DEVELOPMENT OF FORMULATIONS FROM *Samadera indica* Gaetrn. FOR THE MANAGEMENT OF LEAF FEEDING PESTS IN SNAKE GOURD (*Trichosanthes anguina* L.)

by

REMYA, S.
(2018-21-042)

Thesis submitted in partial fulfilment of the requirement for the degree of

DOCTOR OF PHILOSOPHY IN AGRICULTURE

Faculty of Agriculture
Kerala Agricultural University

DEPARTMENT OF AGRICULTURAL ENTOMOLOGY
COLLEGE OF AGRICULTURE
VELLAYANI, THIRUVANANTHAPURAM-695522
KERALA, INDIA

2022

DECLARATION

I, hereby declare that this thesis entitled **"Development of formulations from *Samadera indica* Gaetrn. for the management of leaf feeding pests in snake gourd (*Trichosanthes anguina* L.)"** is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award to me of any degree, diploma, fellowship or other similar title, of any other University or Society.

Vellayani

27/04/2022

Remya, S.

(2018-21-042)

CERTIFICATE

Certified that this thesis entitled **"Development of formulations from *Samadera indica* Gaetrn. for the management of leaf feeding pests in snake gourd (*Trichosanthes anguina* L.)"** is a record of bonafide research work done independently by Mrs. Remya. S (2018-21-042) under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

Vellayani

27/04/2022

Dr. Nisha M. S.

(Chairperson, Advisory Committee)

Assistant Professor and Head

Dept. of Nematology

College of Agriculture, Vellayani

CERTIFICATE

We, the undersigned members of the Advisory Committee of Mrs. Remya. S (2018-21-042), a candidate for the degree of **Doctor of Philosophy in Agriculture** with major in Agricultural Entomology agree that this thesis entitled **"Development of formulations from *Samadera indica* Gaetrn. for the management of leaf feeding pests in snake gourd (*Trichosanthes anguina* L.)"** may be submitted by Mrs. Remya. S in partial fulfilment of the requirement for the degree.

27/4/22

Dr. Nisha M. S.
Assistant Professor and Head
Department of Nematology
College of Agriculture, Vellayani
Thiruvananthapuram-695 522

27/4/22

Dr. N. Anitha
Professor and Head
Department of Agricultural Entomology
College of Agriculture, Vellayani
Thiruvananthapuram-695 522

Dr. M. H. Faizal
Professor
Department of Agricultural Entomology
College of Agriculture, Vellayani
Thiruvananthapuram-695 522

Dr. Thomas George
Principal Investigator
AINP on Pesticide Residues, PRRAL
College of Agriculture, Vellayani
Thiruvananthapuram-695 522

Dr. K. V. Radhakrishnan
Principal Scientist and Head, Organic Chemistry
CSIR – NIIST
National Institute for Interdisciplinary Science and Technology
Thiruvananthapuram – 695 019

EXTERNAL EXAMINER
27/04/2022

Principal Scientist
केन्द्रीय कन्द फसल अनुसंधान संस्थान
Central Tuber Crops Research Institute
श्रीकार्यम/ Sreekariyam
तिरुवनन्तपुरम/ Thiruvananthapuram - 695 017

ACKNOWLEDGEMENT

First and foremost, I am obliged to thank the God Almighty, whose blessings makes humanly impossible things possible thus helped me to finish this endeavour successfully.

I would like to express my sincere and heartfelt utmost gratitude to Dr. Nisha M. S., Chairperson of my Advisory Committee, for her selfless support, constant inspiration and constructive suggestions all through this journey without which this endeavour would not have been successful.

I extend my utmost gratitude to Dr. N. Anitha, Professor & Head, Department of Agricultural Entomology, for her valuable guidance and encouragement during the course of my research work.

I extend my sincere gratitude to Dr. M. H. Faizal, Professor, Department of Agricultural Entomology for his guidance and suggestions during the period of study.

I am extremely thankful and pay my deep sense of gratitude to Dr. Thomas George, Principal Investigator, AINP on Pesticide residues, PRRAL for his unhesitated guidance and generous support on laboratory facilities during the course of the study.

I also acknowledge with a deep sense of reverence, my gratitude to Dr. K. V. Radhakrishnan, Principal Scientist and Head, Organic Chemistry, CSIR – NIIST for his timely help rendered during the work.

I owe my heartfelt gratitude to Smt. Brigit Joseph, Associate Professor and Dr. Pratheesh, Assistant Professor, Department of Agricultural Statistics for their valuable advices which helped me to complete the statistical analysis.

I wish to express my earnest thanks to Dr. K. D. Prathapan for his active help and cooperation for the successful completion of this venture.

I am deeply indebted to my teachers Dr. Malini Nilamudeen, Dr. Amritha V. S., Dr. Ambily Paul, Dr. Reji Rani, Dr. Santhosh Kumar, Dr. Thania Sara Varghese, Dr. Vijayasree, Dr. Krishnakumar and Dr. Narayana.

I express my greatest acknowledgement to Dr. Shanas, Dr. P R Shashank and Dr. J Poorani for the help rendered during identification of test insects.

I extend my sincere thanks to lab assistants Kiran and Abin for their cooperation and help during the period of study.

I owe my deep sense of gratitude to my seniors Anusree chechi, Mithra chichi, Chinchu chechi and Viswajyothi chechi. Special word of thanks to my fellow lab mates and friends Athira Anil, Rupini, Shabana, Sailaja, Gayathri, Hari and Manu Ma'am. Completion of this project could not have been accomplished without the help extended by my dear friends Athira, Sree, Neethu, Pooja and Geethu. Unforgettable was the help rendered by Biji chechi, Leema, Vignesh and Vrinda chechi during crisis situation. A special thanks to you all.

I am extremely grateful to my parents and younger sister for their love, prayers, care and sacrifice for my future. I am very much thankful to my husband for his love, understanding, prayers and incessant support to complete this research work.

Remya S

CONTENTS

LIST OF TABLES

LIST OF PLATES

LIST OF FIGURES

LIST OF ABBREVIATIONS

CD	Critical Difference
CRD	Completely Randomised Design
DAS	Days After Storage
DAT	Days After Treatment
DMSO	Dimethyl Sulfoxide
et al.	And others
Fig.	Figure
g	Gram
g^{-1}	Per gram
h	Hour
HAT	Hours After Treatment
HPLC	High Performance Liquid Chromatography
IPM	Integrated Pest Management
KAU	Kerala Agricultural University
LD_{50}	Median Lethal Dose
LD_{90}	Lethal Dose 90
mg	Milli gram
mL	Milli Litre
mL^{-1}	Per milli Litre
NS	Non Significant
NSKE	Neem Seed Kernel Extract
O/W	Oil in Water
R_f	Retention Factor
SE (m)	Standard Error of Mean
viz.	Namely

Introduction

1. INTRODUCTION

Green revolution, a concept which had arisen in 1960s has resulted in bumper yield in food grain production. This superfluous yield, which was due to heavy usage of chemical fertilizers and pesticides had eventually lead to several deleterious episodes of environmental and health effects. Realization of unhealthy effects of chemicals has diverted people's attention towards homestead farming wherein organic means of pest control are being adopted. Furthermore, to attain self sufficiency in food production after the dreadful event of Covid-19, people are now adopting 'pesticide free' vegetable cultivation for self consumption.

Indian Council of Medical Research (ICMR) recommendes consumption of 300 g of vegetables per day by per person. This indicates the importance of vegetables in our diet. Snake gourd (*Trichosanthesanguina* L.) is a cucurbitaceous vegetable that is commonly grown in Kerala. The consumption of snake gourd offers several beneficial health effects like detoxification of body, improvement of digestion, management of diabetes etc. as it is a source of various minerals and dietary fibre. The major pests in snake gourd are pumpkin caterpillar (*Diaphania indica* Saund.), epilachna beetle (*Henosepilachna septima* (Dieke), snake gourd caterpillar (*Anadevidia peponis* F.), fruit fly (*Bactrocera cucurbitae* (Coquillett) etc. Pumpkin caterpillar, besides feeding on leaves, feeds on flowers, tender shoots, young and mature fruits too. Epilachna beetle causes damage by scraping the surface of leaves resulting in skeletonization and drying. Being a crop generally grown in the homesteads, the pests could be managed only in an environmentally feasible manner. However, in the view of a commercial cultivator, pest damage results in low productivity and fetches less income. This forces the farmer to adopt chemical methods of pest management, which paves way to the problems of pest resurgence, pesticide resistance and pest replacement, besides causing environmental and health hazards. All these factors reinforce the fact that organic control measures are ideal to ward off pest problems in snake gourd.

In this scenario, botanicals offer a satiable means of pest control owing to their eco-friendly approach. Kerala is well reputed for its diverse tropical flora. Unfortunately this floral diversity remains virtually untapped for pest control purposes and many potential plants await discovery. There are several plants which can be exploited successfully in IPM (Integrated Pest Management) programmes for controlling insect pests. Among these, the plants like *Samadera indica* Gaetrn. (Niepa bark Tree), *Quisqualis indica* L. (Rangoon creeper), *Chromolaena odorata* (L.) R. M. King & H. Robinson (Siam weed), *Annona squamosa* L. (Sweetsop), *Mikania micrantha* Kunth. (Mile-a-minute vine) etc. are worth mentioning.

S. indica is a tree belonging to the family Simaroubaceae. Bitter principles known as quassinoids present in this tree possess promising anti-insect properties. The extracts should be converted into ideal formulations so that the effect of active ingredient gets increased. An ideal formulation consists of active ingredient, surfactant and organic solvents whose ratio differs based on HLB (Hydrophilic Lipophilic Balance) values of the bioactive compound. Commonly available formulations include Wettable Powder (WP), Dusts (D), Granules (G) etc. The efficiency of formulation gets increased with its reduced size as that in micro and nano sized formulations.

In Kerala, existing fallow lands can be used for cultivation of potential plants with anti-insect properties and the raw material can be provided to industries for developing them into suitable insecticidal formulations. Identification and exploitation of indigenous sources of botanical pesticides will result in effective substitution of huge quantities of synthetic insecticides now being used. Hence, the present study aims to isolate and characterize the insecticidal principles present in *S. indica* and to evaluate the efficacy of its formulations in managing leaf feeding pests in snake gourd.

Considering the above aspects, the present study entitled “Development of formulations from *Samadera indica* Gaetrn. for the management of leaf feeding

pests in snake gourd (*Trichosanthes anguina* L.)" has been taken up with the following objectives:

- Screening of bark and seeds of *S. indica* for insecticidal properties
- Bioefficacy study of chromatographic fractions containing bioactive compounds against *D. indica* and *H. septima*
- Structural characterisation of the bioactive compounds
- Development of suitable formulations of bioactive compounds
- Field evaluation of effective formulation against *D. indica* and *H. septima*

Review of Literature

2. REVIEW OF LITERATURE

The present investigation focused on exploration of anti-insect properties of bark and seed extracts from *Samadera indica* Gaetrn., identification of bioactive compounds in the effective extract and development of suitable formulations from the extract for the management of leaf feeding pests in snake gourd both under *in vivo* and *in vitro* conditions. A comprehensive review on efficacy of botanicals against insect pests in terms of antifeedant effect, insecticidal effect and effect on biology of insects and pest management using botanical insecticide formulations and chemical is given below.

2.1. BIOEFFICACY OF BOTANICALS AGAINST INSECT PESTS

2.1.1. Antifeedant Effect

2.1.1.1. Leaf Feeders

Ventura and Ito (2000) observed that feeding of cucurbit beetle, *Diabrotica speciosa* (Genn.) decreased significantly when provided with increasing concentrations of flower extracts from chinaberry, *Melia azedarach* (L.), ranging from 1 to 7 g per 100 mL. The root extracts of large caltrops, *Pedalium murex* L. exhibited good antifeedant activity against tobacco cutworm, *Spodoptera litura* (Fabricius) (Sahayaraj *et al*., 2003). Methanol, acetone and chloroform extracts of Malabar nut, *Justicia adhatoda* L., goatweed, *Ageratum conyzoides* L. and Ceylon leadwort, *Plumbago zeylanica* L. strongly deterred the feeding of Bihar hairy caterpillar, *Spilarctia obliqua* Walker at a dose of 10 mg mL^{-1} (Prajapati *et al*., 2003).

Han *et al.* (2006) reported that methanol extract from roots of Chinese angelica, *Angelica dahurica* Fish. Ex Hoffm. @ 1.30 mg cm^{-2} exhibited complete antifeedant activity against larvae of black carpet beetle, *Attagenus unicolor (Brahm*) over a 30-day period. Abdullah and Subramanian (2008) reported that application of methanolic extracts of neem @ 100 to 200 ppm caused antifeedant effect on epilachna beetle, *Epilachna indica* Mulsant which is the pest of egg plant, *Solanum melongena* L. Root extracts (500 ppm) of *Tylophora indica* R. Br.

exhibited antifeedant activity of 56.06 per cent against *S. litura* after 24 h (Reddy *et al*., 2009).

The research findings by Zapata *et al*. (2009) revealed that n-hexane extract from stem bark of canelo, *Drimys winteri* J.R. Forster et G. Forster @ 5000 ppm exhibited the strongest antifeedant activity against sixth instar larvae of Egyptian cotton leaf worm, *Spodoptera littoralis* (Boisduval) with a Feeding Deterrence Index (FDI) value of 75.50 per cent.

More than 95 per cent antifeedant activity was exhibited by extracts from Norwegian angelica, *Angelica archangelica* L., masterwort, *Imperatoria ostruthium* (L.) W. D. J. Koch, Arabian pea, *Psoralea bituminosa* (L.) C. H. Stirt. and white swallow-wort, *Vincetoxicum hirundinaria* Medik. against *S. littoralis* (Pavela, 2010). Baskar *et al*. (2011) unveiled that greater degree of antifeedant activity (56.06 per cent) was exhibited by root extracts of Indian birthwort, *Aristolochia tagala* Cham. against *S. litura*. As per the findings by Arivoli and Arivoli and Tennyson (2013), maximum antifeedant activity was observed in ethyl acetate extract of poison nut, *Strychnos nux-vomica* L. (88.98 per cent), followed by hexane extracts of Chinese chastetree, *Vitex negundo* L. (86.41 per cent) and curry leaf tree, *Murraya koeingii* (L.) Sprengel (81.46 per cent), ethyl acetate extract of prickly ash, *Zanthoxylum limonella* (Dennst.) Alston. (80.58 per cent) and hexane extract of rosary pea, *Abrus precatorius* L. (78.61 per cent) in *S. litura*.

Application of methanolic extract of leaves from Malabar nut, *Adathoda vasika* (L.) Nees @ 1000 ppm resulted in feeding repellance in neonate larvae of *S. littoralis* (Antifeedant index – 72) (Sadek, 2003). Lingathurai *et al*. (2011) observed maximum antifeedant activity (84.30 per cent) in third instar larvae of diamondback moth, *Plutella xylostella* (L.) when treated with fractions from chloroform extract of birch-leaved cat tail, *Acalypha fruticosa* Forssk. at 1000 ppm concentration. Petroleum ether extract from bark of worm-cure, *Albizia anthelmintica* Brong. @ one per cent exhibited antifeedant activity against Mopani emperor moth, *Nudaurelia belina* (Westw.) (Kaoneka and Mollel, 2012).

The work by Ulrichs *et al*. (2008) revealed that hexane extract of leaves from wild rice, *Porteresia coarctata* Takeoka @ 1000 to 2000 ppm exhibited antifeedant action against *S. litura*. Incorporation of methanolic extract of wild bishop, *Bifora radians* M. Bieb. into the diet of third instar larvae of oblique banded leaf roller *Choristoneura rosaceana* (Harris) @ one per cent deterred feeding by larvae (Gokse *et al*., 2010). Bakavathiappan *et al.* (2012) observed that chloroform extract from leaves of Apple of Sodom, *Calotropis procera* (Aiton) W. T. Aiton @ five per cent exhibited antifeedant activity of 63.42 per cent against *S. litura*.

Wheeler and Isman (2001) observed 50 per cent feeding deterrence in *S. litura* when fed with diet treated with methanolic extract of *Trichilia americana* (Sesse and Mocino) Pennington. Antifeedant activity of methanol extract of leaves from bitterweed, *Hymenoxys robusta* (Rusby) K. F. Parker on beet armyworm, *Spodoptera exigua* (Hubner) was illustrated by Juarez *et al*. (2014). Five per cent extract from leaves of holy basil, *Ocimum sanctum* L. showed antifeedant activity towards fourth instar larvae of *S. litura*, with an antifeedant index of 57.61 (Summarwar and Pandey, 2015).

Kutas and Nadasy (2005) observed that five per cent methanolic extract of scentless Mayweed, *Matricaria inodora* L. caused feeding aversion in larvae of Colorado potato beetle, *Leptinotarsa decemlineata* Say. Kashiwagi *et al*. (2007) observed that the activity of migratory locust, *Locusta migratoria* L. was deterred by crude methanol extracts of Japanese cedar, *Cryptomeria japonica* (L. f.) D. Don. Magierowicz *et al.* (2020) observed that application of aqueous extracts of tansy-plant (*Tanacetum vulgare* L.), yarrow-plant (*Achillea millefolium* L.) and summer savory (*Satureja hortensis* L.) caused the larvae of snout moth, *Acrobasis advenella* (Zinck.) to avoid treated feeding sites as indicated by lesser number of larvae on treated inflorescences. The percentage of larvae observed in these treatments was 2.09, 4.19 and 4.69 percent respectively.

Gramine, the principal alkaloid in barley, *Hordeum vulgare* L. inhibited the feeding behaviour of *L. migratoria* (Ishikawa and Kanke, 2000). Methylene chloride extracts of sand heath, *Ceratiola ericoides* and christmas berry, *Ardisia*

crenata deterred feeding in root weevil, *Diaprepus abbreviates* L. (Sandoval-Mojica and Capinera, 2011). Anusree *et al*. (2018) reported that third instar larvae of *S. litura* exhibited maximum antifeedant effect with 5 per cent methanolic crude extracts from leaves of *Samadera indica* (Gaetrn.) (45.62 per cent) and flowers of rangoon creeper, *Quisqualis indica* L. (31.87 per cent).

Calcagno *et al*. (2002) observed feeding deterrent effect in *S. littoralis* upon treatment with coumarin. The diterpene scutecyprol A isolated from *Scutellaria valdiviana* (Clos) Epling reduced the feeding activity of *S. exigua* in choice and no choice bioassays (Caballero *et al*., 2008). Azadirachtin reduced food consumption in larvae of gypsy moth, *Lymantria dispar* L. (Kostic *et al*., 2008). Sandoval-Mojica and Capinera (2011) reported that the application of ryanodine caused feeding deterrence in American grasshopper, *Schistocerca americana* (Drury) and *D. abbreviates*.

2.1.1.2. Sucking Pests

Abtew *et al*. (2015) reported that the plants black pepper, *Piper nigrum* L., true cinnamon tree, *Cinnamomum zeylanicum* J. Presl. and Chinese cinnamon, *Cinnamomum cassia* (L.) J. Presl. repelled the thrips *Megalurothrips sjostedti* Trybom with degree of repellency being 0.01 to 1 per cent. Methanol extract from false ashoka, *Polyalthia longifolia* (Sonn.) Thwaites (0.1%) repelled half of the population of mustard aphid, *Lipaphis erysimi* (Kaltenbach) (Arora *et al*., 2017).

2.1.1.3. Storage Pests

Khanam *et al*. (2006) observed that application of lignin from sugarcane bagasse resulted in strong repellent activity towards confused flour beetle, *Tribolium confusum* Jacquelin du Val at concentrations of 471.57 and 628.76 µg cm^{-2}. The findings by Omar *et al*. (2007) shows that terpenes from langsat, *Lansium domesticum* Corr. Serr. exhibited antifeedant activity against rice weevil, *Sitophilus oryzae* L. Wang *et al*. (2015) reported that methanol extract from stem bark of mastic-leaf prickly ash, *Zanthoxylum schinifolium* Siebold & Zucc.

exhibited repellency against red flour beetle, *Tribolium castaneum* (Herbst) as evident with the FDI value of 41.12 per cent.

2.1.1.4. Other Pests

Saric *et al*. (2007) observed a decrease in the larval duration of common fruit fly, *Drosophila melanogaster* Meigen flies raised on diet containing 1.75% quercetin. Sandoval-Mojica and Capinera (2011) reported that the application of ryanodine caused feeding deterrence in American grasshopper, *Schistocerca americana* (Drury) and root weevil, *Diaprepes abbreviates* L.

2.1.2. Insecticidal Effect

2.1.2.1. Leaf Feeders

Lenin (2011) found that contact application of seed extracts of annona and neem, oil from neem and three commercial formulations of annona, milletia and neem (Anosom, Derisom and NeemAzal T/S, respectively @ 2 mLL^{-1}) resulted in 100 per cent mortality of *Diaphania indica* Saund. Stem bark extracts from the meliaceous plants *Aglaia* spp., cotton fruit (*Sandoricum koetjape* Merr.) and cedar mangrove (*Xylocarpus moluccensis* (Lamk.) M. Roem) in chloroform brought about mortality in third instar larvae of *S. litura* (Tukiran, 2013). Anusree *et al.* (2016) reported that the crude methanol extract of *Q. indica* flower at 5 per cent concentration was equally effective as chemical pesticides against *S. litura*.

Ramya *et al*. (2008) recorded mortality rates of 72.80, 67.80 and 62.20 per cent with aqueous leaf extracts of the plants green chiretta (*Andrographis paniculata* Nees.), periwinkle (*Catharanthus roseus* L. (G) Don.) and thornapple (*Datura metal* L.) respectively against sixth instar larvae of cotton bollworm, *Helicoverpa armigera* (Hubner). Aqueous leaf extract of bintaro (*Cerbera odollam*) resulted in mortality of 75 per cent larvae of *S. litura* after one week (Purwani *et al*., 2014). Application of methanol extracts from leaves of patawali, *Tinospora crispa* (L.) Hook. f. & Thomson (160 ppm) and guava, *Psidium*

guajava L. (200 ppm) resulted in complete mortality in eggs of *S. litura* (Elanchezhiyan *et al*., 2015).

Samuel *et al*. (2009) observed that pongam leaf extracts caused toxicity in second instar larvae of *S. litura* and LC_{50} value was 5.44% after 72 hours of exposure. Application of ethanolic extract of leaves of custard apple, *Annona squamosa* L. @ 1 and 5% resulted in 100 per cent mortality after 40 and 15 min respectively against *P. xylostella* (Kumar *et al*., 2010). The investigations by Moreno *et al*. (2012) revealed that ethanol extract of toothache plant, *Acmella oleracea* (L.) R. K. Jansen exhibited 88.30 per cent mortality against tomato leaf miner, *Tuta absoluta* (Meyrick).

2.1.2.2. Sucking Pests

Coelho *et al*. (2006) reported that ethanolic extract from root of *Simarouba versicolor* and hexane extract from root of American muskwood, *Guarea guidonia* Allam were responsible for 95.00 and 75.00 per cent mortality each in fourth instar nymphs of assassin bugs, *Rhodnius milesi* Stal. The findings by Bhutto *et al*. (2017) revealed that tobacco extracts reduced the population of whiteflies, aphids and jassids in okra by 58.02, 47.31 and 54.71 per cent respectively. *Milletia* leaf extract showed acute toxicity to turnip aphid, *Lipaphis pseudobrassicae* (Davis) with LC_{50} values of 0.585, 0.151 and 0.113% at 24, 48 and 72 hours respectively (Tran *et al*., 2016).

Ethanol extract from stem bark of cerrado plant exhibited mortality in fourth instar nymphs of *R. milesi* to the tune of 5 to 15 per cent (Coelho *et al*., 2006). The lethality of root extracts of bitter apple, *Citrullus colocynthis* on bird cherry-oat aphid, *Rhopalosiphum padi* L. was established by Asiry (2015).

Kim *et al*. (2001) observed that application of methanolic extract of Korean mint, *Agastache rugosa* Kuntze @ 5000 ppm resulted in elimination of more than 90 per cent of adults of brown plant hopper, *Nilaparvata lugens* (Stal). As per the findings by Madanat *et al*. (2016), leaf extracts of castor, *Ricinus communis* L. and *Lantana camara* L. in acetone exhibited lethality towards green peach aphid, *Myzus persicae* Sulzer with LC_{50} values of 1150 and 6660 ppm

respectively after 24 hours. The toxicity of water extracts of neem leaves towards various larval instars of mustard aphid, *L. erysimi* was proven by Chattree *et al.* (2016).

N'Guessan *et al*. (2006) reported high mortality rates of 88.20, 96.60 and 98.50 per cent against cocoa mirids with neem seed aqueous crude extracts @ 10, 20 and 30 per cent respectively at 96 HAT. Magsi *et al*. (2017) reported that the mean reduction in population of white fly observed with application of aqueous leaf extracts of Gurakhu tobacco, Balkhi tobacco, Desi tobacco and Hazaropattar tobacco was 86.58, 85.95, 83.71 and 79.83 per cent respectively. The crude methanolic extract of wrinkled leaf isodon, *Isodon rugosus* (Wall. Ex Benth.) resulted in mortality of pea aphid, *Acyrthosiphon pisum* Haris and the LC_{50} and LC_{90} values were 36 and 102 ppm respectively (Khan *et al*., 2017).

