statistical constants of novaluron and lambda cyhalothrin

The statistical constants obtained due to the analysis of persistence data as per Hoskins (1961) of novaluron and lambda cyhalothrin tested on tomato are given in Table 4.18.

Table 4.18 Statistical constants of test insecticides in/on tomato fruits

Pesticide	Dose (g a.i. /ha)	Statistical Constants			
		Regression equation (y = a + bx)	Correlation cofficient (R^2)	RL_{50} (Days)	Waiting period (Days)
Novaluron	71	y = -0.1614 - 0.2717x	- 0.9945	1.86	10.90
	142	y = -0.1392 - 0.0503 x	- 0.9917	2.16	15.04
Lambda cyhalothrin	14	y = -0.1681 - 0.5800 x	- 0.9841	1.79	2.67
	28	y = -0.1503 - 0.3217x	- 0.9883	2.00	4.82

4.3.1 Residue half-life value (RL_{50})

The half life values are influenced by the volatility of the compounds, initial deposits, growth and physiology of plants, weather conditions, oxidation, reduction and hydrolytic biodegradation factors. Half life within vegetation were found to be degraded four times faster compared with plant surface half lives (Juraske *et al.*, 2008). Insecticides with shorter half lives tend to build up less because they are much less likely to persist in the environment while those having longer half lives are more likely to build up after repeated applications. This may increase the risk of contaminating nearby surface water, ground water, plants, and animals (Hanson *et al*., 2015).

4.3.1.1 Novaluron

Novaluron initial deposits dissipated to half at standard dose in 1.86 days as compared to 2.16 days at double dose from tomato fruits, indicating first order kinetic behaviour of novaluron (Table 4.18). Similarly, Malhat *et al*. (2014) found that novaluron residues tend to dissipate following first-order rate kinetics with half life of 2.08 days in tomato. In chickpea, novaluron dissipated in green pods with half life of 1.40 days at 37.5 g a.i. ha^{-1}, 1.63 days at 75 g a.i. ha^{-1} and 2.73 days at 150.0 g a.i. ha^{-1}, respectively following first order kinetics (Ahlawat *et al*., 2017). Kumar *et al*. (2018) observed the half life of novaluron applied at 37.5 and 75 g a.i. ha^{-1} as 2.1 and 2.3 days for fresh chilli peppers and 2.4 and 2.3 days for dry chilli peppers

4.3.1.2 Lambda cyhalothrin

The data presented in the Table 4.18 indicated that lambda cyhalothrin deposits dissipated to half at standard dose (14 g a.i./ha) in 1.79 days as compared to 2.00 days at double dose (28 g a.i./ha) from tomato fruits indicating difference in dissipation behavior of insecticide at both doses. Our findings are in line with Sharma and Awasthi (2002) who evaluated the half life of lambda cyhaothrin residues in cauliflowe ranging between 2.0 to 2.4 days. Singh and Singh (2003) found that the initial residues of lambda cyhalothrin at the rate of 25 and 50 g a.i./ ha on chickpea was degraded with half life values of 4.9 and 5.0 days. In another study, Fan *et al*. (2013) examined the half-lives of lambda cyhalothrin in leafy vegetables which was found in the range of 1.77 - 6.24 days. Gupta *et al*. (2015) reported half-life of lambda cyhalothrin deposits on brinjal fruits as 2.65 days at dosage of 15 g a.i./ha.

4.3.2 Waiting Period

The time between last application of insecticide on the crop and harvest is referred as waiting period. These values are used in calculation for predicting residue concentration in harvested produce and for detecting the time interval needed between crop spraying and harvesting or potential processing/ consumption in order to minimize residue concentrations (Lewis and Tzilivakis, 2017). Thus considering the safety of consumer, waiting periods has been calculated at FSSAI MRL i.e. 0.01 and 0.1 mg/kg (FSSAI, 2017) for novaluron and lambda cyhalothrin, respectively.

4.3.2.1 Novaluron

The waiting period of novaluron as a component of combination product (novaluron and lambda cyhalothrin) has been calculated as 10.90 and 15.04 days for safe harvesting of tomato fruits after application at standard (X) and double (2X) doses, respectively. Kumar *et al*. (2018) reported waiting period of 8.4 and 9.4 days for novaluron in fresh chilli peppers at 37.5 g a.i. ha^{-1} and 75 g a.i. ha^{-1}, respectively, whereas it was 11.3 and 13.0 days respectively in dry chilli peppers.