Treating adults of apple woolly aphid, *Eriosoma lanigerum* (Hosmann) with quercetin dehydrate, naringin and rutin hydrate @ 1000 ppm resulted in 86, 80 and 96.67 per cent mortality after 72 hours (Ateyyat *et al*., 2012). Morehead (2016) claimed that use of alkaloids from sabadilla witnessed greater mortality (70 per cent) in nymphs of brown marmorated stink bug, *Halyomorpha halys* Stal. Application of 3.09 ppb of azadirachtin resulted in 50 per cent mortality of black watermelon bug, *Coridius viduatus* (Fabricius) (Aljedani, 2018).

As per the findings by Aswin *et al*. (2016), use of neem oil and azadirachtin recorded mortality of 66.60 per cent against red spider mite at 72 HAT. Aljedani (2018) opined that application of azadirachtin took approximately 28 h to reduce the population of black watermelon bug *C. viduatus* to half of the initial. Khan *et al.* (2019) explored the possibility of rosmarinic acid to be used as a potential insecticide as a very low concentration of this compound (LC_{90} - 5.4 ppm) caused significant mortality in pea aphid, *Acyrthosiphon pisum* Mordvilko within 24 hours.

Aphidicidal effect of oil from *Bifora radians* Bieb. with LC_{50} value of 0.3 $mgmL^{-1}$ was demonstrated by Sampson *et al*. (2005). Essential oil from *Artemisia seiberi* caused significant mortality of *E. lanigerum* and LD_{50} value was 6.16

μLmL^{-1}(Ateyyat *et al.*, 2012). Jahan *et al*. (2016) reported the insecticidal activity of limonene present in sweet orange, *Citrus sinensis* (L.) Osbeck on *M. persicae* with LC_{50} value of 57.70 μLL^{-1}.

Application of essential oil from Greek juniper (*Juniperus excels* M. Bieb.), prickly juniper (*Juniperus oxycedrus* L.), sweet fennel (*Foeniculum vulgare* Mill.) and bay laurel (*Laurus nobilis* L.) significantly reduced the reproduction potential of *B. brassicae* and brought about mortality (Mustafa and Gorur, 2009). Motazedian *et al*. (2014) noticed that artemisia ketone from same plant showed mortality in cabbage aphid, *Brevicoryne brassicae* (L.) at a concentration of 25 μL mL^{-1}. Czerniewicz *et al*. (2018) observed that sabinene and β-myrecen in *Artemisia absinthium* L. caused mortality in *M. persicae* at a concentration of 6.90 μL mL^{-1}.

2.1.2.3. Storage Pests

Mollah and Islam (2002) evaluated the toxic effect of hexane extract of akanda root against adults of cowpea seed beetle, *Callosobruchus maculatus* Pic. and observed that the lowest LD_{50} value (538.35 μgcm^{-2}) was recorded after 72 hours. Jayasekara *et al.* (2005) confirmed the efficacy of methanol extract from roots of *Securidaca longipedunculata* Fers. against maize weevil, *Sitophilus zeamais* (Motschulsky). The findings by Kumar *et al.* (2010) disclosed that application of ethanolic extracts of custard apple, *A. squamosa* @ one per cent and five per cent resulted in complete mortality of *S. oryzae* after 39.6±1.4 and 14.5±1.1 minutes respectively.

There was 63.33 per cent reduction in adults of pulse beetle, *Callosobruchus chinensis* L. when treated with 10% ether extracts of Ashwagandha (*Withania somnifera* (L.) Dunal) roots (Gupta and Srivastava, 2008). The findings by Rajashekar *et al*. (2010) revealed that methanol extract from roots of swallow-root, *Decalepis hamiltonii* Wight & Arn. has greater potency as insecticide against stored product pests. Arora *et al.* (2011) established that application of extracts from roots of *W. somnifera* (10%) showed significant effect on mortality of *T. castaneum* larvae (46.20 per cent).

The efficacy of extract from *W. somnifera* was also studied by Suvanthini *et al*. (2012) who observed complete mortality of *S. oryzae* on 10th day of exposure when treated with 86.07 per cent of extract. Exposure of stored grain pests, *S. oryzae*, lesser grain borer (*Rhyzopertha dominica* (F.)) and *T. castaneum* to hexane extract of giant taro, *Alocasia indica* (Linn.) @ 300 µg L^{-1} for a period of 72 hours resulted in cent per cent mortality (Rajashekar and Tonsing, 2014). Hexane extract from roots of wild pepper, *Piper sarmentosum* Roxb. exhibited mortality towards *S. oryzae*, *R. dominica* and Indian mealmoth, *Plodia interpunctella* (Hübner) (Hematpoor *et al*., 2017).

Ethanol extract from bark of *V. negundo* caused mortality in larval and adult stages of *T. castaneum* (Herbst) (Chowdhury *et al*., 2009). Methanolic extract from root of Senegal prickly-ash, *Zanthoxylum zanthoxyloides* (Lam.) @ five per cent resulted in mortality of *S. zeamais* and *Callosobruchus maculates* (Fabricius) at 96 HAT (Udo, 2011). The findings by Gomathi and Rathinam (2017) pointed out that bark extract from arjun tree, *Terminalia arjuna* (Roxb.) Wight & Arn. showed higher mortality in larvae of *S. oryzae.*

The results by Loko *et al.* (2017) revealed that acetone extract from leaves of Senegal mahagony, *Khaya senegalensis* (Desr.) A. Juss. is toxic towards bamboo powder post beetle, *Dinoderus porcellus* L. with LC_{50} value of 0.29 μLinsect^{-1}. The findings by Remi-Esan and Bankole (2020) revealed that application of methanolic extract from stem bark of red ironwood tree, *Lophira alata* (Ekki) @ 2 g per 20 g and 3 g per 20 g of cowpea seeds eliminated entire population of *C. maculatus* within 48 and 96 hours of application.

The extracts from Syrian rue (*Peganum harmala* L.), ground pine (*Ajuga iva* (L.) Schreb), Dutchman's pipe (*Aristolochia baetica* L.) and radish (*Raphanus raphanistrum* L.) disrupted the normal biology of *T. castaneum*. Moreover, both the larvae and adults exhibited mortality too (Jbilou *et al*., 2006). Khoshnoud *et al*. (2008) disclosed that application of extracts from mullein *Verbascum* spp. resulted in cent per cent mortality in adults of *S. oryzae* at 21st day of exposure. Krishnan and Murugan (2015) demonstrated that flavonoids in liverwort,

Marchantia linearis Lehm. & Lindenb. @ 3% showed 45.00 per cent larvicidal and 40.80 per cent pupicidal effects against *S. litura*. Ingestion of saline extract from leaves of Brazilian pepper tree, *Schinus terebinthifolius* Raddi (100 and 250 mg of extract per g of *wheat* flour) resulted in death of maize weevil, *S. zeamais*. The mortality rates ranged between 94 and 97 per cent after 12 days of incubation (Camaroti *et al*., 2018).

LC_{50} values for aqueous extracts of neem, bishkatali and akanda towards *S. oryzae* were 2.27, 2.19 and 2.54 µL per insect after 72 hours of treatment (Shahjahan and Amin, 2000). Zhong *et al.* (2017) reported that azadirachtin affected the survival of neonate larvae from treated eggs, prolonged larval development and duration of the pupal stage in coconut spike moth, *Tirathaba rufivena* Walker. Moreover, there was also a reduction in the percentage of adult emergence and longevity of emerged adults.

The findings by Germinara *et al.* (2017) revealed the fumigant and toxic effects of essential oil from English lavender, *Lavandula angustifolia* Mill. against adults of granary weevil, *Sitophilus granarius* (L). Application of essential oils from pepper (200 µL per 500 cm^3 of treated surface), cinnamon (30 µL per 500 cm^3), clove (30 µL per 500 cm^3) and lemongrass (200 µL per 500 cm^3) resulted in 100, 95.55, 76.67 and 68.89 per cent mortality, respectively in *S. oryzae* (Binseena *et al*., 2018). Contact application of essential oil from Indian borage, *Plectranthus amboinicus* @ 1200 ppm on cowpea bruchids exhibited 100 per cent mortality after 168 hours and fumigation with 12 µL L^{-1} air resulted in elimination of entire population after 72 hours (Wanna and Kwang-Ngoen, 2019).

2.1.2.4. Other Pests

As per the findings by Tian *et al.* (2010), application of root powder of *Derris hancei* containing 7% rotenone caused 95.32 per cent population of red imported fire ant, *Solenopsis invicta* Buren to eliminate the nest. LC_{50} of extracts of *A. squamosa* and soursop (*Annona muricata* L.) were less than one per cent for adult mosquitoes, 0.5 per cent for larvae of *Aedes albopictus* (Skuse), 1 to 5 per

cent for adult mosquitoes and 0.5 to 1 per cent for larvae of *Culex quinquefasciatus* Say (Ravaomanarivo *et al.*, 2014). Application of root extracts of *Derris elliptica* (Wall.) Benth @ 0.09, 0.13, 0.17, 0.21 and 0.25 per cent resulted in 32.80, 49.60, 72.00, 95.20 and 100.00 per cent mortality in larvae of mosquito, *Aedes aegypti* (L.) (Permatasari and Sumanto, 2019).

Methanolic extracts from bark of pummelo, *Citrus grandis* @ 40 and 60 mg mL^{-1} were proved to be effective against third instar larvae of *A. aegypti* (Gutierrez *et al.*, 2014). Overgaard *et al.* (2014) observed that hexane extract from bark of olon tree, *Zanthoxylum heitzii* was effective against females of *Anopheles gambiae* Giles in causing mortality with LD_{50} value of 102 ng mg^{-1}. Application of bark extract of *Cinnamosma* spp. eliminated 90 per cent of adult *A. aegypti* mosquitoes within 24 hours (Inocente *et al*., 2019).

Jeyabalan *et al.* (2003) observed that methanol extract from leaves of *Pelargonium citrosa* exhibited toxicity towards *Anopheles stephensi* Liston. Ali *et al*. (2014) proved that bark extract of *Rhizophora mucronata* Lam. in ethanol was effective against fourth instar larvae of *A. aegypti* with LC_{50} value of 0.03 and LC_{90} value of 0.0915 µg mL^{-1}. Ezemuoka *et al.* (2019) investigated the efficacy of aqueous extract of stem bark in bringing about mortality in *A. aegypti*. It was observed that with 12.50 µg mL^{-1} of the extract, there was 38.70 per cent mortality and the mortality was 87.10 per cent when the extract was concentrated to 200 µg mL^{-1}.

Kaushik and Saini (2008) recorded a mortality of 98.33 per cent in second instar larvae of *C. quinquefasciatus* on application of leaf extract of Indian cork tree, *Millingtonia hortensis* L. f. in acetone (300 ppm). Leaf extract of *Zanthoxylum heitzii* L. @ one per cent exhibited mortality in larvae of *A. gambiae* (Overgaard *et al*., 2014).

Rahuman *et al.* (2009) reported that extracts of bush morning glory, *Ipomea carnea* Jacq. leaf in acetone, hot water, methanol and petroleum ether showed larvicidal activity against fourth stage larvae of *C. quinquefasciatus*. As per the research by Kim and Ahne (2017), LC_{50} values of extracts of Japanese pepper, *Zanthoxylum piperitum* (L.) DC bark in methanol, hexane and chloroform

towards third instar larvae of *A. aegypti* after 24 hours of exposure were 3.95, 4.21 and 5.68 mg L^{-1} respectively. Permatasari and Sumanto (2019) claimed that LC_{50} and LC_{90} values observed with methanol extracts of roots of *D. elliptica* in controlling larvae of *A. aegypti* were 1600 and 2040 ppm.

The investigations by Thomas *et al*. (2004) demonstrated 100 per cent larvicidal property in essential oil of *I. cairica* in *Culex tritaeniorhynchus* Giles, *A. aegypti*, *A. stephensi* and *C. quinquefasciatus* at concentrations of 100, 120, 120 and 170 ppm respectively. Kang *et al.* (2009) observed that rosemary oil resulted in nearly 50 per cent mortality in caged *Culex pipiens pallens* Coquillett, while citronella and lemongrass oil provided only 20 per cent kill, when all were applied in aerosol form at 1000 ppm. Cardol exhibited the strongest larvicidal and pupicidal effects and presented the most prolonged residual activity against larvae and pupae of *A. aegypti* and *C. quinquefasciatus* under field conditions (de Carvalho *et al*., 2019).

2.1.3. Effect on Biology of Pests

2.1.3.1. Leaf Feeders

Cespedes *et al.* (2005) observed that application of methanolic root extract of bilberry cactus, *Myrtillocactus geometrizans* (Mart.) Console regulated growth in fall army worm, *Spodoptera frugiperda* (J. E. Smith). Application of Allyl Isothiocyanate (AITC), breakdown product of glucosinolates in cruciferous plants resulted in 100 per cent mortality of *S. litura* @ 3125 ppm. There was also reduction in adult emergence and larval growth indices with the application of the compound (Bhushan *et al.*, 2016). Kovarikova and Pavela (2019) suggested that a blend of neem and karanj oil in the ratio 1:1 was more effective against *L. decemlineata* (LC_{50} value of 0.065 g L^{-1}) than either of them applying alone.

According to Pavunraj *et al*. (2006), hexane extract from leaves of blinding tree, *Excoecaria agallocha* L. @ 5% deterred oviposition and caused ovicidal activity in *S. litura*. Baskar *et al*. (2011) noticed that larval period was extended in *S. litura* with the application of leaf extract of *A. tagala* in ethyl

acetate. Application of root extracts of *W. somnifera* resulted in disruption of moulting and changes in normal metamorphosis leading to a number of developmental aberrations such as prolongation of the pre-pupal life span, delay in pupal-adult ecdysis, reduced rate of pupation and adult emergence, formation of larval-pupal and pupal-adult intermediates, abnormal pupae and adultoids in *S. litura* and castor hairy caterpillar, *Pericallia ricini* F. (Gaur and Kumar, 2020).

Methanol extract from leaves of yellow oleander, *Thevetia nerifolia* Juss. ex A. Dc. caused inhibition in pupation and adult emergence by 29.60 and 22.30 per cent respectively in *S. litura* (Ray *et al*., 2012). Yogesh *et al*. (2013) reported that methanolic extracts from leaves of carrot grass, *Parthenium hysterophorus* L. and croftonweed, *Ageratina adenophora* (Spreng.) @ 10% each caused deformations in all instars of *S. frugiperda*. Methanolic extract from leaves of felt bush, *Kalanchoe beharensis* Drake and long-flower kalanchoe, *Kalanchoe longiflora* Schltr. inhibited adult emergence in *S. littoralis* by 73.30 and 93.30 per cent respectively (Ghaly *et al*., 2014).

Selvamuthukumaran and Aruvudainambi (2010) reported that application of lactone glycoside present in garari, *Cleistanthus collinus* (Roxb.) Benth reduced adult emergence in *S. litura*. The percent emergence inhibition ranged from 40.00 to 86.67 per cent, 33.33 to 73.33 per cent and 26.67 to 60.00 per cent at 0.1 to 0.5 ppm against third, fourth and fifth instars each. Ge *et al*. (2015) observed that application of 10 to 40 mg L^{-1} of alkaloids from dog strangling-vine, *Cynanchum mongolicum* Yang reduced the rates of pupation and emergence rates of *S. litura* and mortality rate increased on third day. Magierowicz *et al*. (2020) noticed that oviposition of snout moth, *Acrobasis advenella* (Zinck.) was detrimentally affected when the fruits of black chokeberry (*Aronia melanocarpa* [Michx.] Elliot) were treated with aqueous extracts of *T. vulgare* (2.43 per cent) and summer savory, *Satureja hortensis* L. (3.89 per cent).

Adverse effect of essential oil from clove on fecundity and egg viability in *S. frugiperda* was reported by Cruz *et al*. (2016). Zhong *et al.* (2017) observed that azadirachtin caused prolongation in larval development and pupal duration of coconut spike moth, *T. rufivena*. It was seen that the development of second

instars fed with leaves treated with LC_{25} (11.35 mg L^{-1}), LC_{50} (28.79 mg L^{-1}) and LC_{90} (160 mg L^{-1}) of azadirachtin was prolonged by 8.50, 11.20 and 18.40 per cent respectively.

2.1.3.2. Sucking Pests

Azadirachtin @ 200 ppm affected the survival of pupae of green lacewing, *Mallada signatus* (Schneider) (Qi *et al*., 2001).

2.1.3.3. Storage Pests

Keita *et al.* (2001) reported application of essential oil from clove basil, *Ocimum gratissimum* L. detrimentally affected egg hatching and adult emergence in *C. maculates*. The findings by Germinara *et al.* (2017) revealed the fumigant and toxic effects of essential oil of English lavender, *Lavandula angustifolia* Mill. against adults of *S. granarius*.

2.1.3.4. Other Pests

Aarthi and Murugan (2012) observed that the eggs of *A. stephensi* did not hatch at all on application of crude ethanol extract of vetiver grass, *Vettiveria zizanoides* (L.) Roberty @ 375 ppm. Saranya *et al.* (2013) observed that aqueous leaf extract of African tulip tree, *Spathodea campanulata* P. Beav. affected the larval morphology of *A. aegypti* resulting in dechitinized larva with damaged digestive tract and attachment of exuvia of the proceeding instar with the cadaver. Ahbirami *et al*. (2014) reported that acetone extract of *I. cairica* leaf resulted in darkening or blackening of abdomen and twisted abdomen in larvae of *A. aegypti* and *A. albopictus* respectively and LC_{50} values were observed to be 101.94 and 105.59 ppm each against both the pests.

Use of leaf extracts of Alexandrian laurel balltree (*Calophyllum inophyllum* L.), wild eggplant (*Solanum surattense*) and snake jasmine (*Rhinocanthus nasutus* (L.) Kurz) inhibited adult emergence in mosquitoes by 50 per cent (Muthukrishnan and Puspalatha, 2001). The findings by Arivoli and Samuel (2011) reported that dichloromethane extract of the plant bitter apple,

Citrullus colocynthis (L.) Schrad was found to be effective against larval *C. quinquefascaitus* (LC_{50} value - 240.36 ppm). They also witnessed stunted larval and pupal development, increase in larval and pupal periods, decrease in pupal transformation and adult emergence. There were ovicidal effect and delay in egg hatching in *A. stephensi*. The findings by Al-Rahimy and Al-Essa (2019) indicated that aqueous and chloroform extracts of roots, stem and leaves of English mint, *Mentha spicata* L. inhibited adult emergence in the mosquito *Culex molestus* Forskal when treated on fourth instar larvae of the insect.

The findings by Arivoli and Samuel (2011) reported that dichloromethane extract of the plant *C. colocynthis* was found to be effective against larval *C. quinquefascaitus* (LC_{50} value - 240.36 ppm). They also witnessed stunted larval and pupal development, increase in larval and pupal periods, decrease in pupal transformation and adult emergence. There were ovicidal effect and delay in egg hatching in *A. stephensi*. Ojiako *et al*. (2015) observed that application of pyrethrum @ 0.25 g, 0.5 g and 0.75 g per 100 mL of water reduced the population of termite white grub (*Holotrichia serrata* F.) and grasshopper (*Oedaleus nigeriensis* Uvarov) in groundnut field to 3.25, 2.50, 2.10 when compared to 4.09, 3.62, 3.42 respectively in unsprayed plots at 4, 6 and 8 Weeks After Planting (WAP). Treatment of melon fly, *Bactrocera cucurbitae* (Coquillett) with extracts of gum Arabic tree, *Acacia nilotica* (Linn.) bark in ethanol and acetone in various concentrations from 1 to 625 ppm witnessed changes in developmental period of the pest (Vasudev *et al*., 2015).

Fatima *et al*. (2011) reported that in *C. quinquefasciatus*, the highest Oviposition Deterrent Index (ODI) was observed with 0.5% *Azadirachta indica* A. Juss. (neem) extract and the lowest ODI was in 0.1% *Momordica charantia* L. extract. Application of sublethal concentration of acetone and hexane extracts of *Ruta chalepensis* L. induced sterility in larvae of *C. pipiens*, which was indicated by sterility indices. These values were 61 and 55 per cent respectively (El-Bokl, 2016). Hexane extract of citronella grass, *Cymbopogon nardus* (L.) Rendle deterred oviposition and inhibited adult emergence in *C. quinquefasciatus* (Ilahi *et al*., 2019).

2.2. MANAGEMENT OF INSECT PESTS

2.2.1. Botanical Insecticide Formulations

2.2.1.1. Emulsifiable Concentrate (EC)

Puripattanavong *et al*. (2013) reported that concentrated emulsion containing 0.1% w/w of nicotine developed using tween 80 and span 80 as surfactants showed aphidicidal activity under field conditions. Zuleta-Castro *et al.* (2017) developed an emulsion using ethanol extract of neem and reported that 0.3% of the formulation resulted in 1.90 per cent reduction in leaf area attack by *S. frugiperda*. Sundaran and Faizal (2018) observed that the treatments Cashew Nut Shell Liquid (CNSL) 0.2% and neem oil emulsion 2% resulted in 64 and 58 per cent mortality respectively in chilli aphid, *Aphis gossypii* Glover at 24 HAT.

Application of Emulsifiable Concentrate (EC) formulation from bitter bean, *Sophora alopecuroides* L. extract at 20 and 30 mg mL^{-1} each resulted in 15.97 and 31.97 per cent reduction in the number of eggs hatched in Asian citrus psyllid (Rizvi *et al*., 2019). There was also reduction in honeydew production (72.86 and 85.50 per cent each) in comparison with control. Purkait *et al*. (2019) reported the efficacy of EC formulations from *A. squamosa* extract against *B. brassicae* (4000 ppm) in terms of mortality (80.70 per cent). Meanwhile, mortality rates were 70.60 and 45.10 per cent each with pongamia, *Milletia pinnata* (L.) Panigrahi and yam bean, *Pachyrhizus erosus* (L.) Urb. Application of emulsion containing *A. paniculata*, pongamia oil and triton X-100 (7:2:10) @ 5 per cent witnessed the lowest population of aphids, thrips and mites in chilli (Bhavyashree, 2019).

Tang and Hou (2008) reported that microemulsion developed from chamaejasmin exhibited insecticidal activity against groundnut aphid, *Aphis craccivora* C. L. Koch and *C. pipiens*, with LC_{50} values being 47.93 and 31.78 mg L^{-1} respectively. According to Min and Yueguan (2009), microemulsion developed from *Piper sarmentosum* Roxb. was toxic to aphids with LC_{50} value of 7.110 mg L^{-1}. Fernandes *et al*. (2014) developed microemulsion from ethanolic extract of fruits of *Manilkara subsericea* (Mart.) Dubard and claimed that it

exhibited insecticidal activity in cotton stainer, *Dysdercus peruvianus* Guerin-Meneville. Microemulsions prepared by encapsulating essential oils from anise burnet (*Pimpinella anisum L.*), ajwain (*Trachyspermum ammi* Sprague) and sea fennel (*Crithmum maritimum L.*) *exhibited* toxicity against mosquito larvae, with LC_{50} values in the range 1.45 to 4.01 mL L^{-1}. There was also high larval mortality and lower adult emergence (Pavela *et al*., 2019). Microemulsion of cinnamon essential oil prepared using tween 80 and anhydrous ethanol repelled rice weevil completely after 48 hours of treatment. Mortality rates observed by contact and fumigant action were 96.67 and 86.67 per cent respectively, after 96^{th} h of treatment (Shi *et al*., 2021).

Campolo *et al.* (2017) developed nanoemulsions using essential oil from citrus peel and established its lethal and sublethal effects on *T. absoluta*. Microemulsions containing essential oils from rosemary (*Rosmarinus officinalis* L.), peppermint (*Mentha piperita* L.) and gum tree (*Eucalyptus globules* L.) and extract of garden thyme (*Thymus vulgaris* L.) showed insecticidal activity and oviposition deterrent effects on *B. tabaci* were tested under greenhouse conditions (Bolandnazar *et al*., 2018). As per the findings by Sharma *et al.* (2019), application of neem oil based nanoemulsion caused mortality in *S. litura* with LD_{50} value of 10%. Essential oil from roots of silver thistle, *Carlina acaulis* L. containing 0.5% of essential oil showed LC_{50} value of 579.10 μLL^{-1} against mosquitoes (Pavela *et al*., 2019). Nanoemulsions of azadirachtin exhibited 86.6 per cent insecticidal effect against the mosquito, *C. quinquefasciatus* (Anjali *et al*., 2012). Nanoemulsions developed using crude extract from *D. elliptica* was toxic to the adults of B. tabaci with LC_{50} value of 3.70 ppm at 96 HAT (Omar et al., 2016). Lina *et al*. (2020) developed nanoemulsions from T. vogelii and claimed that it caused more than 50 per cent mortality in C. pavonana.

2.2.1.2. Wettable Powder (WP)

Lina *et al*. (2018) reported cent per cent mortality in cabbage cluster caterpillar, *Crocidolomia pavonana* (F.) when treated with formulation of WP formulations prepared using the extracts from *T. vogelii* and spiked pepper, *Piper aduncum* L.

2.2.1.3. Soluble Powder (SP)

The bioactive compounds azadirachtin and β-asarone developed as WP formulation using β- methyl cyclodextrin exhibited mortality (20.00 per cent) and weight loss (43.50 per cent) in larvae of S. litura (Chandrashekara *et al*., 2014).

2.2.1.4. Water Dispersible Powder (WDP)

Kala et al. (2017) opined that WDG formulation prepared using neem oil and metallic nanoparticles exhibited 42 per cent antifeedant effect against S. litura.

2.2.1.5. Hydrogels

Hydrogels containing essential oils entrapped in zein nanoparticles presented more than 80 per cent repellency against whitefly (*Bemisia tabaci* (Gennadius)) and two-spotted spider mite (*Tetranychus urticae* Koch) (de Oliveira *et al*., 2019).

2.2.1.6. Capsules

Purkait *et al*. (2021) developed capsule formulations from pongamia oil by coating with polyurea and established its lethality on 2nd instar larvae of silk moth, *Bombyx mori* (L.) (LC_{50} - 1.10% and LC_{90} - 5.90%). Application of 4% of the formulation also reduced the population of aphids from 67.00 to 71.80 per cent and white fly from 62.40 to 74.80 per cent in aubergine after 7 to 14 days of application.

Nanocapsules developed from extract of eucalyptus exhibited mortality in adults of *M. persicae* with LC_{50} value of 14.93 mg mL^{-1} (Khoshraftar *et al*., 2019).

2.2.1.7. Granules

Wiwattanapatapee *et al*. (2009) developed water dispersible granules using *Derris* extract and claimed that it was effective in reducing the population of *S. litura*.

2.2.1.8. Aerosols

Misni *et al*. (2011) reported 80 per cent mortality in *A. aegypti* and 71.60 per cent mortality in *A. albopictus* with the application of aerosol formulation of essential oil from *P. aduncum*. Meanwhile, Bakar *et al*. (2012) opined that

essential oil from paper bark tree, *Melaleuca cajuputi* Powell @ 5% when formulated into aerosols resulted in mortality of 22.90 and 20.00 per cent each in both these insects.

2.2.1.9. Pellets

Pellet formulation containing 1,8-cineole was prepared by Marzanghi *et al*. (2013) and reported it was lethal to *C. chinensis* and LC_{50} and LC_{95} values were 0.017 and 0.050 mL L^{-1} air respectively.

2.2.2. Botanical Insecticides

2.2.2.1. Neem based Insecticides

The commercial formulation of neem, NeemAzal, exhibited 83 per cent mortality in melon thrips, *Thrips palmi* Karny when it was applied at 0.05% v/v (Avilés *et al*., 2001). Susaimanickam *et al*. (2012) determined the effectiveness of a formulation based on neem and pungam oils (PONNEEM), against *H. armigera* and *S. litura*.

Islam *et al.* (2011) observed that application of neemazal @ 5mL^{-1} of water reduced the population of hadda beetle, *Henosepilachna vigintioctopunctata* F. to the extent of 64 per cent. Packiam *et al*. (2012) reported that 20 µL L^{-1} of PONNEEM (a formulation containing azadirachtin and karanjin) resulted in 77.48 per cent deterrence in feeding activities by *S. litura* and *H. armigera*. Pinto *et al.* (2013) reported that commercial product Neemseto® controlled cotton aphid (*Aphis gossypii* Glover) completely. The LC_{50} value of NeemAzal T/S, containing 1% of Azadirachtin A was 0.075 g L^{-1} and that of Rock Effect, another formulation was 0.582 g L^{-1} on 2 DAT (Kovarikova and Pavela, 2019).

2.2.3. Chemical Insecticides

2.2.3.1. Malathion 50 EC

Treatment with malathion 50 EC (250 g a.i. ha^{-1}) during 50 per cent flowering stage resulted in 98.70 per cent mortality in blister beetles, *Mylabris pustulata* Thunberg and *Epicauta* spp. (Boopathi *et al.*, 2009). Sisay *et al*. (2019) claimed that malathion 50 EC was moderately effective against *S. frugiperda* with a mortality of 51.70 per cent at 72 HAT. Application of malathion 50 EC @ 0.05% along with the usage of yellow sticky traps reduced the population of white

fly (2.08 per cent) and jassids (3.98 per cent) in okra (Nath et al., 2020). Kumar *et al*. (2020) observed that application of malathion 50 EC reduced the incidence of H. armigera in fruits of tomato by 85.04 per cent.

Materials and Methods

3. MATERIALS AND METHODS

The present study was carried out in the Department of Agricultural Entomology, College of Agriculture, Vellayani, Kerala and Council of Scientific and Industrial Research – National Institute for Interdisciplinary Science and Technology (CSIR-NIIST), Thiruvananthapuram during 2018 to 2021. The study was conducted with the objectives of isolation and and identification of the bioactive compounds from the bark and seed of *S. indica*, and to develop bioformulations against *Diaphania indica* Saund. and *Henosepilachna septima* (Dieke).

3.1. SCREENING OF VARIOUS PARTS OF THE PLANT FOR INSECTICIDAL PROPERTIES

3.1.1. Maintenance of Insect Culture

3.1.1.1. Diaphania indica

First instar larvae of *D. indica* were collected from snake gourd growing fields of Thiruvananthapuram district and transferred to a container (30 × 15 cm) covered with muslin cloth. The larvae were provided with fresh tender leaves of snake gourd daily till pupation. Pupae were left undisturbed for the emergence of adults and these adults were provided with 10% honey solution as food. The container was covered with a black cloth to facilitate easiness in oviposition. Whitish coloured eggs were seen scattered around the sides of the container and on the cloth. The insect culture thus developed was used for further studies (Plate 1). The species was confirmed with Indian Agricultural Research Institute (IARI), New Delhi (Plate 2).

3.1.1.2.Henosepilachna septima

Egg masses of *H. septima* were collected from host plants at various parts of Thiruvananthapuram district and kept in a Petri plate for hatching. The hatched out grubs were transferred to a container (30 × 15 cm) covered with muslin cloth and were provided with tender leaves from snake gourd daily. Fresh leaves were provided to the grubs till pupation. Further, pupae were left undisturbed for

emerging as adults. Emerged adults were fed with leaves smeared with droplets of vitamin E tablets to facilitate egg laying. Cigar shaped yellowish eggs were laid on the sides and top of the container. Eggs were transferred into Petri plate, and on hatching the grubs were transferred into a rearing jar (size). The insect culture thus developed was used for further studies (Plate 3). The species identity was confirmed with National Research Centre for Banana (NRCB), Trichi (Plate 4).

3.1.2. Processing of Plant Parts

Niepa bark tree (*Samadera indica* (Gaertn.) (family Simaroubaceae) was used for the isolation of active principles to prepare bioformulation againt the targeted pests. The identity of the tree was confirmed by the Department of Botany, University of Kerala, Kariavattom, Kerala, India, and a voucher specimen was deposited in the herbarium of this Department with the Accession Number KUBH 11062 (Plate 5).

3.1.2.1. Collection and Processing of Samadera indica Bark

Bark of *S. indica* (Plate 6) was collected from various parts of Alappuzha district in polythene bags free of moisture. It was chopped into small pieces of approximately three to five cm and shade-dried at 28±5ºC for three weeks. This was then ground into powder and stored in airtight containers for making aqueous and solvent extracts.

3.1.2.1.1. Extracts from Bark

Different concentrations of the extracts from the powdered bark of *S. indica* were prepared by cold extraction method (Wetwitayaklung *et al*., 2007) (Plate 7). Sequential extraction method was employed wherein 20 g of powder of the bark was dissolved in 150 mL of n-hexane, acetone, ethyl acetate, ethanol and distilled water successively. The solvents were added in the order of increasing polarity. All the solvents chosen for the experiment were of HPLC grade. These were then stirred using a Remi rotary shaker for 72 h and the filtrates were separated out through cheese cloth and Whatman No. 1 filter paper successively.

Plate 1A. Larvae kept within rearing jar

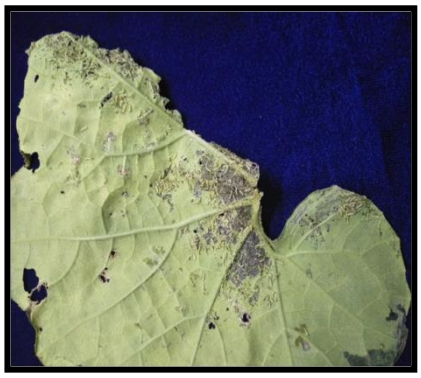


Plate 1B. First instar larvae of *D. indica*

Plate 1C. Pupation of *D. indica* within container

Plate 1D. Pupae

Plate 1E. Adults feeding on honey solution

Plate 1. Rearing of *Diaphania indica*

A. Egg

B. Larvae

C. Pupa

D1. Adult male

D2. Adult female

Plate 2. Developmental stages of *Diaphania indica*

Plate 3A. Grubs of *H. septima* within rearing jar

Plate 3B. Egg mass

Plate 3C. Egg mass prior to hatching

Plate 3D. Second instar grubs

Plate 3E. Pre-pupa

Plate 3F. Pupa

Plate 3. Rearing of *Henosepilachna septima*

A. Egg mass

B. Grub

C. Pupa

D. Adult

Plate 4. Developmental stages of *Henosepilachna septima*

Plate 5. *Samadera indica*

Plate 6. Dried bark of *Samadera indica*

Plate 8. Seeds of *Samadera indica*

A. Powdered bark/seeds

F. Solvent-free extract used for extract level studies

B. Addition of plant powder to solvents

E. Evapouration of solvents using rotary vacuum flash evapourator

C. Shaken using rotary shaker

D. Filtration using cheese cloth and Whatman No. 1 filter paper

Plate 7. Cold extraction method

The filtrate was further subjected to evaporation using Heidolph rotary vacuum flash evaporator to obtain solvent-free extract.

3.1.2.2. Collection and Processing of Samadera indica Seeds

Seeds of *S. indica* (Plate 8) collected from trees during June – July from various parts of Alappuzha district were decorticated and shade-dried at 28±5ºC for three weeks. Grinding these into powder, those were stored in airtight containers for preparing extracts.

3.1.2.2.1. Extracts from Seeds

Extracts from seeds were prepared by cold extraction method as described in 3.1.2.1.1. Further experiments were carried out using this extract, which was preserved in refrigerated condition.

3.1.3. Preparation of Neem Seed Kernel Extract (NSKE)

Neem seeds (50 g) ground into coarse powder were stored in muslin cloth bag. It was immersed in 1 L of water and after keeping it overnight, the cloth bag was squeezed thoroughly till clear liquid is obtained.

3.1.4. Effect of Bark Extracts of *Samadera indica* on *Diaphania indica*

3.1.4.1. Estimation of Lethal Doses

Solvent extracts at varying concentrations, ranging from 0.5 to 5% as fixed by trial and error method were sprayed on the larvae (20 nos.) taken in a Petri plate using potter precision laboratory spray tower. The larvae were provided with fresh leaves of snake gourd daily. Mortality of the larvae were observed for a period of 72 h and expressed in percentage (Paramasivam and Selvi, 2017).The data was subjected to probit-dose analysis to obtain LD_{50} and LD_{90} values using the software GRAPES (General R-shiny based Analysis Platform Empowered by Statistics), developed by KAU (Kerala Agricultural University).

The extracts at LD_{50} and LD_{90} were taken for further experiments involving the assessment of antifeedant effect, insecticidal effect and effect of extracts on biology of the test insect, *D. indica*.

3.1.4.2.Assessment of Antifeedant Effect

Antifeedant effect of *S. indica* extract at LD_{50} and LD_{90} of the extracts were tested by no-choice method (Bentley *et al.*, 1984). Leaf discs of approximately 5 cm diameter were taken and sprayed with LD_{50} and LD_{90} of the extracts. A third instar larva pre starved for four hours was kept in the leaf disc after removing excess moisture from the leaves. Care was taken to avoid drying of leaves by wrapping moist cotton on the petioles. The experiment was conducted in CRD with 10 treatments and three replications.

Quantity of leaf consumed by the larvae (W) at 24, 48 and 72 HAT was calculated by the formula

$$W = W_1 – W_2$$

where

W_1 - Weight of remaining leaf disc after consumption by larvae on previous day.W_2 – Weight of leaf disc after consumption by larvae on that day

The following were the treatments applied:
T1: LD_{50} of hexane extract
T2: LD_{90} of hexane extract
T3: LD_{50} of acetone extract
T4: LD_{90} of acetone extract
T5: LD_{50} of ethanol extract
T6: LD_{90} of ethanol extract
T7: LD_{50} of aqueous extract
T8: LD_{90} of aqueous extract
T9: NSKE 5% (Check)
T10: Distilled water

3.1.4.3.Assessment of Insecticidal Effect

Insecticidal effect of *S. indica* extract at LD_{50} and LD_{90} was estimated, wherein second instar larvae were treated with the extract topically (Pung and Srimongkolchai, 2011).Three replications of each treatment were done with twenty insects in one replication. Percentage mortality was calculated using the formula given by Abbot (1925).

Treatments given were as, except T9 that was Malathion 50 EC @ 0.1% (Check).

3.1.4.4. Assessment of Effect on Biology of Diaphania indica

The effect of bark extracts of *S. indica* on the biology of *D.indica* was studied by observing deformed larvae/pupae/adults, larval duration (days), pupal weight (g), pupal duration (days), adult longevity (days), fecundity (nos.), egg hatchability (nos.), pre-oviposition period (days) and incubation period of eggs (days). Here, topical application of the extract was carried out. Second instar larvae of *D. indica*, 20 nos. each, were topically applied with the insecticides mentioned under 3.1.4.2.

3.1.4.4.1. Deformities in Larvae, Pupae and Adults

Larvae, pupae and adults emergence were observed for malformations due to the treatments.

3.1.4.4.2. Effect on Larval Duration

Mean number of days required from second instar larval stage till the completion of last larval instar was observed.

3.1.4.4.3. Effect on Pupal Weight

Mean weight of pupa (g) was recorded from those that have been developed from plant extract treated larvae.

3.1.4.4.4. Effect on Pupal Period

Mean time period between the onset of pupal development and adult emergence was estimated.

3.1.4.4.5. Effect on Adult Longevity

The effect of *S. indica* extracts in altering the life span of adults of *D. indica* was assessed.

3.1.4.4.6. Effect on Fecundity, Egg Hatchability, Pre-oviposition Period and Incubation Period

The effect of *S. indica* extracts on fecundity, hatching of eggs, alteration in pre-oviposition period and incubation period of eggs in comparison to control was studied on adults emerging from topically treated larvae.

3.1.5. Effect of Bark Extracts of *Samadera indica* on *Henosepilachna septima*

3.1.5.1.Estimation of Lethal Doses

The grubs (20 nos.) were sprayed with *S. indica* seed extracts at varying concentrations, ranging from 0.5 to 15% as fixed by trial and error method. Mortality of the insects was observed for a period of 72 h and expressed in percentage. LD_{50} and LD_{90} of the extract were obtained from probit-dose analysis done using the software GRAPES.

The extracts at LD_{50} and LD_{90} were taken for further experiments involving the assessment of antifeedant effect, insecticidal effect and effect of extracts on biology of the insects as in 3.1.4.

3.1.5.2.Assessment of Antifeedant Effect, Insecticidal Effect and Effect on Biology of Insects

Antifeedant effect of *S. indica* bark extract at LD_{50} and LD_{90} was tested by no-choice method (Bentley *et al.,* 1984) as in 3.1.4.2. Insecticidal effect of *S. indica* extract at LD_{50} and $LD_{90,}$ expressed as percentage mortality was estimated as per the method described in 3.1.4.3. The effect of bark extract of *S. indica* on various parameters of insect biology was studied as in 3.1.4.4.

3.1.6. Effect of Seed Extracts of *Samadera indica* on *Diaphania indica*

3.1.6.1. Lethal Doses

Various doses of aqueous and crude extracts ranging from 0.5 to 15 per cent were checked for mortality in second instar larvae of *D. indica* as in 3.1.4.1. and LD_{50} and LD_{90} were estimated using the software GRAPES. These values were used for further estimation of antifeedant effect, insecticidal effect and effect on biology of insects.

3.1.6.2. Antifeedant Effect

Antifeedant effect of *S. indica* extract was assessed by no-choice method by Bentley *et al.* (1984) as in 3.1.4.2.

3.1.6.3. Insecticidal Effect

Insecticidal effect of *S. indica* seed extracts on *D. indica* was studied by topical application as in 3.1.4.3.

3.1.6.4. Effect on Biology of Insects

The effect of seed extract of *S. indica* on various parameters of insect biology was studied as in 3.1.4.4.

3.1.7. Effect of Seed Extracts of *Samadera indica* on *Henosepilachna septima*

3.1.7.1. Lethal Doses

Aqueous and crude extracts of various doses concentrations ranging from 0.5 to 15% were tested for mortality in second instar grubs of *H. septima* as in 3.1.4.1. and LD_{50} and LD_{90} values were estimated using the software GRAPES. Studies on antifeedant effect, insecticidal effect and biology of insects were studied using these values.

3.1.7.2. Antifeedant Effect

Feeding deterrence of *H. septima* towards seed extract of *S. indica* was evaluated by no-choice method developed by Bentley *et al.* (1984). Experimental set up and observations were as in 3.1.5.2.

3.1.7.3. Insecticidal Effect

Mortality of *H. septima* on the application of *S. indica* seed extract was studied as in 3.1.5.3.

3.1.7.4. Effect on Biology of Insect

The effect of seed extract of *S. indica* on various parameters of insect biology was studied as in 3.1.5.4.

3.1.8.Fractionation of Effective Extract

To isolate the bioactive compounds from the bark and seed of *S. indica*, the solvent extract was subjected to column chromatography followed by Thin Layer Chromatography (TLC). Column chromatography was performed with a column of 90 cm length and 4.50 cm width. The base of the column was closed with a small bit of cotton and it was placed in position using a long glass rod. Silica gel (100 to 200 mesh) was mixed with n-hexane to form slurry and this was poured into the column using a funnel. Sides of the column were tapped intermittently for better adsorption of hexane onto silica and excess hexane was removed through nozzle at the bottom. After filling three-fourth of the column with hexane slurry, 1 g of sample was loaded on the top and a layer of cotton was kept above it. Hexane that was retained above cotton was eluted out. Then the packed column was left as such for half an hour for better adsorption of compounds with silica gel. After this, the column was eluted using a solvent system comprising of non-polar solvents followed by polar solvents (gradient elution technique). Elution rate was 5 to 10 drops per minute.

The following were the mobile phases used in column chromatography:

Hexane – 100%

Hexane: Ethyl acetate – 95:5

Hexane: Ethyl acetate – 90:10

Hexane: Ethyl acetate – 85:15

Hexane: Ethyl acetate – 80:20

Hexane: Ethyl acetate – 75:25

Hexane: Ethyl acetate – 70:30

Hexane: Ethyl acetate – 65:35

Hexane: Ethyl acetate – 60:40

Hexane: Ethyl acetate – 50:50

Hexane: Ethyl acetate – 40:60

Hexane: Ethyl acetate – 20:80

Hexane: Ethyl acetate – 10:90

Ethyl acetate – 100%

Ethyl acetate: Methanol – 95:5

Ethyl acetate: Methanol – 90:10

Ethyl acetate: Methanol – 40:60

Methanol – 100%

From each eluent system, certain number of fractions (10 mL each) were collected (based on colour change) in 15 mL test tubes. These fractions were then concentrated using a rotary evaporator at 50°C to obtain dry powder.

Few drops of DMSO (Dimethyl sulfoxide) were added to dry powder to solubilise it and the powder in dissolved form was spotted onto a TLC (silica coated) plate using capillary tube and the plate was left to dry. This plate was kept in a solvent chamber containing solvent combination from which the dry powder was obtained. Once the solvent rose to three-fourth of TLC plate by capillary action, the plate was removed from solvent, dried under room temperature and visualized under Ultra Violet (UV) torch to observe the fractions containing UV active compounds.

3.2.BIOEFFICACY STUDY OF ISOLATED BIOACTIVE COMPOUND

Detection of Retention Factor (R_f) is important for identifying and combining UV active fractions which contain bioactive compounds. Retention Factor (R_f) was calculated for UV active fractions using the equation

R_f = Distance travelled by the solute (spot) (cm)/Distance travelled by solvent front (cm)

The concentrated sub-fractions from column chromatography with same R_f values, in combination, were clubbed together and checked for their efficacy on test insects.

3.2.1. On *Diaphania indica*

Concentrated fractions with same R_f value and no residual solvent were clubbed together and made upto 5, 2.5 and 1.25% levels each, using distilled water. Addition of DMSO facilitated easy mixing of the fractions in distilled water. Bioefficacy of the prepared extract fractions were assessed on second instar larvae of *D. indica*. The experiment was conducted in CRD with four replications. Check treatments were neemazal @ 0.2% and malathion 50 EC @ 0.1% 0.1%. Observations were taken on quantity of leaf consumed by the treated larvae at 1, 3, 5 and 7 days after treatment (DAT) and mortalityat 24, 48 and 72 HAT.

3.2.2. On *Henosepilachna septima*

Bioefficacy of chromatographic fractions was tested on second instar grubs of *H. septima* as in 3.2.1.

3.3. STRUCTURAL CHARACTERISATION OF THE BIOACTIVE COMPOUND

The chromatographic fraction containing effective bioactive compound/compounds isolated from 3.2. were compared for their similarities with the marker compounds (quassinoids). Quassinoids were identified using NMR technique. For NMR, proton (^{1}H) and carbon (^{13}C) NMR experiments were employed. Structures of the compounds were drawn using ChemDraw software, developed by PerkinElmer Informatics.

3.4. DEVELOPMENT OF SUITABLE FORMULATIONS OF BIOACTIVE COMPOUNDS

Formulations of extracts containing bioactive compounds were developed with the objectives of enhanced availability at the target site, easiness in delivery and better storage life. Suitable formulations containing effective bioactive

compound/compounds were developed by temperature of inversion phase technique (Fernandes *et al*., 2013). Formulations were prepared by adding extract containing active ingredient/bioactive compound, emulsifier/surfactant and solvent in suitable proportions. The emulsifiers and suitable proportions were standardized based on HLB (Hydrophilic Lipophilic Balance) values.

3.4.1. Stability of Formulation

The stability of the formulation was assessed for a period of one week by physical tests described by BIS (Bureau of Indian Standards), 1987.

3.4.1.1. Colour of Formulation

Colour of the formulations was noticed for a period of one week.

3.4.1.2. Appearance of Formulation

The formulations were checked for their oily or viscous nature.

3.4.1.3. Filter Paper Test

The formulations were added to filter paper and checked for the pattern of spreading.

3.4.1.4. Acidity

Acidity of the formulations was estimated using pH meter.

3.4.1.5. Electrical Conductivity (EC)

Electrical Conductivity (EC) was estimated using EC meter and expressed in µS.

3.4.1.6. Cold Test

Hundred mL of formulations each were taken in a beaker and cooled to 10ºC. These were stirred intermittently for 1h and thereafter examined for turbidity or separated oily/solid matter.

3.4.1.7. Heat Stability

Test for heat stability was performed as per the procedure by Allawzi (2016). Formulations for analysis (100 mL) were taken in clean transparent containers and heated to 25, 35, 45and 55ºC on 7, 14, 21, 28 and 35th day. Then, they were cooled to room temperature and further examined for the presence of turbidity or separated oil/solid matter.

3.4.1.8. Bloom Test

Bloom test was done to rate the quality of emulsions in terms of creaming, sedimentation and presence of oil particles. It was done as per the procedure given by Bessette (2007). One mL of each of the formulations was added to 99 mL of distilled water by dipping the tip of the micropipette two cm into distilled water. The quality of emulsions was rated based on the bloom observed and emulsion was coded accordingly.

Bloom rating	Code	Observations
Excellent	5	White bulging emulsion with no oil droplets
Good	4	White bulging emulsion with very few oil droplets
Fair	3	White emulsion with some oil droplets
Poor	2	Poor emulsion with many oil droplets
Unacceptable	1 - 0	No emulsion with only oil droplets

3.4.2. Bioefficacy Studies of Formulation

The insecticidal properties of formulation were assessed by subjecting the formulation to bioefficacy studies on test insects.

3.4.2.1. On Diaphania indica

Based on LD_{50} and LD_{90} of effective extract, two effective doses of each of the two formulations were selected and tested for their efficacy against second instar larvae of *D.indica* by assessing mortality rate at 72 HAT. Design adopted for the experiment was CRD, with three replications. Neemazal @ 0.2% and malathion 50 EC @ 0.1% were taken as checks. Untreated larvae were taken as control.

3.4.2.2. On Henosepilachna septima

Efficacy of two effective doses of each of the two formulations was assessed on second instar grubs of *H. septima* in terms of mortality at 72 HAT. Experimental set up and treatments adopted were same as in 3.4.2.1.

3.4.3. Safety Evaluation of Formulation

Safety assay was carried out on pollinators and parasitoids.

3.4.3.1. Evaluation of Safety to Pollinators

Among pollinators, safety test was done in Indian honey bees (*Apis cerana indica* (Fabricius)). For the experiment, formulations at effective dose and double the effective dose were smeared at the base of the round bottom container using cotton. Fifty per cent honey was also smeared on the sides of the container as food (Sharma and Abrol, 2005). Neemazal @ 0.2% and malathion 50 EC @ 0.1% have served as check treatments and the experiment was replicated thrice. There were 10 insects per replication and observations were taken on the number of dead bees at 6, 12 and 24 HAT and expressed as percentage mortality.