4.3.2.2 Lambda cyhalothrin

For safe consumption of tomato, the waiting period of lambda cyhalothrin was calculated as 2.67 and 4.82 days at standard (X) and double (2X) doses, respectively on the basis of FSSAI MRL of 0.1 mg/kg. Mathirajan *et al.* (2000) observed the waiting periods of 2.51 to 3.41 days on chilli fruits for lambda cyhalothrin at the lower dose (15 g a.i./ha) and 4.19 to 4.89 days at the highest dose (30 g a.i./ha), these findings support our results. On the basis of European Union maximum residue limit of 1 mg/kg for lambda cyhalothrin, Seenivasan and Muraleedharan (2009) evaluated the safely harvest interval of 5 days for tea at the recommended dosage. Safe waiting period reported by Bouri *et al*. (2012) for lambda cyhalothrin in green bean was 8 days in winter and 7.5 days in the spring.

Chapter-5

SUMMARY AND CONCLUSIONS

The present investigation **"Bioefficacy and residue dynamics of a combination product of novaluron and lambda cyhalothrin against insect pests of tomato"** was carried out in the Department of Entomology, Dr. Y.S. Parmar University of Horticulture and Forestry, Nauni, Solan (H.P.). The work was carried out to check the efficacy of a combination product of novaluron and lambda cyhalothrin (GPI 1316) against insect pests of tomato substantiated with residue data for its utilization in safe consumption of tomato fruits. The obtained experimental results are summarized as:

- Bioefficacy studies carried out against fruit borer, *Helicoverpa armigera* revealed that double dose of combination product novaluron + lambda cyhalothrin @ 142 +28 g a.i./ha was the most effective treatment showing highest larval reduction of 83.82 and 92.42 per cent followed by standard dose of same insecticide combination (novaluron + lambda cyhalothrin @ 71 + 14 g a.i./ha) with 80.61 and 89.61 per cent reduction after first and second spray, respectively. Minimum fruit damage of 6.19 and 3.61 per cent was noticed with novaluron + lambda cyhalothrin @ 142 + 28 g a.i./ha which was found statistically at par with novaluron + lambda cyhalothrin @ 71 + 14 g a.i./ha with 7.66 and 5.49 per cent fruit damage after first and second spray, respectively.
- Double dose of combination product (novaluron + lambda cyhalothrin @ 142 + 28 g a.i./ha) was found to be the most effective treatment against pin worm (*Tuta absoluta*) causing maximum reduction of 76.31 and 91.31 per cent followed by standard dose of same insecticide combination (novaluron + lambda cyhalothrin @ 71 + 14 g a.i./ha) with 70.18 and 85.18 per cent reduction after first and second spray, respectively. Minimum fruit damage of 3.66 and 2.33 per cent was noticed with novaluron + lambda cyhalothrin @ 142 + 28 g a.i./ha which was found statistically at par with novaluron + lambda cyhalothrin @ 71 + 14 g a.i./ha causing 4.66 and 2.66 per cent fruit damage after first and second spray, respectively.
- No phytotoxicity symptoms like leaf injury, wilting, vein clearing, necrosis, epinasty and hyponasty were observed on the tomato plants following two foliar applications of

standard (novaluron + lambda cyhalothrin @ 71 + 14 g a.i./ha) and double dose (novaluron + lambda cyhalothrin @ 142 + 28 g a.i./ha) of test insecticide.

- QuEChERS technique was used for extraction and cleanup of tomato fruit and soil samples. Method was validated by spiking matrix at different levels *viz.,* 0.01, 0.05, 0.10, 0.50 and 1.00 mg/kg. Recoveries of novaluron and lambda cyhalothrin from tomato fruit samples fortified at five levels were in the range of 83.60 to 110.00 and 90.00 to 113.00 per cent, respectively. In tomato field soil, the recoveries of novaluron and lambda cyhalothrin were found in between 98.00 to 102.00 and 83.00 to 90.80 per cent, respectively at five fortification levels (0.01, 0.05, 0.10, 0.50 and 1.00 mg/kg).
- Initial deposits of novaluron when sprayed at standard dose (71 g a.i./ha) as a component of combination product of novaluron and lambda cyhalothrin was 0.567 mg/kg which dissipated to 93.58 per cent at 7th day of spray with half life of 1.86 days. At double dose of novaluron (142 g a.i./ha), initial deposit was 1.241 mg/kg which dissipated to 97.82 per cent at 10th day of spray with half life of 2.16 days. The residues of novaluron were found below the limit of determination at 10th and 15th day after the application of respective doses in tomato fruits.
- Initial deposit of lambda cyhalothrin when sprayed at standard dose (14 g a.i./ha) as a component of combination product of novaluron + lambda cyhalothrin was 0.282 mg/kg which dissipated to 87.46 per cent at 5th day of spray with half life of 1.79 days. At double dose of lambda cyhalothrin (28 g a.i./ha), initial deposit was 0.531 mg/kg which dissipated to 92.85 per cent at 7th day of spray with half life of 2.00 days. The residues of lambda cyhalothrin were found below the limit of determination at 7th and 10th day after the application of respective doses in tomato fruits.
- The residues of novaluron and lambda cyhalothrin in tomato field soil were found below the limit of determination at 20th day after the last spray at both the doses.
- Waiting period of 15 and 5 days was calculated on the basis of FSSAI MRL for novaluron and lambda cyhalothrin, respectively for safe consumption of tomato fruits.

CONCLUSIONS

- The investigating test insecticide combination product of novaluron + lambda cyhalothrin at double dose (142 + 28 g a.i./ha) and standerd dose (71 + 14 g a.i./ha) was proved to be most effective against both pests, fruit borer (*Helicoverpa armigera*) and pin worm (*Tuta absoluta*). No phytotoxicity symptoms were observed on tomato plants following two applications of standard and double dose of the test insecticide.
- Combination product of novaluron and lambda cyhalothrin persisted in tomato fruits up to 10 days at both the doses.
- In soil, the residues of novaluron and lambda cyhalothrin were detected at BDL at 20th day after last spraying at both the doses.
- The time period between the last spray and harvest (waiting period) of tomato fruits was suggested to be 15 days for the test insecticide combination.

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Appendix

Bioefficacy of GPI 1316 (novaluron and lambda cyhalothrin) against *H. armigera* after first spray

Source of Variation	DF	Mean Squares
Replication	2	
Factor A	4	78.15
Factor B	5	377.513
Intraction A X B	20	8.844
Error	58	4.205
Total	89	

Bioefficacy of GPI 1316 (novaluron and lambda cyhalothrin) against *H. armigera* on tomato after second spray

Source of Variation	DF	Mean Squares
Replication	2	
Factor A	4	145.579
Factor B	5	802.021
Intraction A X B	20	14.801
Error	58	8.393
Total	89	

Effect of GPI 1316 on per cent fruit damage due to *H. armigera* after first spray

Source of Variation	DF	Mean Squares
Replication	2	
Treatment	6	311.571
Error	12	4.436
Total	20	

Effect of GPI 1316 on per cent fruit damage due to *H. armigera* after first spray

Source of Variation	DF	Mean Squares
Replication	2	
Treatment	6	436.841
Error	12	6.351
Total	20	

Bioefficacy of GPI 1316 (novaluron and lambda cyhalothrin) against *T. absoluta* on tomato after first spray

Source of Variation	**DF**	**Mean Squares**
Replication	2	
Factor A	4	227.595
Factor B	5	603.864
Intraction A X B	20	29.901
Error	58	10.347
Total	89	

Bioefficacy of GPI 1316 (novaluron and lambda cyhalothrin) against *T. absoluta* on tomato after second spray

Source of Variation	**DF**	**Mean Squares**
Replication	2	
Factor A	4	212.821
Factor B	5	542.882
Intraction A X B	20	24.12
Error	58	10.346
Total	89	

Effect of GPI 1316 on per cent fruit damage due to *T. absoluta* after first spray

Source of Variation	**DF**	**Mean Squares**
Replication	2	
Treatment	6	49.19
Error	12	2.833
Total	20	

Effect of GPI 1316 on per cent fruit damage due to *T. absoluta* after first spray

Source of Variation	**DF**	**Mean Squares**
Replication	2	
Treatment	6	55.381
Error	12	3.262
Total	20	