3.4.3.2. Evaluation of Safety to Parasitoids

Among parasitoids, the test insects chosen for safety assay were those that parasitize *D. indica* viz., *Apanteles taragamae* Viereck (Hymenoptera: Braconidae). Adults of *A. taragamae* were obtained from cocoons present on parasitised larvae of *D. indica* and the identity of the specimen was confirmed at Integrated Farming Systems Research Station, Karamana, Kerala. The test insects

were taken in a test tube and the mouth was covered with cheese cloth. The insects were treated by 'dry film technique' wherein the bottom of the test tube was coated with thin film of the extract and it was left to dry under air for a few seconds. The parasitoids were fed with 10% honey solution. Treatments were given at effective dose and double the effective dose (Desneux *et al*., 2006). Check treatments adopted were neemazal @ 0.2% and malathion 50 EC @ 0.1%. The experiment was replicated thrice with ten insects per replication. Observations were taken on the number of dead insects at 1, 3 and 5 DAT and expressed as percentage mortality.

3.4.4. Shelf Life of Formulations

3.4.4.1. Against Diaphania indica

Formulations A and B @ 0.5 and 1% were subjected to shelf life assessment by observing mortality of insects at 72 HAT for a period of six months at monthly intervals. The experiment was conducted in CRD with three replications, each containing twenty insects.

3.4.4.2. Against Henosepilachna septima

Formulations A and B @ 0.5 and 1% were subjected to shelf life evaluation by observing mortality of grubs at 72 HAT for a period of six months at monthly intervals. Experimental design was CRD with three replications, each containing twenty insects.

3.5. FIELD EVALUATION OF FORMULATION AGAINST LEAF FEEDING PESTS IN SNAKE GOURD

For field evaluation of effective formulation, snake gourd plants of variety Kaumudi were raised in pits with a spacing of 2 × 2 m (Plate 9). Seeds of snake gourd were procured from Department of Vegetable Science, College of Agriculture, Vellayani. The crop was raised in plots of size 6 × 2 m, as per Package of Practices recommendations of Kerala Agricultural University (KAU, 2016).

Plate 9. Layout of experimental field

Effective formulations developed in 3.4. at double the dose were evaluated for their efficacy against leaf feeding pests in snake gourd *viz*., *D. indica* and *H. septima* at vegetative stage and 50 per cent flowering.

3.5.1. Efficacy of Formulation

3.5.1.1. At Vegetative Stage

3.5.1.1.1. Pest Population

The effective dose of formulation standardized from 3.4.2.1. was tested in field at vegetative stage for its efficacy against leaf feeding pests by taking pre-count and post-count of insects on alternate days of treatment upto one week. The experiment was laid in RBD with four treatments and five replications.

The following were the set of treatments:

T1: Effective treatment from 3.6.2.1.

T2: Neemazal 1 % @ 0.2%

T3: Malathion 50EC @ 0.1%

T4: Untreated

3.5.1.1.2. Damage on Leaves

Damage caused by pests was assessed on alternate days of treatment by counting the number of infested leaves out of 10 randomly selected leaves and expressed as percentage.

3.5.1.2. At 50 per cent Flowering

3.5.1.2.1. Pest Count

Data was collected on pre-count and post-count of insects as in 3.5.1.1.1.

3.5.1.2.2. Damage on Leaves

Damage caused by leaf feeding pests was assessed on alternate days of treatment as in 3.5.1.1.2.

3.6. STATISTICAL ANALYSIS

The tabulated data were subjected to statistical analysis using one way analysis of variance after suitable transformations (Panse and Sukhatme, 1967).

Results

4. RESULTS

The results of the work entitled "Development of formulations from *Samadera indica* Gaetrn. for the management of leaf feeding pests in snake gourd (*Trichosanthes anguina* L.)" carried out during 2018 to 2021 in the Department of Agricultural Entomology, College of Agriculture, Vellayani and Council of Scientific and Industrial Research – National Institute for Interdisciplinary Science and Technology (CSIR-NIIST), Thiruvananthapuram is presented below. The work was conducted under four main modules *viz.*, bioefficacy of extracts from seeds and bark of *S. indica*, identification of bioactive compounds in effective extract, development of effective formulations containing bioactive compounds, field evaluation of the formulations against pumpkin caterpillar, *Diaphania indica* Saund. and epilachna beetle, *Henosepilachna septima* (Dieke).

4.1. SCREENING OF BOTANICAL PREPARATIONS FOR INSECTICIDAL PROPERTIES

4.1.1. Effect of Bark Extracts of *Samadera indica* on *Diaphania indica*

4.1.1.1. Estimation of Lethal Doses

Lethal doses of bark extracts of *S. indica* effective against *D. indica*, expressed as LD_{50} (Median Lethal Dose) and LD_{90} (Lethal Dose 90) are given in Table 1. It was observed that LD_{50} values of the extract in hexane, acetone, ethanol and aqueous extract were 1.66, 1.03, 1.51 and 2.38% respectively. Meanwhile, values of LD_{90} were 6.49, 7.05, 5.24 and 9.17% respectively. Dose-response curve for *D. indica* in ethanol which exhibited lowest LD_{50} value is depicted in Fig.1.

4.1.1.2. Antifeedant Effect

Data on antifeedant effect showed significant difference among all the treatments. Among the treatments NSKE 5% showed maximum antifeedant activity at 24 HAT followed by ethanol extract @ 5.24%. The mean quantity of leaves consumed by larvae in these treatments was 0.19 and 0.21 g respectively. This was followed by acetone extract @ 7.05% with mean leaf consumption of

Table 1. Lethal doses of *Samadera indica* bark extracts in different solvents against *Diaphania indica*

Solvents	Lethal Doses (%)		χ^2	df	P value
	LD_{50}	LD_{90}			
Hexane	1.66±0.88	6.49±0.29	0.013	8	1
Acetone	1.03±0.035	7.05±0.89	0.035	8	1
Ethanol	1.51±0.89	5.24±0.31	0.033	8	1
Water	2.38±0.88	9.17±0.29	0.017	8	1

χ^2 - chi-square value, not significant at $p > 0.05$ level, df - degrees of freedom, LD_{50} – Median Lethal Dose, LD_{90} – Lethal Dose 90

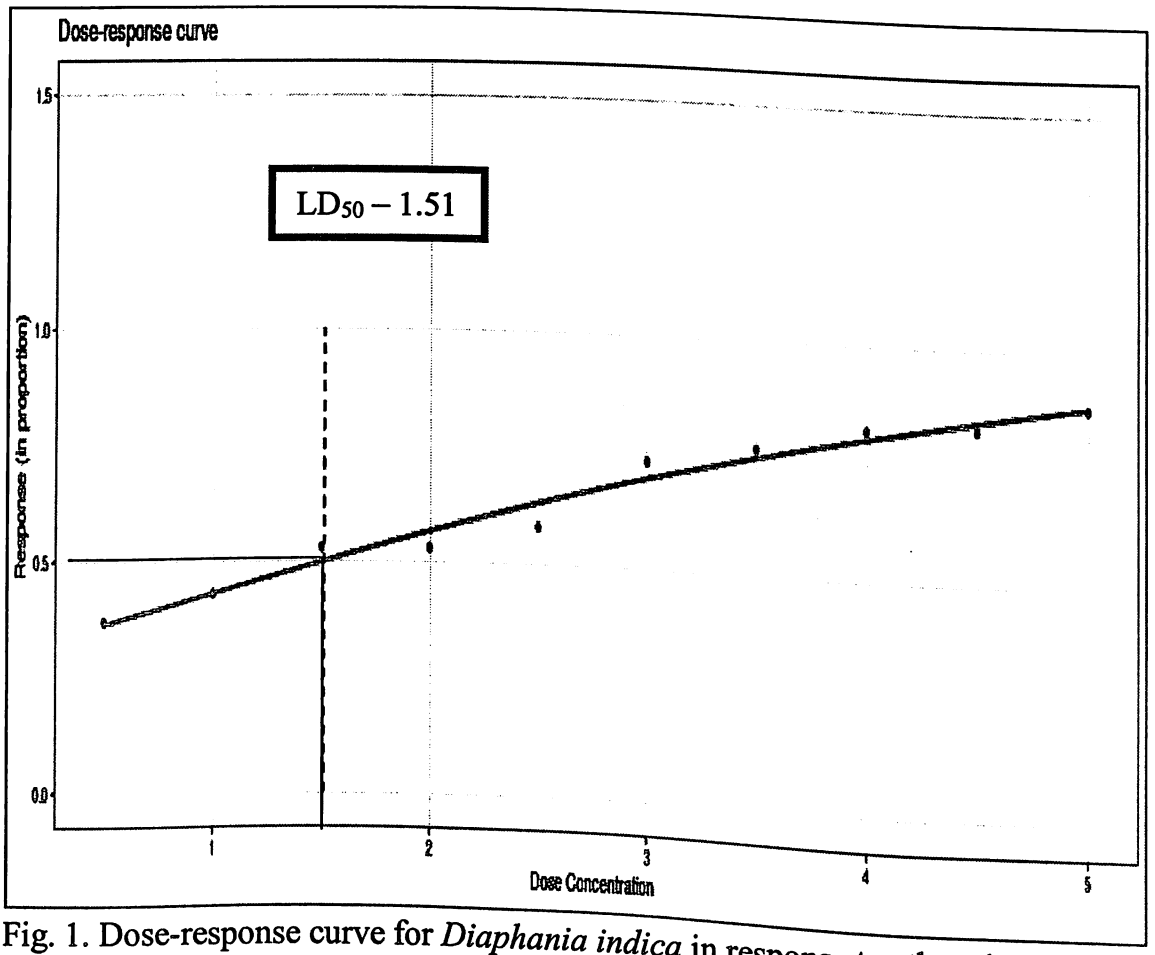


Fig. 1. Dose-response curve for *Diaphania indica* in response to ethanol extract from *Samadera indica* bark

0.23 g. Next effective treatments were hexane extract @ 6.49% and aqueous extract @ 9.17% which were on par. Mean quantity of leaf consumption in these treatments was 0.25 and 0.26 g, each. In treatment with acetone extract @ 1.03%, mean leaf consumption was 0.29 g. Followed by these were the treatments hexane extract @ 1.66%, ethanol extract @ 1.51% and aqueous extract @ 2.38% which exhibited statistical similarity. Mean leaf consumption in these treatments ranged from 0.30 to 0.31 g. Larvae treated with distilled water consumed 0.34 g leaves (Table 2).

At 48 HAT, it was observed that the extracts of *S. indica* in ethanol @ 5.24% and acetone @ 7.05% exhibited similar degree of feeding repellence. The mean quantity of leaves consumed by treated larvae was 0.18 g each. These treatments were next to NSKE 5%, in which the mean leaf consumption was 0.16 g. Effect of hexane extract @ 6.49% and aqueous extract @ 9.17% was statistically similar to each other, with values 0.21 and 0.20 g respectively. Next best treatment was ethanol extract @ 1.51% and it showed statistically significant superiority to hexane extract @ 1.66%, ethanol extract @ 1.51% and aqueous extract @ 2.38%. The mean leaf consumption in these treatments ranged from 0.23 to 0.26 g. Larvae consumed an average of 0.37 g leaves in control.

At 72 HAT, it was noticed that the extracts of *S. indica* in hexane @ 6.49%, ethanol @ 5.24%, acetone @ 7.05% and aqueous extract @ 9.17% were statistically similar and the mean values were in the range of 0.16 to 0.17 g. These were next to NSKE 5% with mean value of 0.12 g. Next was the treatment with ethanol extract @ 1.51% (0.19 g), which exhibited statistical similarity with aqueous extract @ 9.17% (0.17 g). This was followed by hexane extract @ 1.66%, acetone extract @ 1.03% and aqueous extract @ 2.38% with average leaf consumption of 0.22 g each. Larvae in control treatment consumed an average of 0.43 g of leaves.

4.1.1.3. Insecticidal Effect

Insecticidal effect assessed in terms of percentage mortality at 24, 48 and 72 HAT is shown in Table 3. The data revealed statistically significant difference among all treatments. At 24 HAT, it was observed that the treatments with ethanol

Table 2. Antifeedant effect of bark extracts of *Samadera indica* in different solvents on larvae of *Diaphania indica*

Solvents (%)	*Mean leaf consumption (g)		
	HAT		
	24	48	72
Hexane (1.66)	0.31^{b}	0.26^{b}	0.22^{b}
Hexane (6.49)	0.25^{d}	0.21^{d}	0.16^{d}
Acetone (1.03)	0.29^{c}	0.26^{b}	0.22^{b}
Acetone (7.05)	0.23^{e}	0.18^{f}	0.16^{d}
Ethanol (1.51)	0.30^{b}	0.23^{c}	0.19^{c}
Ethanol (5.24)	0.21^{f}	0.18^{f}	0.17^{d}
Aqueous (2.38)	0.31^{b}	0.25^{b}	0.22^{b}
Aqueous (9.17)	0.26^{d}	0.20^{de}	0.17^{cd}
Check (NSKE 5%)	0.19^{g}	0.16^{g}	0.12^{e}
Control	0.34^{a}	0.37^{a}	0.43^{a}
SE (m)	0.006	0.004	0.007
CD (0.05)	0.016	0.018	0.018

HAT – Hours After Treatment; NSKE – Neem Seed Kernel Extract

Table 3. Insecticidal effect of bark extracts of *Samadera indica* in different solvents on larvae of *Diaphania indica*

Solvents (%)	***Mean larval mortality (%)**		
	HAT		
	24	48	72
Hexane (1.66)	11.67 (19.89)^{d}	46.67 (43.09)^{ef}	51.67 (45.96)^{c}
Hexane (6.49)	35.00 (36.27)^{b}	61.67 (51.76)^{bc}	90.00 (71.57)^{ab}
Acetone (1.03)	11.67 (19.89)^{d}	48.33 (44.04)^{e}	53.33 (46.91)^{c}
Acetone (7.05)	41.67 (40.20)^{a}	63.33 (52.74)^{bc}	91.67 (73.40)^{ab}
Ethanol (1.51)	20.00 (26.57)^{c}	53.33 (46.91)^{d}	53.33 (46.91)^{c}
Ethanol (5.24)	38.33 (38.25)^{ab}	65.00 (53.73)^{ab}	93.33 (75.24)^{a}
Aqueous (2.38)	10.00 (18.05)^{d}	43.33 (41.16)^{f}	50.00 (45.00)^{c}
Aqueous (9.17)	33.33 (35.25)^{b}	60.00 (50.77)^{c}	88.33 (70.12)^{b}
Malathion 50 EC @ 0.1%	43.33 (41.16)^{a}	68.33 (55.77)^{a}	93.33 (75.24)^{a}
SE (m)	0.99	1.40	1.06
Control	0^{e}	0^{g}	0^{d}
CD (0.05)	(3.759)	(2.409)	(3.928)

HAT – Hours After Treatment; Figures in parentheses are arc sine transformed values

extract @ 5.24% and acetone extract @ 7.05% were statistically similar to malathion 50 EC @ 0.1% and the mean percentage mortality in the above treatments were 38.33, 41.67 and 43.33 respectively. This was followed by treatments with aqueous extract @ 9.17% and hexane extract @ 6.49% which were statistically on par with ethanol extract @ 5.24%. Mean larval mortality in these three treatments ranged from 33.33 to 38.33 per cent. In treatment with ethanol extract @ 1.51%, mean mortality was 20.00 per cent. This was succeeded by the treatments with hexane extract @ 1.66%, acetone extract @ 1.03% and aqueous extract @ 2.38%, which were statistically on par and mean mortality percentage ranged from 10.00 to 11.67 per cent.

At 48 HAT, effect of ethanol extract @ 5.24%, acetone extract @ 7.05% and hexane extract @ 6.49% were statistically on par with malathion 50 EC @ 0.1%. In these treatments mean mortality rate varied from 61.67 to 68.33 per cent. This was followed by the treatments acetone extract @ 7.05%, hexane extract @ 6.49% and aqueous extract @ 9.17%, which were statistically similar. Here, mean mortality rates observed were 63.33, 61.67 and 60.00 per cent respectively. Ethanol extract @ 1.51% exhibited larval mortality of 53.33 per cent and it was significantly superior to hexane extract @ 1.66%, acetone extract @ 1.03% and aqueous extract @ 2.38%. Mean mortality percentage in these three treatments ranged from 43.33 to 48.33.

At 72 HAT, hexane extract @ 6.49%, acetone extract @ 7.05% and ethanol extract @ 5.24% were found as effective as chemical control, malathion 50 EC @ 0.1%. Here, mortality percentage varied from 90.00 to 93.33. Next was the treatment with aqueous extract @ 9.17% which was also statistically similar to hexane extract @ 6.49% and acetone extract @ 7.05%. These were followed by hexane extract @ 1.66%, acetone extract @ 1.03%, ethanol extract @ 1.51% and aqueous extract @ 2.38%. Effect of these four treatments was statistically on par and mortality in these treatments ranged from 43.33 to 53.33 per cent.

4.1.1.4. Effect on Biology of Insect

The effect of bark extracts of *S. indica* on insect biology was studied by observing malformed larvae/pupae/adults, larval duration, pupal duration, pupal weight, adult longevity, fecundity, egg hatchability, preoviposition period and incubation period of eggs. The data pertaining to these observations are presented in Table 4.

4.1.1.4.1. Deformities in Larvae, Pupae and Adults

It was observed that larvae of *D. indica* treated with various plant extracts have undergone pupal discolouration and the adults got malformed.

4.1.1.4.2. Effect on Larval Duration

There was a general increase in larval duration with the application of extracts and all treatments showed statistically significant variation among themselves. All solvent extracts @ 5.24 to 9.17% exhibited increase in larval duration and it was statistically on par with NSKE 5%, in which the mean values ranged from 14.00 to 15.33 days. Solvent extracts (except aqueous) @ 1.03 to 1.66% were significantly inferior to the above treatments and the mean larval duration observed with these treatments ranged from 11.67 to 12.67 days. In treatment with aqueous extract @ 2.38%, larval duration was 11.33 days, while in control larvae survived for 9.67 days only.

4.1.1.4.3. Effect on Pupal Weight

All treatments exhibited significant variation in mean pupal weight. Treatment with ethanol extract @ 5.24% resulted in mean pupal weight of 0.19 g each and it was statistically equivalent to NSKE 5% with mean pupal weight of 0.18 g. This was followed by ethanol extract @ 1.51% (0.23 g) which was statistically similar to acetone extract @ 7.05% and hexane extract @ 6.49%, with mean pupal weights being 0.21 and 0.24 g each. Treatment with aqueous extract @ 9.17% was statistically on par with hexane extract @ 6.49% and acetone extract @ 1.03%. Mean weight of pupae ranged from 0.24 to 0.27 g in these treatments. Application of hexane extract @ 1.66% resulted in mean pupal weight of 0.27 g and was on par with acetone extract @ 1.03% and aqueous extract @ 9.17%, with mean pupal weight of 0.27 and 0.25 g respectively. Pupal weight noticed in control was 0.31 g.

Table 4. Effect of bark extracts of *Samadera indica* on biology of larvae of *Diaphania indica*

Solvents (%)	Mean					
	No. of malformed insects		Larval duration (days)	Pupal weight (g)	Pupal period (days)	Longevity of adults (days)
	P	A				
Hexane (1.66)	0	0	11.67^{c}	0.27^{bc}	3.67^{d}	5.67^{bcd}
Hexane (6.49)	0.66	0.33	14.33^{ab}	0.24^{e}	4.00^{bc}	5.00^{de}
Acetone (1.03)	0.33	0	12.33^{c}	0.27^{cd}	4.33^{abc}	5.33^{cd}
Acetone (7.05)	0.66	0.33	14.33^{ab}	0.21^{fg}	4.33^{abc}	4.67^{de}
Ethanol (1.51)	0.33	0.66	12.67^{c}	0.23^{ef}	4.67^{ab}	5.33^{cd}
Ethanol (5.24)	1.00	0.66	15.00^{ab}	0.19^{gh}	5.33^{a}	4.33^{e}
Aqueous (2.38)	0	0	11.33^{d}	0.29^{ab}	2.67^{d}	6.67^{ab}
Aqueous (9.17)	0	0	14.33^{ab}	0.25^{de}	3.33^{cd}	6.33^{bc}
NSKE 5%	1.00	0.66	15.33^{a}	0.18^{h}	5.33^{a}	4.33^{e}
Control	0	0	9.67^{d}	0.31^{a}	2.67^{d}	7.67^{a}
SE (m)	0.22	0.24	0.39	0.32	0.24	0.30
CD (0.05)	NS	NS	1.164	0.021	1.077	1.077

NSKE – Neem Seed Kernel Extract; P – Pupae, A – Adults

4.1.1.4.4. Effect on Pupal Period

The application of botanicals resulted in lengthening of pupal period with significant variation among the treatments. Highest mean pupal period was observed in NSKE 5% and it was superior to all other treatments. In treatments with extracts in acetone @ 1.03 and 7.05% and ethanol @ 1.51 and 5.24%, mean pupal period ranged from 4.33 to 5.33 days. Treatment with hexane extract @ 6.49% was statistically on par with acetone extract @ 1.03 and 7.05%, ethanol extract @ 1.51% and aqueous extract @ 7.90%. In these treatments, pupal period lasted for an average of 3.33 to 4.67 days. The treatment with aqueous extract @ 9.17% was statistically on par with hexane extract @ 1.66% and aqueous extract @ 2.38%, with mean pupal duration of 3.33, 3.67 and 2.67 respectively. In control treatment, pupae existed for 2.67 days only.

4.1.1.4.5. Effect on Adult Longevity

Regarding adult longevity, there was statistically significant difference among various treatments. Application of hexane extract @ 6.49% was statistically on par with NSKE 5%, ethanol extract @ 1.51 and 5.24%, acetone extract @ 1.58 and 5.63%, ethanol extract @ 1.51% and hexane extract @ 1.66%. Mean adult longevity in these five treatments varied from 4.33 to 5.67. Effect of aqueous extract @ 2.38%, aqueous extract @ 9.17% and hexane extract @ 1.66% was statistically similar, with mean values ranging from 5.67 to 6.67 days. Insects underwent treatment with distilled water had a longevity of 7.67 days.

4.1.1.4.6. Effect on Fecundity, Egg Hatchability, Preoviposition Period and Incubation Period

About 10 to 50 per cent of the larvae survived till adult stage. As the adults emerged from surviving larvae did not lay eggs, further experiments could not be carried out. Adults from larvae treated with distilled water laid an average of 184.33 eggs and 181.33 eggs hatched out of them. These insects took an average of 4.67 days for oviposition and the incubation period of eggs was 4.00 days.

It can be generalised that among various solvent extracts from *S. indica* bark, ethanol extract @ 5.27% was more effective against second instar larvae of *D. indica* as it exhibited good antifeedant property, insecticidal effect and affected insect biology negatively.

4.1.2. Effect of Bark Extracts of *Samadera indica* on *Henosepilachna septima*

4.1.2.1. Estimation of Lethal Doses

The LD_{50} and LD_{90} of bark extracts against *H. septima* are depicted in Table 5. Values of LD_{50} observed in the extracts with n-hexane, acetone, ethanol and aqueous extract were 2.17, 1.95, 1.68 and 2.49%. Values of LD_{90} observed were 9.53, 6.60, 6.17 and 9.02% respectively. Dose-response curve for *H. septima* in ethanol which exhibited lowest LD_{50} value is illustrated in Fig. 2.

4.1.2.2. Antifeedant Effect

All the treatments exhibited statistically significant variation with regard to antifeedant effect against grubs of *H. septima*. At 24 HAT, maximum antifeedant effect was observed in NSKE 5%, followed by ethanol extract @ 6.17% in which mean leaf consumption was 0.16 and 0.19 g respectively. This was followed by acetone extract @ 6.60% and ethanol extract @ 1.68%, with average leaf consumption of 0.26 g, each. Effect of these two treatments was statistically on par. Insects consumed 0.29 g leaf when the leaves were treated with hexane extract @ 9.53%. This exhibited statistical similarity with acetone extract @ 1.95% and aqueous extract @ 9.02% with mean quantity of leaf consumption of 0.31 and 0.33 g respectively. Effect of hexane extract @ 2.17% and aqueous extracts @ 2.49% was statistically on par with average leaf consumption of 0.35 and 0.36 g respectively. Grubs in control consumed 0.37 g leaves (Table 6).

At 48 HAT, ethanol extract @ 6.17% remained as effective extract of *S. indica* and mean quantity of leaf consumption by grubs was 0.17 g. This treatment preceded NSKE 5% (0.13 g). Acetone extract @ 6.60% exhibited statistical similarity with hexane extract @ 9.53% and ethanol extract @ 1.68%,

Table 5. Lethal doses of *Samadera indica* bark extracts in different solvents against *Henosepilachna septima*

Solvents	Lethal Doses (%)		χ^2	df	P Value
	LD_{50}	LD_{90}			
Hexane	2.17±0.88	9.53±0.29	0.009	8	1
Acetone	1.95±0.88	6.60±0.29	0.01	8	1
Ethanol	1.68±0.89	6.17±0.31	0.03	8	1
Aqueous	2.49±0.88	9.02±0.29	0.02	8	1

χ^2 - chi-square value, not significant at $p > 0.05$ level, df - degrees of freedom, LD_{50} – Median Lethal Dose, LD_{90} – Lethal Dose 90

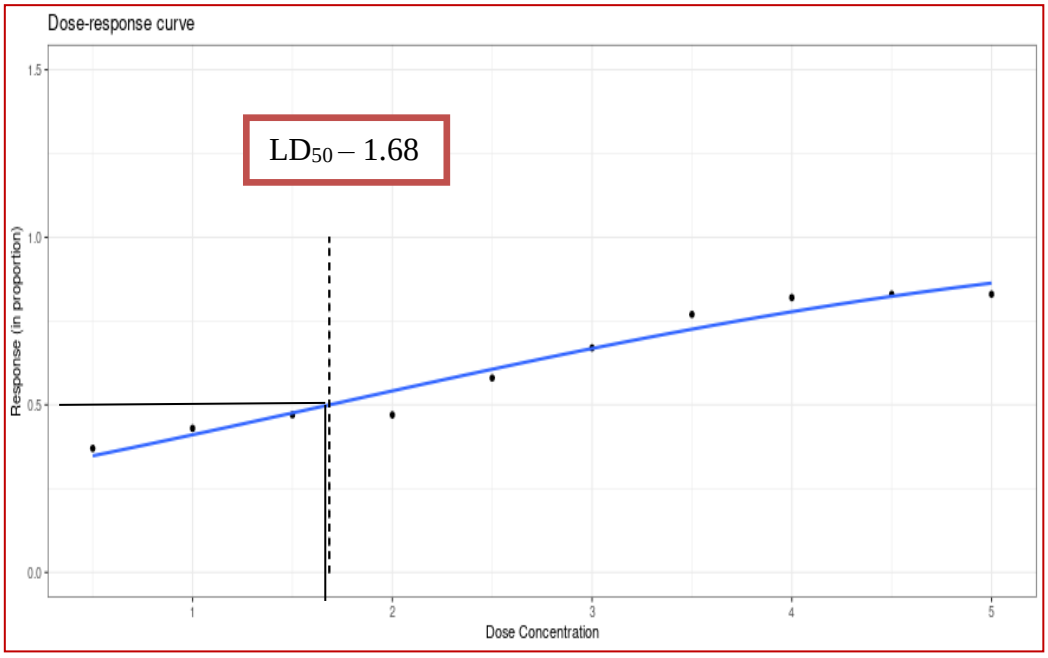


Fig. 2. Dose-response curve for *Henosepilachna septima* in response to ethanol extract from *Samadera indica* bark

Table 6. Antifeedant effect of bark extracts of *Samadera indica* in different solvents on grubs of *Henosepilachna septima*

Solvents (%)	***Mean quantity of leaf consumed by grub (g)**		
	HAT		
	24	48	72
Hexane (2.17)	0.35^{a}	0.26^{bc}	0.22^{b}
Hexane (9.53)	0.29^{c}	0.19^{e}	0.17^{c}
Acetone (1.95)	0.31^{bc}	0.25^{bc}	0.22^{b}
Acetone (6.60)	0.26^{d}	0.20^{de}	0.17^{c}
Ethanol (1.68)	0.26^{d}	0.21^{d}	0.17^{c}
Ethanol (6.17)	0.19^{e}	0.17^{f}	0.14^{d}
Aqueous (2.49)	0.36^{a}	0.25^{bc}	0.21^{b}
Aqueous (9.02)	0.33^{b}	0.27^{b}	0.22^{b}
NSKE 5%	0.16^{f}	0.13^{g}	0.10^{e}
Control	0.37^{a}	0.39^{a}	0.40^{a}
SE (m)	0.007	0.006	0.006
CD (0.05)	0.021	0.018	0.019

HAT – Hours After Treatment; NSKE – Neem Seed Kernel Extract

with mean values of 0.20, 0.19 and 0.21 g. Treatment with hexane extract @ 2.17% was statistically on par with acetone extract @ 1.95% and aqueous extract @ 2.49 and 9.02%. Mean leaf consumption by grubs ranged from 0.25 to 0.27 g in these four treatments. Insects consumed an average quantity of 0.39 g leaves when treated with distilled water.

At 72 HAT also, similar trend was observed with ethanol extract @ 6.17% being the effective bark extract, was preceded by NSKE 5%. Mean leaf consumption in these treatments was 0.14 and 0.10 g respectively. This was followed by the extracts in hexane and acetone @ 9.53 and 6.60% each and ethanol @ 1.68%, which were statistically similar to each other, with mean leaf consumption being 0.17 g each. Next best treatments in the order of effectiveness were hexane extract @ 2.17%, acetone extract @ 1.95% and aqueous extract @ 2.49 and 9.02% each with mean quantity of leaf consumption ranging from 0.21 to 0.22 g. Grubs consumed an average of 0.40 g of leaves when treated with distilled water.

4.1.2.3. Insecticidal Effect

Observations on insect mortality recorded at 24, 48 and 72 HAT witnessed significant variation among the treatments (Table 7).

At 24 HAT, it was noticed that the treatments ethanol extract @ 6.17% and acetone extract @ 6.60% exhibited 56.67 and 51.67 per cent mortality each and were statistically similar to malathion 50 EC @ 0.1% with 56.67 per cent mortality. The treatment acetone extract @ 6.60% also exhibited statistical similarity with hexane extract @ 9.53% and aqueous extract @ 9.02% and the mean mortality values ranged from 51.67 to 48.33 per cent. Next was the treatment with ethanol extract @ 1.68%, which was on parity with acetone extract @ 1.95%, hexane extract @ 2.17% and aqueous extract @ 2.49% with 26.67 to 33.33 per cent mean mortality.

On 48 HAT, the treatments acetone extract @ 6.60%, ethanol extract @ 6.17% and hexane extract @ 9.53% were observed to be statistically equivalent

Table 7. Insecticidal effect of bark extracts of *Samadera indica* in different solvents on grubs of *Henosepilachna septima*

Solvents (%)	***Mean mortality of grubs (%)**		
	HAT		
	24	48	72
Hexane (2.17)	30.00 (33.16)^{cd}	35.00 (36.27)^{de}	48.33 (44.04)^{c}
Hexane (9.53)	48.33 (44.04)^{b}	48.33 (44.04)^{b}	90.00 (71.57)^{ab}
Acetone (1.95)	28.33 (32.14)^{cd}	38.33 (38.25)^{cd}	51.67 (45.96)^{c}
Acetone (6.60)	51.67 (45.96)^{ab}	51.67 (45.96)^{b}	90.00 (71.57)^{ab}
Ethanol (1.68)	33.33 (35.25)^{c}	41.67 (40.20)^{c}	51.67 (45.96)^{c}
Ethanol (6.17)	56.67 (48.84)^{a}	50.00 (45.00)^{b}	91.67 (73.40)^{a}
Aqueous (2.49)	26.67 (31.07)^{d}	33.33 (35.25)^{e}	51.67 (45.96)^{c}
Aqueous (9.02)	46.67 (43.09)^{b}	40.00 (39.23)^{c}	86.67 (68.66)^{b}
Malathion 50 EC @ 0.1%	56.67 (48.85)^{a}	63.33 (52.74)^{a}	91.67 (73.40)^{a}
Control	0^{e}	0^{f}	0^{d}
SE (m)	1.18	0.76	1.12
CD (0.05)	(3.489)	(2.237)	(3.301)

HAT – Hours After Treatment; Figures in parentheses are arc sine transformed values

with mean values being in the range 48.33 to 51.67 per cent. These were preceded by chemical treatment, which witnessed mortality of 63.33 per cent. Next best treatments in the order of effectiveness were aqueous extract @ 9.02% and ethanol extract @ 1.68%, which were observed to be statistically on par with acetone extract @ 1.95%. The mortality percentage in these treatments ranged from 38.33 to 41.67. In hexane extract @ 2.17% and aqueous extract @ 2.49%, mean mortality values varied from 33.33 to 35.33 and were observed to be in parity with acetone extract @ 1.95%.

At 72 HAT, it was observed that treatment with ethanol extract @ 6.17%, acetone extract @ 6.60% and hexane extract @ 9.53% exhibited statistically similar mortality rate as with malathion 50 EC @ 0.1% which ranged from 86.67 to 91.67 per cent. This was followed by hexane extract @ 2.17%, acetone extract @ 1.95%, ethanol extract @ 1.68% and aqueous extract @ 2.49% with mean mortality of 48.33 to 51.67 per cent in treated grubs.

4.1.2.4. Effect on Biology of Insect

The effect of *S. indica* bark extracts on the biology of *H. septima* is presented in Table 8.

4.1.2.4.1. Deformities in Grubs, Pupae and Adults

There was pupal discolouration and poor sclerotization in adults of epilachna beetles treated with solvent extracts.

4.1.2.4.2. Effect on Duration of Grubs

There was significant variation among various treatments with regard to duration of grubs (days). It was observed that in NSKE 5%, mean grub period was 17.33 days and it was on par with ethanol extract @ 6.17% (16.33 days). These were followed by acetone extract @ 6.60% which also exhibited statistical similarity with ethanol extract @ 6.17%, with mean grub duration of 15.33 and 16.33 days respectively. The treatments with hexane extract @ 2.17 and 9.53%, acetone extract @ 1.95%, ethanol extract @ 1.68% and aqueous extract @ 9.02% resulted in grub period of 13.33, 14.33, 12.67, 12.33 and 12.33 respectively.

Table 8. Effect of bark extracts of *Samadera indica* in different solvent extracts on biology of grubs of *Henosepilachna septima*

Solvents (%)	Mean					
	No. of malformed insects		Duration of grubs (days)	Pupal weight (g)	Pupal period (days)	Longevity of adults (days)
	P	A				
Hexane (2.17)	0.33	0	13.33^{c}	1.29	6.00^{e}	15.33^{bc}
Hexane (9.53)	0.66	0.33	14.33^{c}	1.06	7.67^{cd}	13.33^{f}
Acetone (1.95)	0.33	0.33	12.67^{c}	0.89	6.67^{de}	14.33^{de}
Acetone (6.60)	0.66	0.66	15.33^{b}	0.84	8.00^{bc}	13.33^{f}
Ethanol (1.68)	0.66	0.33	12.33^{c}	0.87	7.67^{cd}	14.00^{ef}
Ethanol (6.17)	0.66	0.66	16.33^{ab}	0.74	8.33^{b}	11.33^{g}
Aqueous (2.49)	0	0	11.00^{d}	1.34	5.00^{f}	16.00^{b}
Aqueous (9.02)	0	0	12.33^{c}	1.33	6.00^{e}	15.00^{cd}
Check (NSKE 5%)	0.66	0.33	17.33^{a}	0.55	10.00^{a}	9.33^{h}
Control	0	0	10.33^{d}	1.34	4.33^{g}	19.33^{a}
SE (m)	0.28	0.26	0.45	0.19	0.26	0.28
CD (0.05)	NS	NS	1.077	NS	0.762	0.823

NSKE – Neem Seed Kernel Extract; P – Pupae, A – Adults

Effect of these treatments was statistically on par and was significantly superior to aqueous extract @ 2.49%, with mean value of 11.00. In control, grubs remained in larval period for 10.33 days.

4.1.2.4.3. Effect on Pupal Weight

Regarding mean pupal weight, there was no statistically significant difference among various treatments.

4.1.2.4.4. Effect on Pupal Period

There was an elongation in pupal period in treated insects with considerable variation among different treatments. The treatment acetone extract @ 6.60% was statistically on par with hexane extract @ 9.53% and ethanol extract @ 1.68 and 6.17%, with mean pupal period ranging from 7.33 to 8.33 days. These treatments preceded NSKE 5% with mean pupal duration of 10.00 days. There was statistical similarity between treatments with acetone extract @ 6.60% (8.00 days), hexane extract @ 6.82% (7.67 days) and ethanol extract @ 1.68% (7.67 days). Treatments with extracts in hexane @ 2.17% exhibited statistical similarity with acetone extract @ 1.95% and aqueous extract @ 9.02%. Mean pupal period observed in above treatments were 6.00, 6.67 and 6.00 days respectively. Pupal duration in control was 4.33 days.

4.1.2.4.5. Effect on Adult Longevity

There was statistically significant variation among all treatments in adult longevity. It was observed that the adults developed from grubs treated with various extracts showed significant reduction in their life span when compared to control, wherein adults survived for 19.33 days. Ethanol extract @ 6.17% exhibited mean adult longevity of 11.33 days and was preceded by NSKE 5% (9.33 days). Effect of ethanol extract @ 1.68% was statistically on par with acetone extract @ 6.60% and hexane extract @ 9.53%, with mean adult longevity ranging from 13.33 to 14.00 days. The treatment with ethanol extract @ 1.68% also exhibited similarity with acetone extract @ 1.95% (14.33 days). There was statistical similarity between aqueous extract @ 9.02%, acetone extract @ 1.95% and hexane extract @ 2.17%, with mean longevity of adults varying from 14.33 to 15.33 days.

4.1.2.4.6. Effect on Fecundity, Egg Hatchability, Preoviposition period and Incubation Period

As only less number of treated grubs got transformed into adults, they could not lay eggs. Hence, it was not possible to study the effect of extracts on fecundity and adult emergence. Grubs treated with distilled water laid an average of 168.67 eggs, out of which 157.00 eggs got hatched. Mean preoviposition period was 6.67 days and incubation period was 3.67 days.

It can be concluded from the above experiment that ethanol extract from *S. indica* bark @ 6.17% is effective against *H. septima* in bringing feeding repellency in the pest, mortality and detrimental effects on biology of the pest.

4.1.3. Effect of Seed Extracts of *Samadera indica* on *Diaphania indica*

4.1.3.1. Estimation of Lethal Doses

Lethal doses of *S. indica* seed extract effective against *D. indica* has been illustrated in table 9. The LD_{50} values of hexane, acetone, ethanol and aqueous extract were observed to be 1.79, 0.59, 1.03 and 1.54% respectively. LD_{90} values of extracts were 6.99, 5.17, 7.05 and 7.90% respectively. Dose-response curve for *D. indica* in acetone which exhibited the lowest LD_{50} value is shown in Fig. 3.

4.1.3.2. Antifeedant Effect

The antifeedant effect of *S. indica* seed extracts on *D. indica* assessed in terms of quantity of leaves consumed by the larvae (g) at 24, 48 and 72 HAT is given in Table 10. Among different extracts tested, maximum leaf protection was observed in acetone extract @ 5.17% in which the larvae consumed 0.14 g leaf at 24 HAT. It was statistically on par with NSKE 5% in which the larvae consumed 0.15 g leaf. Next best treatments in the order of effectiveness were 0.59% of acetone extract and 7.05% of ethanol extract with mean leaf consumption of 0.21 and 0.20 g, each. These were followed by treatments with aqueous extract @ 7.90% (0.37 g) and ethanol extract @ 1.03% (0.35 g) which exhibited statistical similarity among them and also with the treatments hexane extract @ 1.79% and

Table 9. Lethal doses of *Samadera indica* seed extracts in different solvents against *Diaphania indica*

Solvents	Lethal Doses (%)		χ^2	df	P Value
	LD_{50}	LD_{90}			
Hexane extract	1.79±0.88	6.99±0.29	0.021	8	1
Acetone extract	0.59±0.88	5.17±0.30	0.019	8	1
Ethanol extract	1.03±0.87	7.05±0.29	0.021	8	1
Aqueous extract	1.54±0.87	7.90±0.28	0.024	8	1

χ^2 - chi-square value, not significant at $p > 0.05$ level, df - degrees of freedom, LD_{50} – Median Lethal Dose, LD_{90} – Lethal Dose 90

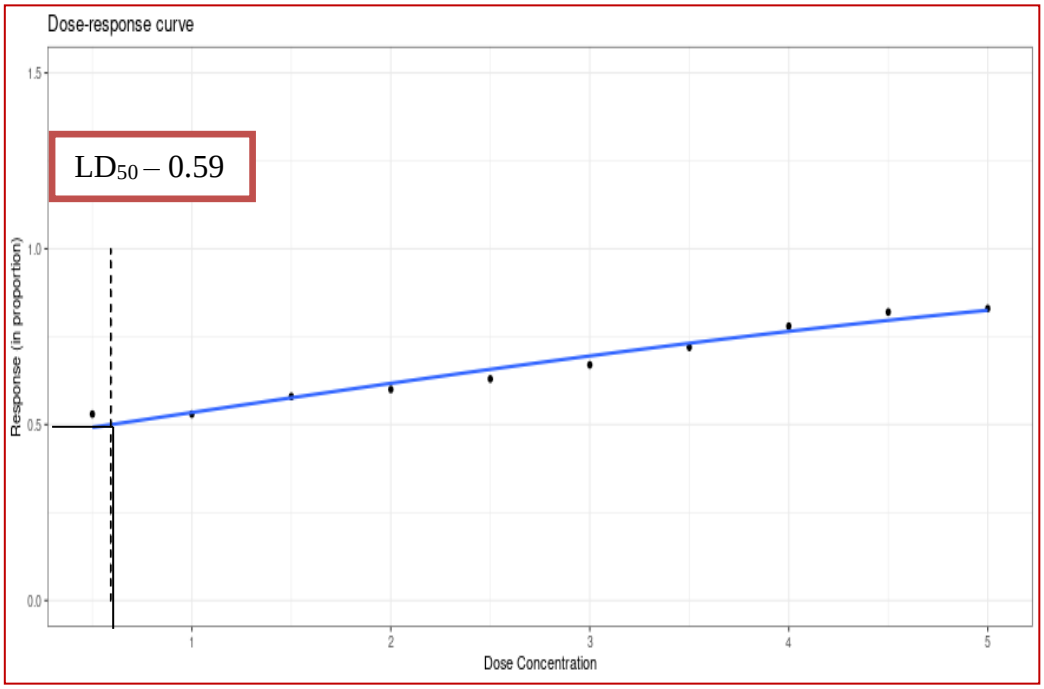


Fig. 3. Dose-response curve for *Diaphania indica* in response to acetone extract from *Samadera indica* seed

Table 10. Antifeedant effect of seed extracts of *Samadera indica* in different solvents on larvae of *Diaphania indica*

Solvents (%)	***Mean leaf consumption (g)**		
	HAT		
	24	48	72
Hexane (1.79)	0.35^{bc}	0.28^{b}	0.19^{c}
Hexane (6.99)	0.34^{c}	0.23^{c}	0.14^{d}
Acetone (0.59)	0.21^{d}	0.15^{e}	0.13^{de}
Acetone (5.17)	0.14^{f}	0.11^{f}	0.11^{ef}
Ethanol (1.03)	0.35^{bc}	0.20^{d}	0.17^{c}
Ethanol (7.05)	0.20^{e}	0.19^{d}	0.13^{de}
Aqueous (1.54)	0.36^{abc}	0.26^{b}	0.22^{b}
Aqueous (7.90)	0.37^{ab}	0.21^{cd}	0.17^{c}
NSKE 5%	0.15^{f}	0.11^{f}	0.10^{f}
Control	0.37^{a}	0.40^{a}	0.43^{a}
SE (m)	0.007	0.009	0.008
CD (0.05)	0.020	0.026	0.023

HAT – Hours After Treatment; NSKE – Neem Seed Kernel Extract

aqueous extract @ 1.54%. Larvae treated with distilled water consumed 0.37 g leaves.

At 48 HAT also, almost similar trend was observed in the case of acetone extract @ 5.17% in which the larvae consumed 0.11 g leaves as it was statistically on par with NSKE 5% (0.11 g). The second best treatment was acetone extract @ 0.59%, with mean leaf consumption of 0.15 g. Effect of ethanol extracts @ 1.03 and 7.05% and aqueous extract @ 7.90% was statistically on par and average leaf consumption by larvae ranged from 0.19 to 0.21 g. Mean leaf consumption in treatments with hexane extract @ 1.79% and aqueous extract @ 1.54% were 0.28 and 0.26 g respectively and effect of these two treatments was statistically on par. Larvae in control consumed 0.40 g leaves.

At 72 HAT, effect of acetone extract @ 5.17% was statistically on par with NSKE 5%, ethanol extract @ 7.05% and acetone extract @ 0.59% and mean leaf consumption in these treatments ranged from of 0.10 to 0.13 g. Larvae fed with leaves treated with hexane extract @ 6.99% consumed 0.14 g and was statistically on par with ethanol extract @ 7.05% and acetone extract @ 0.59%. The treatments hexane extract @ 1.79%, ethanol extract @ 1.03% and aqueous extract @ 7.90% were significantly inferior to other treatments and the mean leaf consumption ranged from 0.17 to 0.19 g. In the treatment with aqueous extract @ 1.54%, larvae consumed 0.22 g leaves and it was significantly superior to control treatment in which leaf consumption was 0.43 g.

4.1.3.3. Insecticidal Effect

The insecticidal effect of *S. indica* seed extracts against *D. indica* is shown in Table 11. The observations indicated that there was statistically significant variation among all treatments. Among solvent extracts, acetone extract @ 5.17% and ethanol extract @ 7.05% exhibited 48.33 and 45.00 per cent mortality respectively and it was statistically on par with malathion 50 EC @ 0.1% (46.67 per cent) at 24 HAT. This was followed by hexane extract @ 6.99%, with mean mortality percentage of 35.00. Acetone extract @ 0.59% exhibited statistical

Table 11. Insecticidal effect of seed extracts of *Samadera indica* in different solvents on larvae of *Diaphania indica*

Solvents (%)	***Mean larval mortality (%)**		
	HAT		
	24	48	72
Hexane (1.79)	18.33 (25.31)^{de}	48.33 (44.04)^{ef}	46.67 (43.08)^{c}
Hexane (6.99)	35.00 (36.27)^{b}	61.67 (51.76)^{bc}	83.33 (66.15)^{ab}
Acetone (0.59)	21.67 (27.71)^{cd}	55.00 (47.87)^{d}	48.33 (44.04)^{c}
Acetone (5.17)	48.33 (44.04)^{a}	63.33 (52.74)^{ab}	86.67 (68.86)^{ab}
Ethanol (1.03)	25.00 (29.93)^{c}	50.00 (45.00)^{e}	46.67 (43.08)^{c}
Ethanol (7.05)	45.00 (42.13)^{a}	63.33 (52.74)^{ab}	86.67 (68.86)^{ab}
Aqueous (1.54)	11.67 (19.89)^{f}	45.00 (42.13)^{f}	46.67 (43.09)^{c}
Aqueous (7.90)	16.67 (24.05)^{e}	58.33 (49.80)^{cd}	68.33 (61.26)^{b}
Malathion 50 EC @ 0.1%	46.67 (43.09)^{a}	66.67 (54.75)^{a}	91.67 (73.40)^{a}
Control	0^{g}	0^{g}	0^{d}
SE (m)	1.10	0.93	1.84
CD (0.05)	(3.245)	(2.729)	(9.436)

HAT – Hours After Treatment; Figures in parentheses are arc sine transformed values

similarity with the treatments ethanol extract @ 1.03% and hexane extract @ 1.79%, with mean percentage mortality of 21.67, 25.00 and 18.33 respectively. Treatment with hexane extract @ 1.79% was also in parity with aqueous extract @ 7.90%, and the mean mortality ranged was 16.67 and 18.33 per cent respectively.

At 48 HAT, acetone extract @ 5.17% and ethanol extract @ 7.05% exhibited similarity with chemical control and mean mortality percentages were 63.33, 63.33 and 66.67 each. Treatment with hexane extract @ 6.99% exhibited statistical similarity with acetone extract @ 5.17%, ethanol extract @ 7.05%, aqueous extract @ 7.90% and acetone extract @ 0.59%. Here, mean mortality rate varied from 55.00 to 63.33 per cent. Effect of hexane extract @ 1.79% was on par with ethanol extract @ 1.03% and aqueous extract @ 1.54%, with percentage mean mortality ranging from 45.00 to 50.00 per cent.

At 72 HAT, hexane extract @ 6.99%, acetone extract @ 5.17% and ethanol extract @ 7.05% exhibited statistically similar percentage of mortality, ranging from 68.33 to 86.67. In chemical control, mortality was 91.67 per cent and was on par with hexane extract @ 6.99%, acetone extract @ 5.17% and ethanol extract @ 7.05%. Followed by these were the treatments hexane extract @ 1.79%, acetone extract @ 0.59%, ethanol extract @ 1.03% and aqueous extract @ 1.54%, which were statistically on par with each.

other. Mean mortality values ranged from 46.67 to 48.33 per cent in these treatments.

4.1.3.4. Effect on Biology of Insect

The effect of seed extracts of *S. indica* on biology of *D. indica* is given in Table 12.

4.1.3.4.1. Deformities in Larvae/Pupae/Adults

Deformities as in 4.1.1.4.1. were noticed in pupae and adults.