Dr. Y S PARMAR UNIVERSITY OF HORTICULTURE AND FORESTRY
NAUNI, SOLAN HP 173 320
DEPARTMENT OF ENTOMOLOGY

Title of thesis	**:**	**"Bioefficacy and residue dynamics of combination product of novaluron and lambda cyhalothrin against insect pests of tomato"**
Name of the student	**:**	Vinit Kumar
Admission number	**:**	H-2017-16-M
Major advisor	**:**	Dr. Sapna Katna
Main field	**:**	Entomology
Minor field	**:**	Vegetable Science
Degree awarded	**:**	M.Sc (Agriculture) Entomology
Year of degree awarded	**:**	2019
Number of pages in thesis	**:**	61+II
Number of words in abstract	**:**	387

ABSTRACT

The present investigations on **"Bioefficacy and residue dynamics of combination product of novaluron and lambda cyhalothrin against insect pests of tomato"** was carried out during 2018 in the department of Entomology, Dr. Y S Parmar University of Horticulture and Forestry, Nauni, Solan. The objectives of the study were to evaluate the bioefficacy of combination product of novaluron and lambda cyhalothrin (GPI 1316) against insect pests of tomato and their persistence on tomato fruits and soil. The study comprised of treatments *viz.*, novaluron + lambda cyhalothrin (9.45+1.9%) ZC @ 47+10 g a.i./ha, novaluron + lambda cyhalothrin (9.45+1.9%) ZC @ 71+14 g a.i./ha, novaluron + lambda cyhalothrin (9.45+1.9%) ZC @ 142+28 g a.i./ha, novaluron 10 EC @ 75 g a.i./ha, lambda cyhalothrin 4.9% CS @ 28 g a.i./ha and chlorantraniliprole 18.5% SC @ 28 g a.i./ha. Bioefficacy results revealed that novaluron + lambda cyhalothrin @ 142+28 g a.i./ha, was most effective against both, fruit borer (*Helicoverpa armigera*) (83.82-92.42 %) and pin worm (*Tuta absoluta*) (76.31-91.31%) showing highest percent larval reduction followed by novaluron + lambda cyhalothrin @ 71+14 g a.i./ha and novaluron + lambda cyhalothrin @ 47+10 g a.i./ha. Minimum fruit damage of fruit borer (3.61%) and pin worm (2.33%) was observed with novaluron + lambda cyhalothrin @ 142+28 g a.i./ha, followed by novaluron + lambda cyhalothrin @ 71+14 g a.i./ha and novaluron + lambda cyhalothrin @ 47+10 g a.i./ha and found statistically at par with each other. Two foliar applications of test insecticide at standard and double dose did not showed any phyto-toxic symptoms. The initial deposits of 0.567 and 1.241 mg/kg of novaluron and 0.282 and 0.531 mg/kg of lambda cyhalothrin were recorded at the standard and double dose after second application of combination product of novaluron and lambda cyhalothrin. Novaluron residues persisted upto 10 and 15 days whereas residues of lambda cyhalothrin persisted upto 5 and 7 days at standard and double dose, respectively in tomato fruits. The residues of both novaluron and lambda cyhalothrin in fruits reduced to half in less than 3 days. Novaluron and lambda cyhalothrin residues were found below determination level in tomato field soil at 20th day after last application of both the doses. From the consumer safety point of view, the safe waiting period of 15 days was suggested for the combination product of novaluron and lambda cyhalothrin for tomato.

Signature of Major Advisor **Signature of the Student**
(Dr. Sapna Katna)

Countersigned

Professor and Head
Department of Entomology
Dr. YS Parmar University of Horticulture & Forestry
Nauni, Solan, (HP)-173 230

BRIEF BIO-DATA

Name	**:**	Vinit Kumar
Father's name	**:**	Sh. Rajesh Kumar
Mother's name	**:**	Smt. Roshani Devi
Date of birth	**:**	7th December 1995
Sex	**:**	Male
Marital status	**:**	Unmarried
Nationality	**:**	Indian

Academic Qualification:

Examination Passed	Year of Passing	University/Board	Division
Matriculation	2010	CBSE	First
10+2	2012	CBSE	First
Graduation	2017	Dr YSP University of Horticulture and Forestry, Nauni, Solan (HP)	First

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(Vinit Kumar)