Table 12. Effect of seed extracts of *Samadera indica* in different solvent extracts on biology of larvae of *Diaphania indica*

Solvents (%)	Mean					
	No. of malformed insects		Larval duration (days)	Pupal weight (g)	Pupal period (days)	Longevity of adults (days)
	P	A				
Hexane (1.79)	0.33	0	15.33^{d}	0.30^{c}	7.67^{bc}	7.33^{ab}
Hexane (6.99)	0.66	0.33	15.67^{cd}	0.28^{de}	8.33^{ab}	6.67^{bc}
Acetone (0.59)	0.33	0.33	16.67^{b}	0.28^{e}	8.00^{abc}	6.67^{bc}
Acetone (5.17)	0.66	0.66	17.00^{b}	0.25^{f}	8.67^{a}	6.33^{c}
Ethanol (1.03)	0.33	0	16.33^{bc}	0.29^{cd}	7.67^{bc}	6.67^{bc}
Ethanol (7.05)	0.33	0.33	16.67^{b}	0.25^{f}	8.33^{ab}	6.67^{bc}
Aqueous (1.54)	0	0	14.33^{e}	0.34^{b}	7.33^{c}	7.33^{ab}
Aqueous (7.90)	0	0	15.33^{d}	0.28^{e}	7.67^{bc}	7.33^{ab}
NSKE 5%	0.66	0.66	18.00^{a}	0.25^{f}	8.67^{a}	5.33^{d}
Control	0	0	12.67^{f}	0.35^{a}	5.33^{d}	7.67^{a}
SE (m)	1.60	0.24	0.30	0.003	0.32	0.33
CD (0.05)	NS	NS	0.880	0.009	0.933	0.983

NSKE – Neem Seed Kernel Extract; P – Pupae, A- Adults

4.1.3.4.2. Effect on Larval Duration

There was an increase in larval duration with the application of solvent extracts of *S. indica* seed and the treatments varied considerably among them. It was noticed that larval duration observed with the application of extracts in acetone (0.59 and 5.17%) and ethanol (1.03 and 7.05% each), were statistically on par with each other and the mean values were in the range of 16.33 to17.00 days. These treatments were preceded by the check, NSKE 5% (18.00 days). When larvae were treated with 6.99% of hexane extract, larval duration was extended to 15.67 days. This treatment exhibited statistical similarity with hexane extract @ 1.79%, ethanol extract @ 1.03% and aqueous extract @ 7.90%, with mean larval duration of 15.33, 16.33 and 15.33 days respectively. In control treatment, larval period was 12.67 days.

4.1.3.4.3. Effect on Pupal Weight

Application of plant extracts resulted decrease in pupal weight in all treatments with considerable variation among them. It was observed that the seed extracts of *S. indica* in acetone @ 6.63% and ethanol @ 7.05% were statistically on par with NSKE 5%, with mean pupal weight of 0.25 g each. This was followed by hexane extract @ 6.99%, acetone extract @ 0.59% and aqueous extract @ 7.90%, wherein the mean pupal weight was 0.28 g, in each. Next best treatments were hexane extract @ 1.79 and ethanol extract @ 1.03%, with mean pupal weight being 0.29 and 0.30 g respectively. These treatments exhibited statistical similarity among them. When larvae were treated with aqueous extract @ 1.54%, pupal weight was 0.34 g. Pupal weight observed in control treatment was 0.35 g.

4.1.3.4.4. Effect on Pupal Period

The observations indicated that there is extension in pupal period with the application of various plant extracts and all the treatments showed statistically significant difference. Application of extracts in hexane @ 6.99%, acetone @ 0.59 and 5.17% and ethanol @ 7.05% witnessed significant increase in pupal duration and were statistically equivalent to NSKE 5%. Mean pupal period varied from 8.00 to 8.67 days in these treatments. Application of hexane extract @ 1.79%, ethanol extract @ 1.03% and aqueous extract @ 7.90% resulted in mean pupal

period of 7.67 days. These treatments showed statistical similarity with hexane extract @ 6.99%, ethanol extract @ 7.05% and acetone extract @ 0.59%. Pupal period in control was 5.33 days.

4.1.3.4.5. Effect on Adult Longevity

There was a reduction in life span of adult insects when solvent extracts of *S. indica* seed were applied topically on insect larvae. Moreover, all the treatments showed statistically significant variation. Application of NSKE 5% resulted in significantly lower longevity in adults (5.33 days). Effect of hexane extract @ 1.79%, aqueous extract @ 1.54 and 7.90% was statistically on par with hexane extract @ 6.99%, acetone extract @ 0.59% and ethanol extract @ 1.03 and 7.05% with mean adult longevity of 6.67 to 7.33 days. Treatment with acetone extract @ 5.17% exhibited statistical similarity with hexane extract @ 6.99%, acetone extract @ 0.59% and ethanol extract @ 1.03 and 7.05%. Mean adult longevity in these five treatments varied from 6.33 to 6.67 days. Adults from larvae treated with distilled water survived for 7.67 days.

4.1.3.4.6. Effect on Fecundity, Egg Hatchability, Preoviposition Period and Incubation Period

As there was very little number of adults, they did not lay eggs. Hence, further experiments could not be done. Meanwhile, adults from larvae treated with distilled water laid 177.00 number of eggs and all the eggs hatched out of them. Mean preoviposition period was 4.33 days with incubation period of 5.00 days.

It can be deduced that seed extract of *S. indica* in acetone @ 5.17% possessed pronounced antifeedant properties, insecticidal effect and resulted in deformities in the developmental stages of *D. indica*.

4.1.4. Seed Extracts of *Samadera indica* on *Henosepilachna septima*

4.1.4.1. Estimation of Lethal Doses

The lethal doses of *S. indica* seed extract effective against *H. septima* is given in Table 13. LD_{50} of the extract in hexane, acetone, ethanol and water were 1.63, 0.83, 1.60 and 2.43% respectively. Meanwhile, LD_{90} of the extract in corresponding solvents were 6.82, 5.27, 7.94 and 7.85% respectively. Dose-

Table 13. Lethal doses of *Samadera indica* seed extracts in different solvents against *Henosepilachna septima*

Extracts	Lethal Doses (%)		χ^2	df	P Value
	LD_{50}	LD_{90}			
Hexane extract	1.63±0.86	6.82±0.28	0.026	8	1
Acetone extract	0.83±0.88	5.27±0.30	0.021	8	1
Ethanol extract	1.60±0.87	7.94±0.29	0.013	8	1
Aqueous extract	2.43±0.87	7.85±0.28	0.020	8	1

χ^2 - chi-square value, not significant at $p > 0.05$ level, df - degrees of freedom, LD_{50} – Median Lethal Dose, LD_{90} – Lethal Dose 90

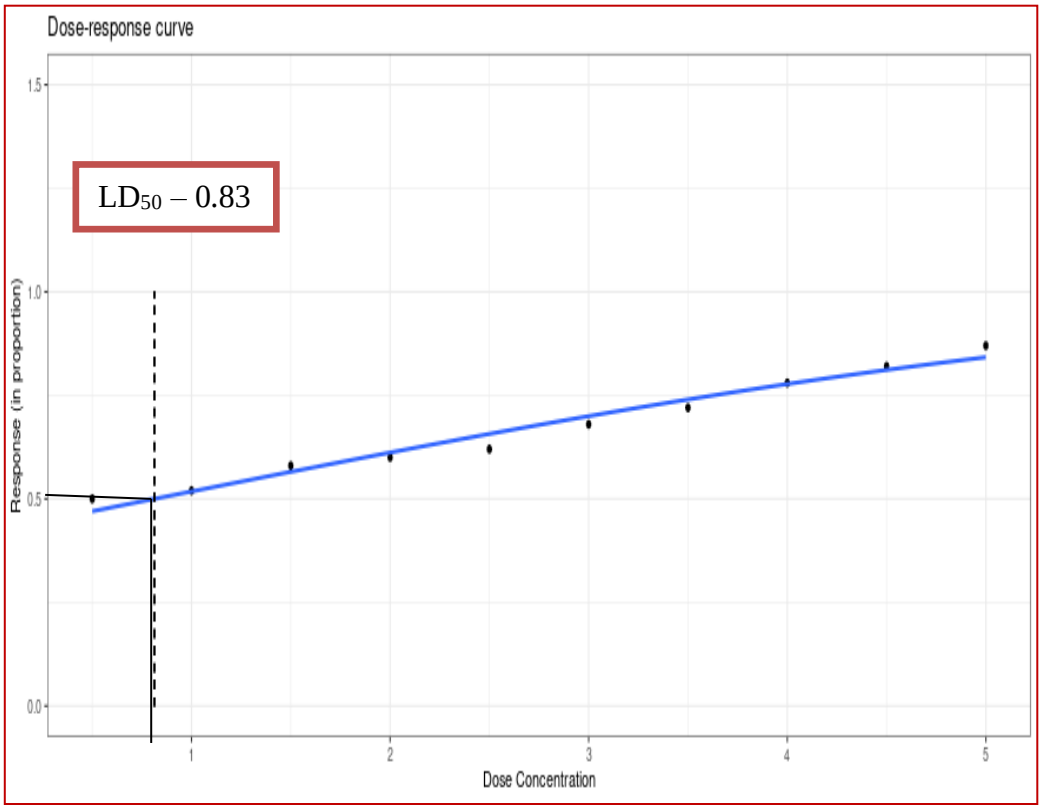


Fig. 4. Dose-response curve for *Henosepilachna septima* in response to acetone extract from *Samadera indica* seed

response curve for *H. septima* in acetone which showed lowest LD_{50} value is depicted in Fig. 4.

4.1.4.2. Antifeedant Effect

The antifeedant effect of *S. indica* seed extract on grubs of *H. septima* expressed as quantity of leaves consumed by the grubs upto third day of treatment is shown in Table 14. The amount of leaf consumption by the grubs varied between treatments. At 24 HAT, it was noticed that the quantity of leaves consumed by the insects when fed by leaves treated with acetone extract @ 0.83 and 5.27% were 0.20 and 0.19 g each, and were statistically on par. This treatment was next to NSKE 5% with 0.16 g of leaf consumption. The treatment ethanol extract @ 7.94% (0.22 g) was also statistically similar to hexane extract @ 6.82% (0.23 g). These treatments were followed by hexane extract @ 1.63% and ethanol extract @ 1.60%, with the mean quantity of leaf consumption being 0.27 g, each. Next best treatments in the order of effectiveness were aqueous extract @ 2.43 and 7.85% with 0.35 and 0.31 g respectively. These treatments statistically differed among them. Grubs in control consumed 0.36 g leaves.

At 48 HAT, it was observed that with acetone extracts @ 0.83 and 7.94% exhibited mean leaf consumption of 0.15 and 0.14 g, each. This was on par with NSKE 5%, wherein mean leaf consumption was 0.14 g. Treatment with ethanol extract @ 7.94% resulted in mean leaf consumption of 0.18 g. The treatments hexane extract @ 1.63 and 6.82% and ethanol extract @ 1.60% with mean leaf consumption of 0.22 to 0.24 g were statistically similar among them. Next best treatments in the order of effectiveness were aqueous extract @ 7.85 and 2.43% which did not exhibit statistical similarity among them. Mean quantity of leaf consumption in these treatments was 0.28 and 0.31 g respectively. Grubs treated with distilled water consumed 0.37 g leaves.

On third day of treatment, it was observed that the quantity of leaves consumed by the grubs fed by leaves treated with the extracts in acetone @ 0.83 and 5.27% and ethanol @ 7.94% were statistically on par with NSKE 5%. Here,

Table 14. Antifeedant effect of seed extracts of *Samadera indica* in different solvents on biology of grubs of *Henosepilachna septima*

Solvents (%)	***Mean leaf consumption (g)**		
	HAT		
	24	48	72
Hexane (1.63)	0.27^{c}	0.23^{d}	0.20^{d}
Hexane (6.82)	0.23^{d}	0.24^{d}	0.19^{d}
Acetone (0.83)	0.20^{ef}	0.15^{f}	0.13^{e}
Acetone (5.27)	0.19^{f}	0.14^{f}	0.12^{e}
Ethanol (1.60)	0.27^{c}	0.22^{d}	0.18^{d}
Ethanol (7.94)	0.22^{de}	0.18^{e}	0.12^{e}
Aqueous (2.43)	0.35^{a}	0.31^{b}	0.29^{b}
Aqueous (7.85)	0.31^{b}	0.28^{c}	0.25^{c}
NSKE 5%	0.16^{g}	0.14^{f}	0.12^{e}
Control	0.36^{a}	0.37^{a}	0.39^{a}
SE (m)	0.007	0.009	0.01
CD (0.05)	0.022	0.026	0.033

HAT – Hours After Treatment; NSKE – Neem Seed Kernel Extract

the mean values were in the range of 0.12 to 0.13 g. The treatments with hexane extract @ 1.63 and 6.82% and ethanol extract @ 1.60% succeeded the above treatments with mean leaf consumption in the range 0.18 to 0.20 g. The treatments with aqueous extracts @ 2.43 and 7.85% differed significantly among themselves and the values observed were 0.29 and 0.25 g respectively. In control, mean leaf consumption by grubs was observed to be 0.39 g.

4.1.4.3. Insecticidal Effect

The mean mortality of insects showed statistically significant variation among treatments. At 24 HAT, it was observed that acetone extract @ 5.27% and ethanol extract @ 7.94% were statistically on par with each other, with mean mortality values of 46.67 and 45.00 per cent respectively. These were in parity with malathion 50 EC @ 0.1% also, with mean mortality of 48.33 per cent. Followed by these were the treatments hexane extract @ 1.63 and 6.82%, acetone extract @ 0.83% and ethanol extract @ 1.60%, and the values ranged from 16.67 to 30.00 per cent. Hexane extract @ 1.63% was equally effective as aqueous extract @ 7.85% also, and the mean values of mortality were observed to be 16.67 and 13.33 per cent, each. Followed by these were aqueous extract @ 2.43 and 7.85%, which were statistically equivalent to each other. Here, mean mortality was 11.67 and 13.33 per cent respectively (Table 15).

At 48 HAT, it was noticed that there was no significant difference between hexane extract @ 6.82%, acetone extract @ 5.27% and ethanol extract @ 7.94%. Mean mortality percentages observed were 63.33, 65.00 and 65.00 respectively. Treatment with aqueous extract @ 7.85% exhibited statistical similarity with acetone extract @ 0.83% and hexane extract @ 6.82%, with mean mortality values of 60.00, 56.67 and 63.33 per cent each. Effect of hexane extract @ 1.63% (50.00 per cent) was statistically similar to ethanol extract @ 1.60% (51.67 per cent) and aqueous extract @ 2.43% (46.67 per cent).

At 72 HAT, it was observed that there was statistical similarity among the treatments hexane extract @ 6.82%, acetone extract @ 5.27%, ethanol extract @ 7.94% and aqueous extract @ 7.85%, with mean values of 85.00, 86.67, 86.67 and 83.33 per cent respectively. These treatments except aqueous extract were

Table 15. Insecticidal effect of seed extracts of *Samadera indica* in different solvents on grubs of *Henosepilachna septima*

Solvents (%)	***Mean larval mortality (%)**		
	HAT		
	24	48	72
Hexane (1.63)	16.67 (24.05)^{cd}	50.00 (45.00)^{ef}	46.67 (43.08)^{c}
Hexane (6.82)	30.00 (33.16)^{b}	63.33 (52.74)^{bc}	85.00 (67.41)^{ab}
Acetone (0.83)	20.00 (26.57)^{c}	56.67 (48.84)^{d}	55.00 (47.88)^{c}
Acetone (5.27)	46.67 (43.09)^{a}	65.00 (53.73)^{b}	86.67 (68.86)^{ab}
Ethanol (1.60)	21.67 (27.71)^{c}	51.67 (45.96)^{e}	53.33 (46.92)^{c}
Ethanol (7.94)	45.00 (42.13)^{a}	65.00 (53.73)^{b}	86.67 (68.86)^{ab}
Aqueous (2.43)	11.67 (19.89)^{e}	46.67 (43.09)^{f}	46.67 (43.09)^{c}
Aqueous (7.85)	13.33 (21.15)^{de}	60.00 (50.77)^{cd}	83.33 (66.15)^{b}
Malathion 50 EC @ 0.1%	48.33 (44.04)^{a}	68.33 (55.77)^{a}	91.67 (73.40)^{a}
Control	0^{f}	0^{g}	0^{d}
SE (m)	1.32	0.69	2.05
CD (0.05)	(3.897)	(2.039)	(6.047)

HAT– Hours After Treatment; Figures in parentheses are arc sine transformed values

statistically on par to chemical treatment (91.67 per cent mean mortality). Effect of hexane extract @ 1.63%, acetone extract @ 0.83%, ethanol extract @ 1.60% and aqueous extract @ 2.43% were statistically similar to each other. Here, mean mortalities were in the range 46.67 to 55.00 per cent.

4.1.4.4. Effect on Biology of Insect

The effect of *S. indica* seed extract on various biological aspects of *H. septima* is expressed in Table 16.

4.1.4.4.1. Deformities in Grubs/Pupae/Adults

Pupae and adults from various treatments showed malformations as in para 4.1.2.4.1.

4.1.4.4.2. Effect on Duration of Grubs

There was an increase in duration of grubs owing to the application of various plant extracts. The treatments exhibited statistically significant variation among them. There was statistical similarity between ethanol extract @ 7.94%, acetone extract @ 5.27% and acetone extract @ 0.83% with NSKE 5%. Here, mean duration of grubs was 13.67 to 14.67 days. The treatments hexane extract @ 1.63 and 6.82% and ethanol extract @ 1.60% were also similar, with mean values in the range of 12.67 to 13.33. Aqueous extracts @ 2.43 and 7.85% exhibited statistical similarity in larval duration (11.33 and 12.33 days each). In control, grubs existed in same stage for 11.33 days.

4.1.4.4.3. Effect on Pupal Weight

There was reduction in mean pupal weight due to the application of plant extracts, with statistical significance among all treatments. Acetone extract @ 5.27% and ethanol extract @ 7.94% formed the best treatments and was found to be statistically on par with NSKE 5% (1.25 g each). These were followed by ethanol extract @ 1.60% and aqueous extract @ 7.85% with mean value of 1.28 days each. In treatment with hexane extract @ 6.82%, pupal weight observed was 1.29 g. Mean pupal weights observed in acetone extract and hexane extract @ 0.83 and 1.63% were 1.29 and 1.30 g respectively and were on par with each

Table 16. Effect of seed extracts of *Samadera indica* in different solvents on biology of grubs of *Henosepilachna septima*

Solvents (%)	Mean					
	No. of malformed insects		Larval period (days)	Pupal weight (g)	Pupal period (days)	Longevity of adults (days)
	P	A				
Hexane (1.63)	0.33	0.33	12.67^{cd}	1.30^{c}	7.00^{bcd}	15.00^{b}
Hexane (6.82)	0.66	0.33	13.33^{bcd}	1.29^{d}	7.33^{abc}	13.67^{c}
Acetone (0.83)	0.33	0.33	13.67^{abc}	1.29^{cd}	7.33^{abc}	13.33^{cd}
Acetone (5.27)	0.66	0.66	14.00^{ab}	1.25^{f}	8.00^{a}	12.33^{d}
Ethanol (1.60)	0	0	13.33^{bcd}	1.28^{e}	7.00^{bcd}	13.67^{c}
Ethanol (7.94)	0.33	0.33	13.67^{abc}	1.25^{f}	7.33^{abc}	12.33^{d}
Aqueous (2.43)	0	0	11.33^{e}	1.34^{b}	6.33^{d}	15.33^{b}
Aqueous (7.85)	0	0	12.33^{de}	1.28^{e}	6.67^{cd}	14.33^{bc}
NSKE 5%	1.00	1.00	14.67^{a}	1.25^{f}	7.67^{ab}	10.67^{e}
Control	0	0	11.33^{e}	1.35^{a}	4.33^{e}	19.67^{a}
SE (m)	0.24	0.24	0.37	0.003	0.28	0.45
CD (0.05)	NS	NS	1.077	0.009	0.823	1.319

P – Pupae, A- Adults; NSKE – Neem Seed Kernel Extract

other. With aqueous extract @ 2.43%, mean pupal weight was observed to be 1.34 g. Mean weight of pupae in control was 1.35 g.

4.1.4.4.4. Effect on Pupal Period

An elongation in pupal period was observed with the application of plant extracts. All treatments differed considerably. The increased pupal duration observed with the application of hexane extract @ 1.63 and 6.82%, acetone extract @ 0.83 and 5.27%, ethanol extract @ 1.60 and 7.94% and aqueous extract @ 7.85% were significantly high and statistically equivalent to NSKE 5%. In these treatments, pupal period was observed to be in the range of 6.67 to 8.00 days. Succeeded by these was the treatment with aqueous extract @ 9.02% (6.67 days), which was statistically on par with aqueous extract @ 2.43% (6.33 days), hexane extract @ 1.63% (7.00 days) and ethanol extract @ 1.60% (7.00 days). Pupae existed for 4.33 days in control.

4.1.4.4.5. Effect on Adult Longevity

There was a decrease in longevity of adults on application of plant extracts and this effect exhibited statistical variation across the treatments. On application of ethanol extract @ 7.94%, acetone extract @ 0.81 and 6.17%, adults were alive upto a mean period of 12.33 to 13.33 days. This was preceded by NSKE 5% with 10.67 days as mean life span in adult beetles. Adult longevity observed with the application of hexane extract @ 6.82%, ethanol extract @ 1.60% and aqueous extract @ 7.85% were statistically similar to acetone extract @ 0.83%. The mean values were noticed to be 13.67, 13.67, 14.33 and 13.33 each. Next were the treatments hexane extract @ 1.63% and aqueous extract @ 2.43% which were on par with aqueous extract @ 7.85%, wherein the mean values ranged from 14.33 to 15.33 days. In treatment with distilled water, adults survived for a mean period of 19.67 days.

4.1.4.4.6. Effect on Fecundity, Egg Hatchability, Preoviposition period and Incubation Period

Only 10 to 50 per cent of grubs were left out after 72 HAT. These were unable to lay eggs. Hence, further experiments on adult emergence could not be carried out. Adults from untreated control laid mean number of 198.20 eggs and 177.00 eggs hatched out of them. It took an average of 5.67 days from adult emergence for oviposition and the mean incubation period was 4.33 days.

The results of this experiment revealed that acetone extracts of *S. indica* seeds @ 5.27% exhibited greater feeding deterrence, mortality and adverse effects on biology of epilachna beetles.

With regard to lethal doses, LD_{50} and LD_{90} observed with seed extract of *S. indcia* in acetone was the lowest, when compared with the bark extract. Hence, seed extract in acetone was used for further experiments.

4.1.5. Fractionation of Effective Extract

Fractionation of acetone extract from seeds of *S. indica* using column chromatography resulted in the development of 86 fractions (Table 17).

4.2. BIOEFFICACY STUDY OF ISOLATED BIOACTIVE COMPOUND

Based on TLC (Thin Layer Chromatography) profile, chromatographic fractions were clubbed into six main fractions *viz*., A, B, C, D, E and F. *Table* 18 shows the list of main fractions, constituent fractions and the corresponding R_f (Retention Factor) values. UV active spots were not detected in Fraction A comprising of 24 sub-fractions. This might be due to the presence of oil in these fractions leading to the assumption that no UV-active compounds were present in these fractions. Hence, all fractions, exempting fraction A were taken for bioefficacy studies on insects.

4.2.1. On *Diaphania indica*

4.2.1.1. Antifeedant Effect

Antifeedant effect of chromatographic fractions, expressed as quantity of leaf fed by treated larvae on one, three, five and seven DAT is given in Table 19. It was observed that all these treatments differed statistically.

Observations on 1st DAT indicated that fractions from acetone extract exhibited significant antifeedant effect in a dose-dependent manner. In treatment

Table 17. Chromatographic fractions collected using different solvent combinations

Sl. No.	Solvent combinations	No. of fractions collected
1	Hexane – 100%	1-5
2	Hexane: Ethyl acetate – 90:10	5-10
3	Hexane: Ethyl acetate – 80:20	10-18
4	Hexane: Ethyl acetate – 70:30	19-23
5	Hexane: Ethyl acetate – 60:40	24-28
6	Hexane: Ethyl acetate – 50:50	29-33
7	Hexane: Ethyl acetate – 40:60	34-38
8	Hexane: Ethyl acetate – 30:70	39-43
9	Hexane: Ethyl acetate – 20:80	44-50
10	Ethyl acetate – 100%	51-57
11	Ethyl acetate: Methanol – 95:5	58-64
12	Ethyl acetate: Methanol – 90:10	65-71
13	Ethyl acetate: Methanol – 60:40	72-78
14	Ethyl acetate: Methanol – 40:60	79-81
15	Methanol – 100%	82-86

Table 18. Chromatographic fractions from acetone extract of *Samadera indica* seeds

Chromatographic fractions	**Constituent fractions**	**R_f value**
Fraction A	1 – 24	UV active spots were not detected
Fraction B	25 – 35	0.86
Fraction C	36 – 43	0.83
Fraction D	44 – 51	0.76
Fraction E	52 – 61	0.33
Fraction F	62 – 86	0.27

R_f – Retention Factor

Table 19. Antifeedant effect of chromatographic fractions from acetone extract of *Samadera indica* seeds on *Diaphania indica*

Treatments	***Mean quantity of leaves consumed by larva (g)**			
	DAT			
	1	3	5	7
Fractions from acetone extract @ 5%	0.14^{cd}	0.10^{c}	0.07^{c}	0.04^{bc}
Fractions from acetone extract @ 2.5%	0.16^{c}	0.10^{c}	0.06^{c}	0.04^{bc}
Fractions from acetone extract @ 1.25%	0.21^{b}	0.16^{b}	0.09^{b}	0.06^{b}
Neemazal @ 0.2%	0.13^{d}	0.09^{d}	0.07^{c}	0.03^{c}
Malathion 50 EC @ 0.1%	0.09^{e}	0.05^{e}	0.02^{d}	0.01^{d}
Control	0.36^{a}	0.41^{a}	0.46^{a}	0.49^{a}
SE (m)	0.006	0.005	0.005	0.006
CD (0.05)	0.017	0.014	0.016	0.018

DAT – Days After Treatment

with malathion 50 EC @ 0.1%, mean leaf consumption was 0.09 g. This was succeeded by treatment with fractions from acetone extract @ 5% (0.14 g) which was on par with neemazal @ 0.2% (0.13 g). Treatments with fractions @ 2.5% (0.16 g) exhibited statistical similarity with fractions @ 5%. In treatment with fractions @ 1.25%, mean quantity of leaf consumption was 0.21 g. Larvae in control consumed 0.36 g leaves.

After 72 h, it was noticed that larvae that consumed the leaves treated with chromatographic fractions of acetone extract @ 5 and 2.5% were statistically similar and the values were 0.10 g each. This was preceded by neemazal @ 0.2% with 0.09 g, being the mean quantity of leaf consumption. In leaves treated with chemical control, average leaf consumption was 0.05 g. Larvae fed an average of 0.16 g of leaves when fed with leaves treated with acetone fractions @ 1.25%. Larvae in control fed an average of 0.41 g leaves.

On the 5^{th} day of treatment, it was observed that the mean quantity of leaf consumption in treatments with acetone fractions @ 5% and 2.5% were in parity with neemazal @ 0.2%, thus proving them to be the effective treatments. The mean values observed were 0.07, 0.06 and 0.07 g, each. When leaves were treated with fractions from acetone extract @ 1.25%, larvae consumed an average of 0.09 g of leaves. Larvae consumed 0.02 g leaves when leaves were treated with chemical insecticide. These treatments differed significantly among each other. Larvae consumed an average of 0.46 g leaf when treated with distilled water.

At one week after treatment, it was observed that the treatments with fractions from acetone extract @ 2.5% was statistically similar in effect with 5 and 1.25% of the extracts and neemazal @ 0.2%, with the mean values of leaf consumption being 0.04, 0.04, 0.06 and 0.03 g respectively. These were preceded by malathion 50 EC @ 0.1% (0.01 g) which differed statistically among them. In treatment with distilled water, larvae consumed an average of 0.49 g leaves.

4.2.1.2. Effect on Mortality of Larvae

The effect of chromatographic fractions on larval mortality at 24, 48 and 72 HAT is expressed in Table 20. The data exhibited statistical variation among the treatments. At 24 HAT, it was observed that there was 53.33 per cent mortality with fractions from acetone extract @ 5% and this was found to be statistically on par with neemazal @ 0.2%, with a mortality of 56.67 per cent. These were preceded by malathion 50 EC @ 0.1%, with 66.67 per cent mortality. The treatments with chromatographic fractions from acetone extract @ 2.5 and 1.25 per cent differed significantly among each other and the values were observed to be 40.00 and 23.33 per cent, each.

At 48 HAT, fractions from acetone extract @ 5% exhibited mortality equivalent to that of neemazal @ 0.2% and the values were 54.78 and 63.33 per cent respectively. These were observed to be just behind malathion 50 EC @ 0.1% with 76.67 per cent mortality. The percentage mortality exhibited by chromatographic fractions from acetone extract @ 2.5 and 1.25% were 46.67 and 40.00 per cent and were statistically on par.

On third day of treatment, it was observed that treatment with malathion 50 EC @ 0.1% resulted in 93.33 per cent mortality in treated larvae. This was followed by fractions @ 5% which was statistically on par with that of neemazal @ 0.2% and fractions from acetone extract @ 2.5%. Mean percentage mortality observed in these treatments was 73.33, 76.67 and 60.00 respectively. Followed by these were the treatments with fractions from acetone extract @ 1.25%, with mean mortality of 53.33 per cent.

4.2.2. On Henosepilachna septima

4.2.2.1. Antifeedant Effect

The repellent effect on feeding action of grubs of epilachna beetles expressed as quantity of leaf consumed by treated grubs on one, three, five and seven DAT is given in Table 21. There was significant variation among various treatments.

On the initial day after treatment, it was noticed that the mean quantity of leaves consumed by grubs when fed with leaves treated with the chemical, malathion 50 EC @ 0.1% was 0.09 g. Next were the treatments with

Table 20. Effect of chromatographic fractions from acetone extract of *Samadera indica* seeds on *Diaphania indica*

Treatments	***Mean mortality of larvae (%)**		
	HAT		
	24	48	72
Fractions from acetone extract @ 5%	53.33 (46.92)^{b}	54.78 (52.78)^{b}	73.33 (59.00)^{bc}
Fractions from acetone extract @ 2.5%	40.00 (39.23)^{c}	46.67 (43.08)^{c}	60.00 (50.77)^{cd}
Fractions from acetone extract @ 1.25%	23.33 (28.78)^{d}	40.00 (39.23)^{c}	53.33 (46.92)^{d}
Neemazal @ 0.2%	56.67 (48.85)^{b}	63.33 (66.67)^{b}	76.67 (61.22)^{b}
Malathion 50 EC @ 0.1%	66.67 (54.78)^{a}	76.67 (61.22)^{a}	93.33 (77.71)^{a}
Control	0^{e}	0^{d}	0^{e}
SE (m)	2.72	1.66	2.93
CD (0.05)	(5.084)	(5.135)	(9.008)

HAT – Hours After Treatment; Figures in parentheses are values after arc sine transformation

Table 21. Antifeedant effect of chromatographic fractions from acetone extract of *Samadera indica* seeds on *Henosepilachna septima*

Treatments	***Mean quantity of leaves consumed by grubs (g)**			
	DAT			
	1	3	5	7
Fractions from acetone extract @ 5%	0.16[d]	0.14[c]	0.09[c]	0.07[c]
Fractions from acetone extract @ 2.5%	0.17[c]	0.14[c]	0.09[c]	0.07[c]
Fractions from acetone extract @ 1.25%	0.20[b]	0.17[b]	0.11[b]	0.09[b]
Neemazal @ 0.2%	0.16[d]	0.12[d]	0.08[d]	0.05[d]
Malathion 50 EC @ 0.1%	0.09[e]	0.06[e]	0.03[e]	0.01[e]
Control	0.40[a]	0.44[a]	0.47[a]	0.49[a]
SE (m)	0.003	0.005	0.004	0.006
CD (0.05)	0.010	0.014	0.013	0.017

DAT – Days After Treatment

chromatographic fractions of acetone extract @ 5% and neemazal @ 0.2%, which were statistically on par. Mean quantity of leaf consumption in these treatments was 0.16 g, each. This was subsequently followed by the treatments with fractions from acetone extract @ 2.5 and 1.25% each, mean leaf consumption of 0.17 and 0.20. These treatments differed significantly among each other. Mean leaf consumption in control was 0.40 g .

On 3rd day of treatment, it was observed that larvae fed with leaves treated with neemazal @ 0.2% and malathion 50 EC @ 0.1% consumed 0.12 and 0.06 g respectively. These were followed by fractions from acetone extract @ 5 and 2.5% with 0.14 g each being the average quantity of leaf consumption by grubs and these treatments were found to be statistically on par. Treatment with these at 1.25% resulted in 0.17 g of leaf consumption. An average of 0.44 g leaves was consumed by grubs when fed with leaves treated with distilled water.

On the 5th day of treatment too, similar trend was seen with fractions from acetone extract @ 5 and 2.5% each exhibiting statistical similarity among each other. The mean values observed were 0.09 g each. These were preceded by treatments with neemazal @ 0.2% and malathion 50 EC @ 0.1%, wherein mean quantity of leaf consumption was 0.08 and 0.03 g respectively. The average quantity of leaves consumed by grubs when fed with leaves treated with chromatographic fractions @ 1.25% was 0.11 g. When treated with distilled water, grubs consumed an average of 0.47 g leaves.

On 7 DAT, the mean quantity of leaf consumption observed with neemazal @ 0.2% and malathion 50 EC @ 0.1% were 0.05 and 0.01 g, each. The mean quantity of leaf consumption in treatments with fractions from acetone extract @ 5 and 2.5% were observed to be statistically similar and the mean values were 0.07 g, each. In treatment with fraction from acetone extract @ 1.25%, mean quantity of leaf consumption was observed as 0.09 g. But, this treatment differed significantly from the above two treatments. In treatment with distilled water, 0.49 g was the mean leaf consumption.

4.2.2.2. Effect on Mortality of Grubs

Data on mortality of grubs due to the application of chromatographic fractions of acetone exhibited significant difference. On first day of treatment, fractions from acetone extract @ 5% and neemazal @ 0.2% exhibited statistically similar mortality and were next to malathion 50 EC @ 0.1% with 73.33 per cent mortality. In the former treatments, the mean mortality was 53.33 and 60.00 per cent each. The mean mortality exhibited by chromatographic fractions @ 2.5 and 1.25 per cent was observed were 36.67 and 26.67 per cent respectively (Table 22).

At 48 HAT, fractions from acetone extract @ 5% were found to exhibit 66.67 per cent mortality and it showed statistical similarity with the check treatment neemazal @ 0.2% with 73.33 per cent mean mortality. Insects treated with chromatographic fractions @ 2.5 and 1.25% showed mean mortality of 53.33 and 36.67 per cent respectively. These treatments differed significantly from each other.

At 72 HAT, it was observed that fractions from acetone extract @ 5 and 2.5% caused 73.33 and 63.33 per cent mortality each in grubs and effect of these two were statistically on par with neemazal @ 0.2% with mean mortality of 73.33 per cent. But, these treatments differed statistically from malathion 50 EC @ 0.1% with 93.33 per cent mortality. In treatment with 1.25% chromatographic fraction, percentage mortality in grubs was 53.33.

The above results indicated that application of chromatographic fractions from seed extracts of *S. indica* @ 5% resulted in higher degree of feeding repellency and higher mortality rate in young instars of *D. indica* and *H. septima*.

4.3. STRUCTURAL CHARACTERISATION OF THE BIOACTIVE COMPOUND

Comparison of TLC (Thin Layer Chromatography) profiles of effective chromatographic fractions from acetone extract and that of the earlier identified marker compounds (quassinoids) available at CSIR-NIIST revealed similarity among the two. Hence, proton (^{1}H) and carbon (^{13}C) NMR data were derived to identify the similar compounds.

Table 22. Effect of chromatographic fractions from acetone extract of *Samadera indica* seeds on *Henosepilachna septima*

Treatments	***Mean mortality of grubs (%)**		
	HAT		
	24	48	72
Fractions from acetone extract @ 5%	53.33 (46.92)^{b}	66.67 (54.78)^{b}	73.33 (59.00)^{b}
Fractions from acetone extract @ 2.5%	36.67 (37.23)^{c}	53.33 (46.92)^{c}	63.33 (52.78)^{bc}
Fractions from acetone extract @ 1.25%	26.67 (31.00)^{d}	36.67 (37.23)^{d}	53.33 (46.92)^{c}
Neemazal @ 0.2%	60.00 (50.77)^{b}	73.33 (59.00)^{ab}	73.33 (59.00)^{b}
Malathion 50 EC @ 0.1%	73.33 (59.00)^{a}	76.67 (61.22)^{a}	93.33 (77.71)^{a}
Control	0^{e}	0^{e}	0^{d}
SE (m)	1.71	1.90	3.04
CD (0.05)	(5.269)	(5.842)	(9.355)

HAT – Hours After Treatment; Figures in parentheses are values after arc sine transformation

Compound 1 was obtained as a colourless solid and showed a molecular ion peak at m/z 331.1211 $[M+H]^+$ (calculated for $C_{18}H_{19}O_6$, 331.12) in the HRESIMS (High Resolution ElectroSpray Ionisation Mass Spectrometry), and the molecular formula was established as $C_{18}H_{18}O_6$. The ^1H NMR spectrum of compound 1 (Fig. 5) displayed peaks at δ 6.24 (d, *J* = 1Hz, 1H), 6.02 (s, 1H), 4.82 (d, *J* = 9 Hz, 1H), 4.30 (d, *J* = 3.5 Hz, 1H), 4.16 (dd, J_1 = 9 Hz, J_2 = 1.5 Hz, 1H), 3.58 (d, *J* = 10.5 Hz, 1H), 3.45 (d, *J*= 1 Hz, 1H), 2.27 (dd, J_1 = 1 Hz, J_2 = 4.5 Hz, 1H), 2.23 (d, *J* = 1.5 Hz, 3H), 1.73 (s, 3H) and 1.60 (s, 3H) ppm. The ^{13}CNMR spectrum of compound 1 (Fig. 6) displaying the presence of 18 carbon atoms was ascertained with the carbon signals at δ 203.5, 193.8, 171.1, 168.8, 163.3, 134.1, 116.8, 89.2, 81.8, 76.1, 69.0, 58.1, 57.4, 48.2, 40.2, 21.4, 20.9 and 13.7 ppm. Compound 1 was assessed to be samaderin A.

Compound 2 was obtained as a colourless crystalline solid and showed a molecular ion peak at m/z 363.38 $[M+H]^+$ (calculated for $C_{19}H_{23}O_7$, 363.14) in the HRESIMS, and the molecular formula was established as $C_{19}H_{22}O_7$. The ^1H NMR spectrum of compound 2 (Fig. 7) exhibited peaks at δ 5.99 (d, *J* = 1Hz, 1H), 5.56 (d, *J*= 4 Hz, 1H), 4.74 (d, *J* = 8.5 Hz, 1H), 4.63 (m, 1H),4.38 (d, *J* = 4.5 Hz, 1H) 4.27 (d, *J*= 3.5 Hz, 1H), 4.23 (d, *J* = 2.5 Hz, 1H), 3.60 (d, *J* = 7 Hz, 1H), 3.42 (d, *J* = 1Hz, 1H), 3.07 (d, *J* = 13.5 Hz, 1H), 2.75 (m, 2H), 2.12 (d, *J* = 3.5 Hz, 1H), 1.88 (s, 3H), 1.41 (s, 3H), 1.24 (s, 3H) ppm. From ^{13}C NMR spectrum of compound 2 (Fig. 8), the presence of 19 carbon atoms was ascertained with the carbon signals at δ 205.3, 197.7, 171.9, 161.2, 124.2, 86.8, 83.6, 80.9, 74.6, 69.5, 60.4, 55.3, 48.5, 46.7, 46.6, 38.4, 21.4, 20.4 and 9.9 ppm. Compound B was assessed to be samaderin B.

Compound 3 was obtained as a colourless crystalline solid and displayed a molecular ion peak at m/z 365.1610 $[M+H]^+$ (calculated for $C_{19}H_{25}O_7$, 367.16) in the HRESIMS, and the molecular formula was established as $C_{19}H_{24}O_7$. The ^1H NMR spectrum of compound 3 (Fig. 9) exhibited peaks at δ 5.67 (d, *J* = 4.5 Hz, 1H), 5.34 (s, 1H), 4.80 (t, *J* = 3 Hz, 2H), 4.66 (dd, J_1 = 9 Hz, J_2 = 5 Hz, 1H), 4.45

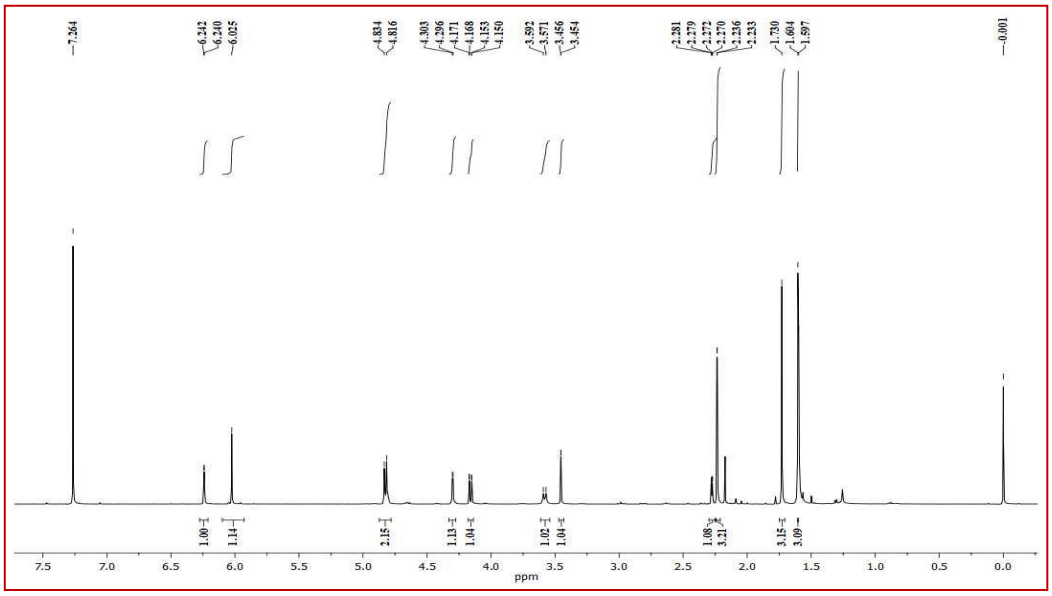


Fig. 5. ^{1}H NMR spectrum of samaderin A

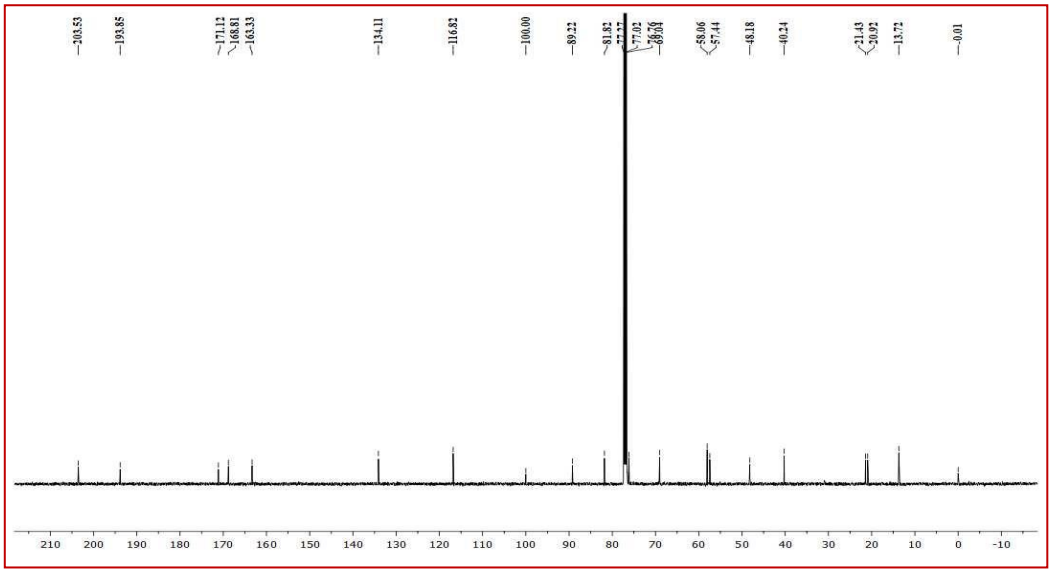


Fig. 6. ^{13}C NMR spectrum of samaderin A

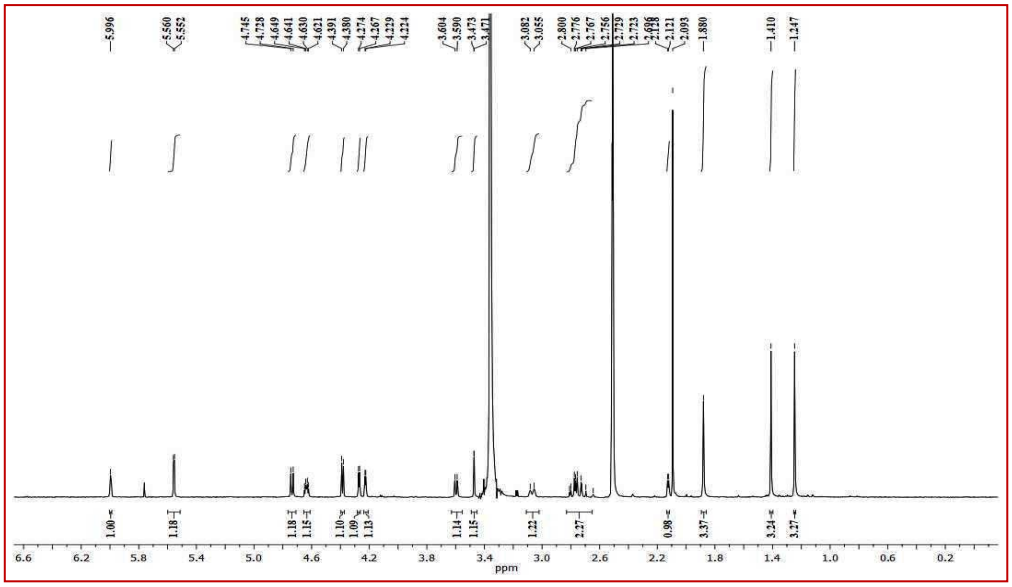


Fig. 7. ^{1}H NMR spectrum of samaderin B

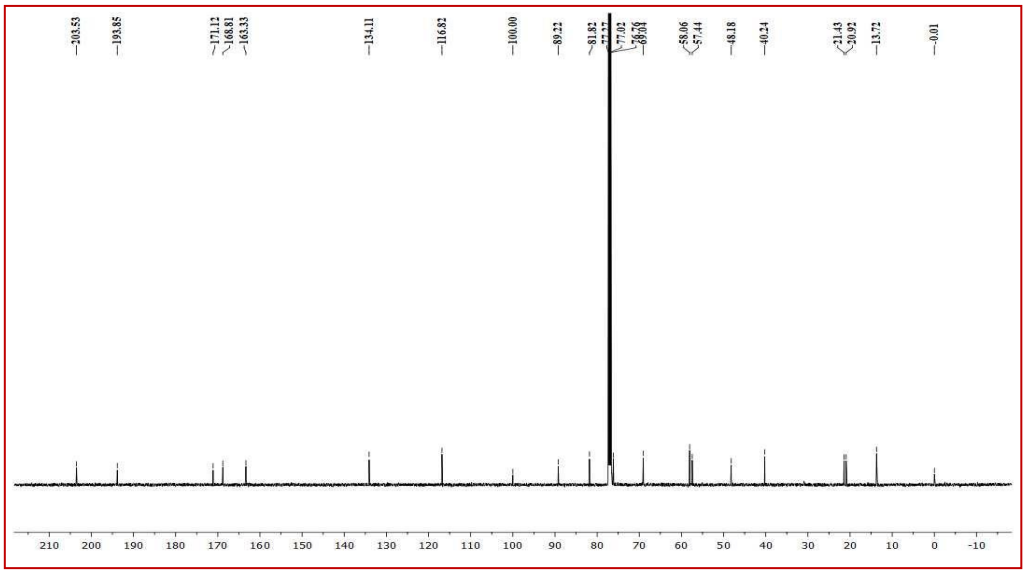


Fig. 8. ^{13}C NMR spectrum of samaderin B

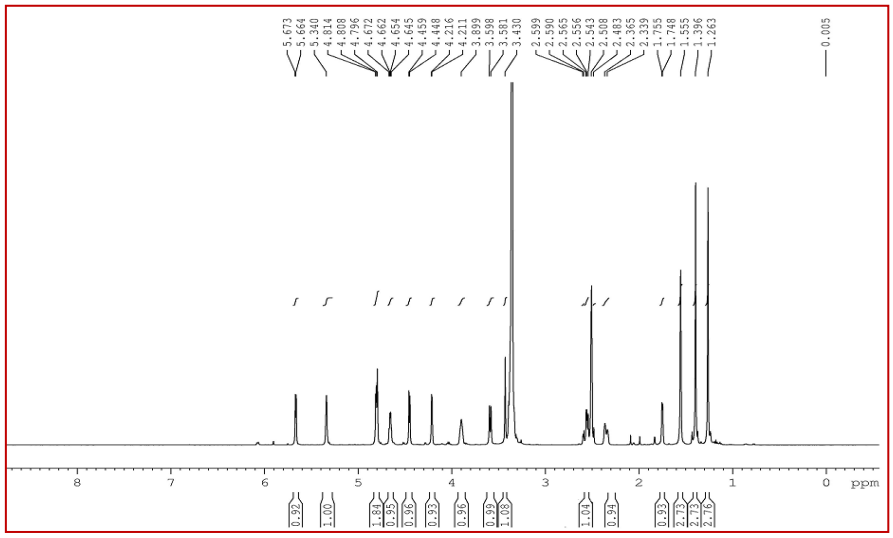


Fig. 9. ^{1}H NMR spectrum of samaderin C

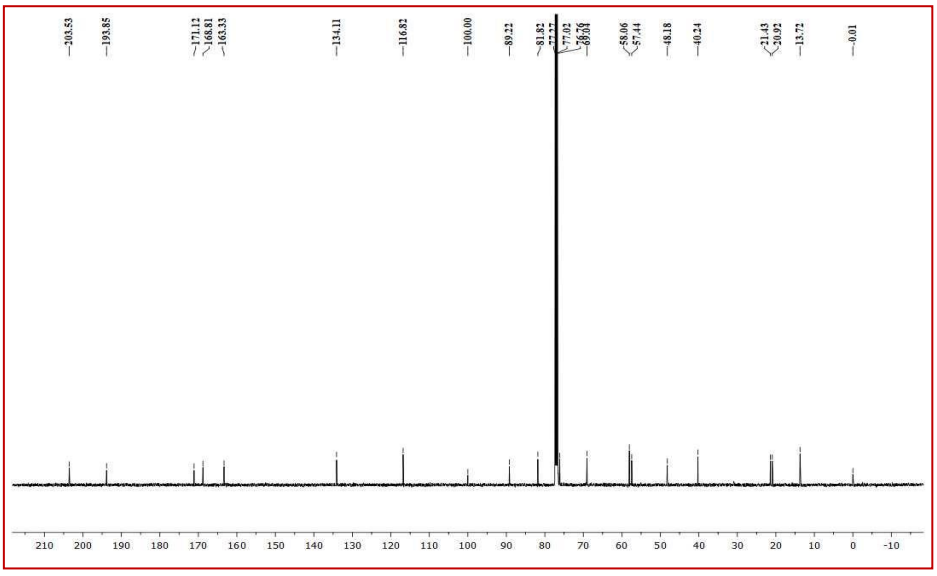


Fig. 10. ^{13}C NMR spectrum of samaderin C

(d, *J* = 5.5 Hz, 1H), 4.21 (d, *J* = 5 Hz, 1H), 3.90 (s, 1H), 3.59 (d, *J* = 8.5 Hz, 1H), 3.43 (s, 1H), 2.55 (t, *J* = 4.5 Hz, 1H), 2.35 (d, *J* = 13 Hz, 1H), 1.75 (d, *J* = 3.5 Hz, 1H), 1.55 (s, 3H), 1.40 (s, 3H), 1.26 (s, 3H) ppm. From ^{13}C NMR spectrum of compound 3 (Fig. 10), the presence of 19 carbon atoms was established with the carbon signals at δ 206.0, 172.2, 132.4, 125.7, 86.8, 83.7, 80.5, 75.1, 71.5, 69.6, 60.5, 55.7, 49.2, 47.5, 46.9, 43.0, 20.4, 20.1 and 10.3 ppm. Samaderin C was inferred to be the third compound.

Compound 4 was obtained as a colourless crystalline solid and showed a molecular ion peak at m/z 365.16 $[M+H]^+$ (calculated for $C_{19}H_{25}O_7$, 365.16) in the HRESIMS, and the molecular formula was established as $C_{19}H_{24}O_7$. The ^1H NMR spectrum of compound 4 (Fig. 11) exhibited peaks at δ 5.96 (d, *J* = 1.5 Hz, 1H), 5.10 (d, *J* = 3.5 Hz, 1H), 5.05 (d, *J* = 4 Hz, 1H), 4.69 (t, *J* = 3 Hz, 1H), 4.47 (d, *J* = 8 Hz, 1H), 4.08 (d, *J* = 6.5 Hz, 1H), 4.04 (d, *J* = 3.5 Hz, 2H), 3.73 (s, 1H), 3.60 (d, *J* = 8.5 Hz, 1H), 3.06 (d, *J* = 12.5 Hz, 1H), 2.70 (s, 1H), 2.09 (t, *J*= 4.5 Hz, 1H), 1.91 (m, 1H), 1.86 (s, 3H), 1.53 (t, *J* = 13 Hz, 1H), 1.33 (s, 3H), 1.12 (s, 3H) ppm. From the ^{13}C NMR spectrum of compound 4 (Fig. 12), the presence of 19 carbon atoms was ascertained with the carbon signals at δ 198.3, 172.1, 164.4, 124.0, 86.6, 83.4, 83.1, 74.4, 69.8, 69.4, 58.65, 54.3, 47.5, 44.0, 42.1, 40.1, 29.1, 22.1, 20.7 and 10.4 ppm. Cedronin was assessed to be compound 4.

The structures of the four bioactive compounds *viz*., samaderins A, B, C and cedronin described above are illustrated in Fig. 13.

4.4. DEVELOPMENT OF SUITABLE FORMULATIONS OF BIOACTIVE COMPOUNDS

Two sets of formulations (Formulation A and Formulation B) were prepared by adding active ingredient/bioactive compound, emulsifier/surfactant and solvent in appropriate proportion. Formulation A (Plate 10) was prepared by adding acetone extract, tween 80 and water in the ratio 15: 5: 80. Acetone extract was added to tween 80 and water heated at 55 ± 5^0C was added to this mixture. Formulation B (Plate 11) was also prepared in a similar manner as that of formulation A, except that tween 80 was replaced by a combination of tween 80

and span 80 in 1: 1 ratio. Crude formulations A and B were termed as Emulsifiable Concentrates (EC) and these became emulsions (Plate 12) when diluted with water prior to application.

4.4.1. Stability of Formulation

The results on assessment of stability of the formulations assessed for a period of one week are illustrated in Table 23.

4.4.2. Bioefficacy Studies of Formulation

4.4.2.1. On Diaphania indica

The efficacy of bioformulations from seed extracts of *S. indica* expressed as mean mortality (%) at 24, 48 and 72 HAT is given in Table 24. There was statistical difference among all treatments. At 24 HAT, application of formulation A @ 1% has resulted 41.67 per cent mortality and was preceded by malathion 50 EC @ 0.1% and neemazal @ 0.2%, with 68.33 and 58.33 per cent mortality each. But, these treatments differed significantly among each other. In treatment with formulation B @ 1%, there was 33.33 per cent mortality in beetles. Treatments with formulations A and B @ 0.5% each showed statistically significant variation and mean mortality in these treatments was 18.33 and 15.00 per cent respectively.

At 48 HAT too, the trend observed was similar. Among formulations of *S. indica*, formulation A @ 1% was the most effective one with 56.67 per cent mortality. This was followed by formulation B @ 1% with 48.33 per cent mortality. Both the formulations A and B @ 0.5% each witnessed similar mortality percentage in second instar larvae of *D. indica* which ranged from 33.33 to 36.67. Mortality observed with the application of neemazal @ 0.2% and malathion 50 EC @ 0.1%was 71.67 and 76.67 per cent each and these two treatments differed significantly from each other.

On third day of treatment, application of check treatment (malathion 50 EC @ 0.1%) resulted in 96.67 per cent larval mortality. Formulation A @ 1%

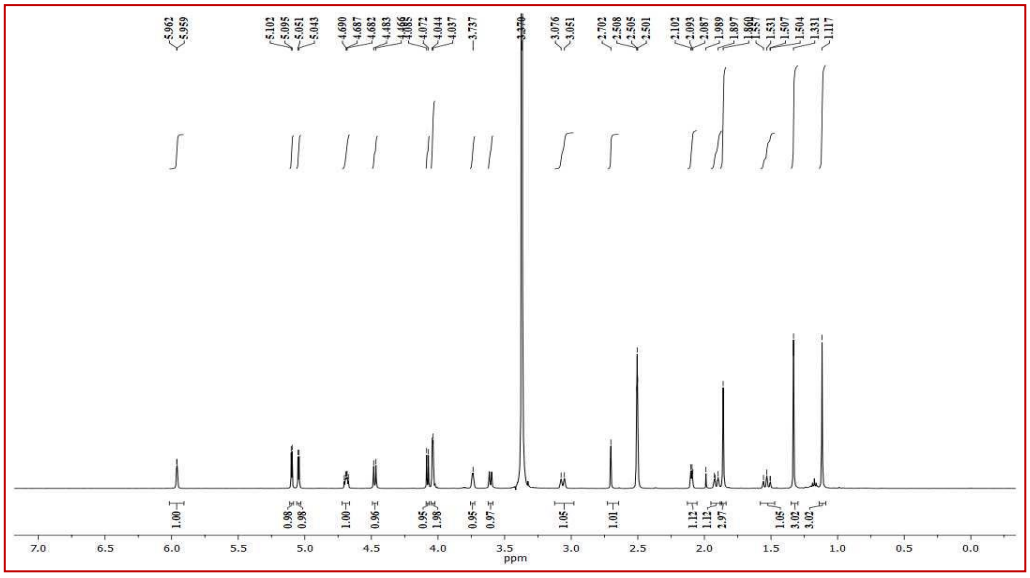


Fig. 11. ^{1}H NMR spectrum of cedronin

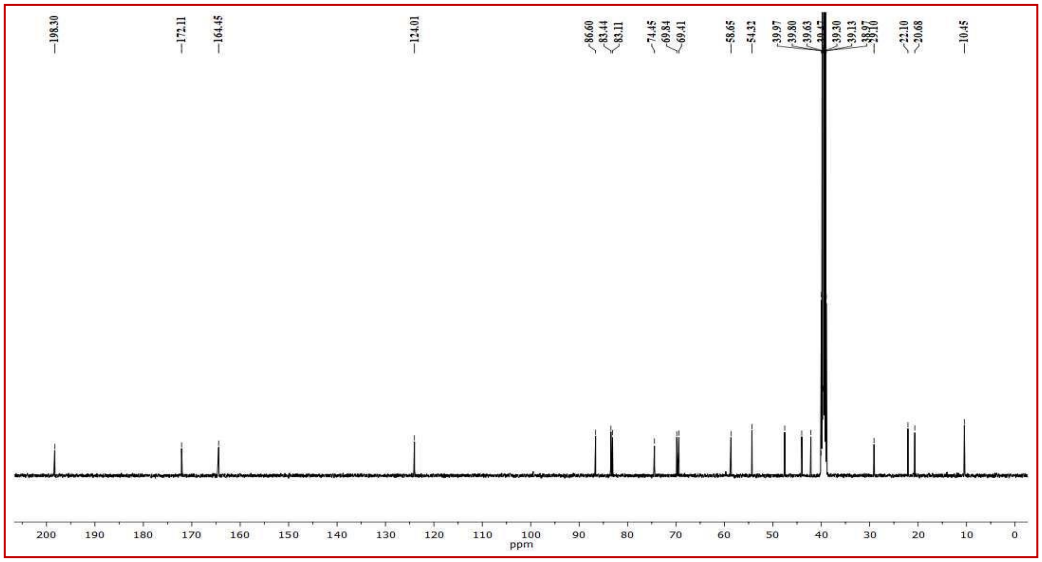


Fig. 12. ^{13}C NMR spectrum of cedronin

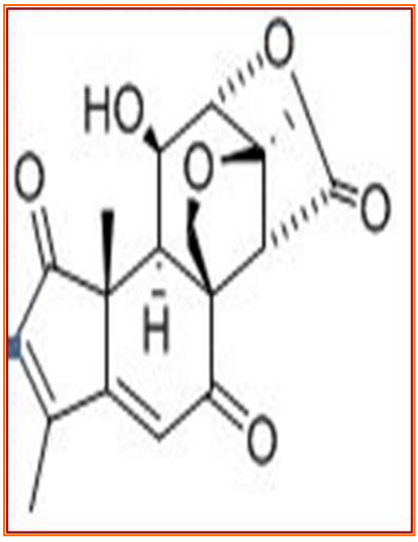


Fig. 13 A. Samaderin A

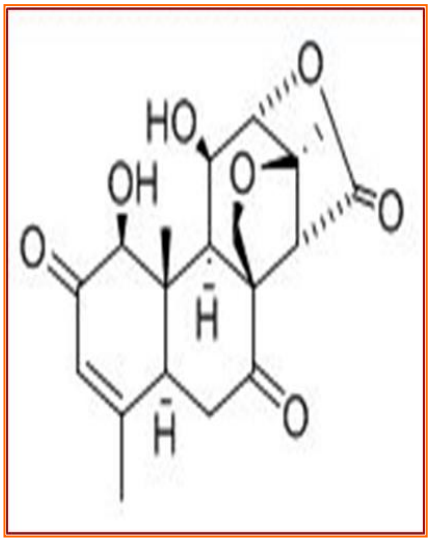


Fig. 13 B. Samaderin B

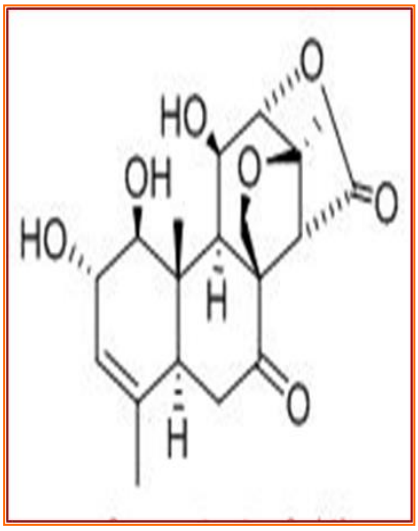


Fig. 13 C. Samaderin C

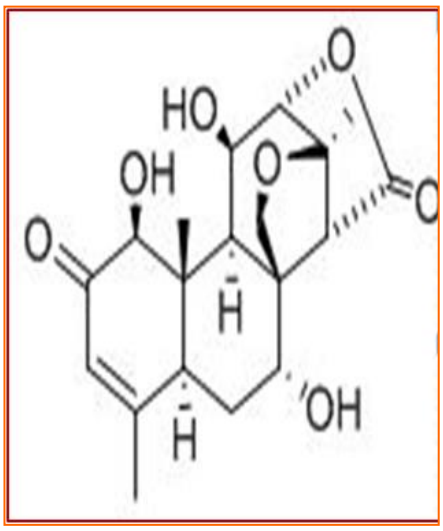


Fig. 13 D. Cedronin

Fig. 13. Structure of bioactive compounds

Table 23. Physical tests for stability of formulations A and B

Physical tests	**Formulation A**	**Formulation B**
Colour	Yellow	Light yellow
Appearance	Free-flowing	Free-flowing
Filter paper test	Spreads out rapidly in filter paper (O/W emulsion)	O/W emulsion
Cold test	No turbidity or separated oily or solid matter	No turbidity or separated oily or solid matter
Test for heat stability	No turbidity	Lesser turbidity
Acidity	pH - 5.87	pH - 6.66
Electrical Conductivity (EC)	20 µS	14 µS
Bloom test	Bloom was rated as excellent with code 5 (Plate 13A)	Bloom was rated as excellent with code 5 (Plate 13B)

Table 24. Effect of formulations from *Samadera indica* seed extracts on *Diaphania indica*

Treatments	***Mean mortality of larvae (%)**		
	HAT		
	24	48	72
Formulation A @ 1%	41.67 (40.20)^{c}	56.67 (48.84)^{c}	80.00 (63.74)^{bc}
Formulation A @ 0.5%	18.33 (25.31)^{e}	36.67 (37.26)^{e}	46.67 (43.09)^{d}
Formulation B @ 1%	33.33 (35.25)^{d}	48.33 (44.04)^{d}	71.67 (57.86)^{c}
Formulation B @ 0.5%	15.00 (22.79)^{e}	33.33 (35.25)^{e}	43.33 (41.16)^{d}
Neemazal @ 0.2%	58.33 (49.80)^{b}	71.67 (57.86)^{b}	88.33 (70.12)^{b}
Malathion 50 EC @ 0.1%	68.33 (55.77)^{a}	76.67 (61.15)^{a}	96.67 (81.30)^{a}
Control	0^{f}	0^{f}	0^{e}
SE (m)	0.89	0.95	2.26
CD (0.05)	(2.697)	(2.882)	(6.774)

HAT – Hours After Treatment; Figures in parentheses are values after arc sine transformation

Plate 10. Formulation A

Plate 11. Formulation B

Plate 12. Emulsion

Plate 13A. Formulation A

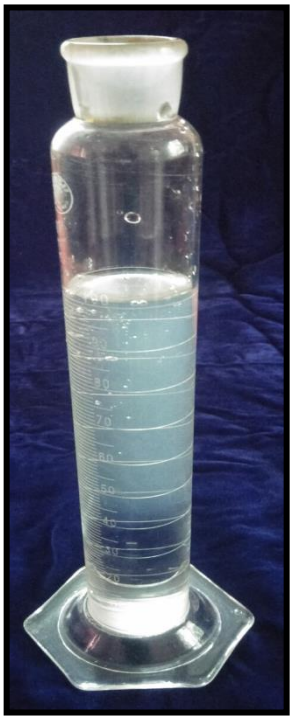


Plate 13B. Formulation B

Plate 13. White bulging emulsion with no oil droplets

witnessed reduction in the population of *D. indica* to 80.00 per cent and mortality was statistically on par with neemazal @ 0.2% and formulation B @ 1%. Mean percentage mortality in these two treatments ranged from 71.67 to 88.33. Next were the treatments with formulations A and B at 0.5% each, which exhibited statistical similarity among them, with mean mortality ranging from 43.33 to 46.67 per cent.

4.4.2.2. On Henosepilachna septima

The data on mortality of *H. septima* witnessed significant difference. On first DAT, application of formulation A and formulation B @ 1% recorded 41.67 per cent each against *H. septima*. Treatments with 0.5 per cent of the above mentioned formulations exhibited 18.33 to 20.00 per cent mortality and these were found to be statistically on par. In treatment with neemazal @ 0.2%, an average of half of the treated population exhibited mortality (50.00 per cent). It was observed that 56.67 per cent of the grubs died when treated with the chemical malathion 50 EC @ 0.1%. These two treatments differed significantly among each other (Table 25).

At 48 HAT, formulation A @ 1% exhibited mean mortality of 58.33 per cent and was next to neemazal @ 0.2% with 66.67 per cent mortality. Meanwhile, formulation B @ 1% and formulations A and B @ 0.5% exhibited mean mortality of 31.67 to 48.33 per cent. These treatments were also found to be statistically on par with each other. The mean mortality of grubs in chemical, malathion 50 EC @ 0.1% was 78.33 per cent.

At 72 HAT, formulation A @ 1% caused mean mortality of 80.00 per cent, which was statistically on par with neemazal @ 0.2%, with 88.33 per cent mean mortality. This was succeeded by formulation B @ 1% with a mean mortality of 71.67 per cent. Subsequent were the treatments with formulations A and B @ 0.5% each, wherein mean values ranged from 43.33 to 46.67 per cent. The effect of these treatments was statistically on par. In chemical control, mortality of grubs was 96.67 per cent.

Table 25. Effect of formulations from *Samadera indica* seed extracts on *Henosepilachna septima*

Treatments	***Mean mortality of grubs (%)**		
	HAT		
	24	48	72
Formulation A @ 1%	41.67 (40.20)^{c}	58.33 (49.80)^{c}	80.00 (63.74)^{b}
Formulation A @ 0.5%	20.00 (26.57)^{d}	31.67 (34.23)^{d}	46.67 (43.09)^{d}
Formulation B @ 1%	41.67 (40.20)^{c}	48.33 (44.04)^{d}	71.67 (57.86)^{c}
Formulation B @ 0.5%	18.33 (25.31)^{d}	33.33 (35.25)^{d}	43.33 (41.16)^{d}
Neemazal @ 0.2%	50.00 (45.00)^{b}	66.67 (54.75)^{b}	88.33 (70.12)^{b}
Malathion 50 EC @ 0.1%	56.67 (48.84)^{a}	78.33 (62.29)^{a}	96.67 (81.39)^{a}
Control	0^{e}	0^{e}	0^{e}
SE (m)	0.89	0.95	2.26
CD (0.05)	(2.402)	(2.878)	(6.853)

HAT – Hours After Treatment; Figures in parentheses are arc sine transformed values

4.4.3. Safety Evaluation of Formulation

The observations on safety assay of formulations of seed extracts of *S. indica* on pollinators and parasitoids are shown below.

4.4.3.1. Evaluation of Safety to Pollinators

The safety of bioformulation was assessed on Indian honey bee (*Apis cerana indica* (Fabricius)) and expressed in terms of mortality of test insect at 6, 12 and 24 HAT (Table 26). All treatments showed statistically significant variation.

Observations at 6 HAT indicated that application of malathion 50 EC @ 0.1% resulted in mortality of 60.00 per cent in honey bees. Application of formulation A @ 2% resulted in mortality of 26.67 per cent bees and was on par with neemazal @ 0.2%, formulation B @ 2% and formulation A @ 1%. Mean mortality in these treatments ranged from 16.67 to 33.33 per cent. This was followed by formulation B @ 1% wherein there was 6.67 per cent mortality of bees only. At 12 HAT, spraying of malathion 50 EC @ 0.1% resulted 66.67 per cent mortality of bees. This was followed by neemazal @ 0.2% in which 36.67 per cent mortality of bees was observed. Application of formulation B @ 2% exhibited statistical similarity with formulation A @ 2% and formulation A and B @ 1% each. Mean mortality percentage in these treatments ranged from 13.33 to 26.67 per cent.

At 24 HAT, treatment with malathion 50 EC @ 0.1% and neemazal @ 0.2% resulted in 76.67 and 46.67 per cent mortality of bees. Spraying of formulation B @ 1% showed 26.67 per cent mortality of bees. This treatment was on par with formulation A and B @ 2% each. Mean mortality of bees in these two treatments was 33.33 and 23.33 respectively.

4.4.3.2. Evaluation of Safety to Parasitoids

The results on safety assessment of bioformulations of *S. indica* seed extracts expressed as mortality of the wasp, *Apanteles taragamae* Viereck at 1, 3

Table 26. Effect of formulations from *Samadera indica* seed extracts on Indian honey bee

Treatments	***Mean mortality of honey bee (%)**		
	HAT		
	6	12	24
Formulation A @ 1%	16.67 (23.86)^{c}	16.67 (23.86)^{d}	30.00 (21.15)^{d}
Formulation A @ 2%	26.67 (31.00)^{bc}	26.67 (31.00)^{c}	33.33 (28.29)^{cd}
Formulation B @ 1%	6.67 (12.29)^{d}	13.33 (21.15)^{d}	26.67 (35.22)^{c}
Formulation B @ 2%	20.00 (26.57)^{bc}	20.00(26.57)^{cd}	23.33 (33.21)^{c}
Neemazal @ 0.2%	33.33 (35.22)^{b}	36.67 (37.23)^{b}	46.67 (43.08)^{b}
Malathion 50 EC @ 0.1%	60.00 (50.85)^{a}	66.67 (54.78)^{a}	76.67 (61.22)^{a}
Control	0^{e}	0^{e}	0^{e}
SE (m)	3.06	1.99	1.79
CD (0.05)	(9.288)	(6.029)	(7.626)

HAT – Hours After Treatment; Figures in parentheses are values after arc sine transformation

and 5 DAT is given in Table 27. All treatments showed statistically significant variation.

On the first day of treatment, spraying of both formulations at 1% and 2% each equally harmed the parasitoid and the mean mortality values ranged from 20.00 to 26.67 per cent. Effect of these treatments was statistically on par. Significantly high mortality rate (53.33 per cent) was observed in chemical, malathion 50 EC @ 0.1%.

Observations at 3 DAT revealed that there was 56.67 per cent mortality in chemical treatment, malathion 50 EC @ 0.1%and it was significantly superior to other treatments. Spraying formulation A @ 2% exhibited statistical similarity with formulation A @ 1% and formulation B @ 1 and 2% each. Here, mean mortality ranged from 20.00 to 33.33 per cent.

The trend observed in 5^{th} DAT was same as in 1^{st} DAT. Both formulations at a concentration of 1% and 2% each showed statistically similar effect in mortality of wasps (33.33 – 36.67 per cent). Treatment with malathion 50 EC @ 0.1% resulted significantly higher mortality rate (76.67 per cent).

It can be generalised that among the formulations prepared from solvent extracts of *S. indica*, both the formulations A and B retained stability. Formulation A @ 1% can be considered as the ideal one among the two as indicated by higher rate of mortality exhibited by the pests, *D. indica* and *H. septima*. Furthermore, both the formulations were safe against honey bees and parasitoids.

4.4.4. Shelf Life Evaluation of Formulations

The storage period of the botanical formulations evaluated in terms of mortality of the test insects is expressed below.

4.4.4.1. On Diaphania indica

Data on shelf life of formulations evaluated on *D. indica* in terms of mortality at monthly intervals for a period of six months is shown in Table 28.

Table 27. Effect of formulations from *Samadera indica* seed extracts on *Apanteles taragamae*

Treatments	***Mean mortality of wasp (%)**		
	DAT		
	1	3	5
Formulation A @ 1%	23.33 (28.78)^{b}	23.33 (28.78)^{cd}	33.33 (35.22)^{b}
Formulation A @ 2%	26.67 (31.00)^{b}	26.67 (31.00)^{bcd}	36.67 (37.23)^{b}
Formulation B @ 1%	20.00 (26.57)^{b}	20.00 (26.57)^{d}	36.67 (37.23)^{b}
Formulation B @ 2%	26.67 (31.00)^{b}	33.33 (35.22)^{b}	36.67 (35.22)^{b}
Neemazal @ 0.2%	26.67 (31.00)^{b}	30.00 (33.21)^{bc}	36.67 (37.23)^{b}
Malathion 50 EC @ 0.1%	53.33 (46.92)^{a}	56.67 (48.84)^{a}	76.67 (61.22)^{a}
Control	0^{c}	0^{e}	0^{c}
SE (m)	1.83	1.58	1.89
CD (0.05)	(5.538)	(4.802)	(5.738)

DAT – Days After Treatment; Figures in parentheses are values after arc sine transformation

Table 28. Shelf life evaluation of formulations from *Samadera indica* seed extracts on larvae of *Diaphania indica*

Treatments	***Mean mortality of larvae (%)**						
	0^{th} DAP	1^{st} MAP	2^{nd} MAP	3^{rd} MAP	4^{th} MAP	5^{th} MAP	6^{th} MAP
Formulation A @ 0.5%	46.67	46.67	46.67	46.67	46.67	46.67	45.00
Formulation A @ 1%	80.00	80.00	78.33	76.67	76.67	76.67	71.67
Formulation B @ 0.5%	43.33	43.33	43.00	43.00	41.67	41.67	40.00
Formulation B @ 1%	75.00	75.00	73.33	73.33	71.67	71.67	71.67

DAP – Day After Preparation; MAP – Month After Preparation

The mean mortality rate observed with formulation A @ 1% was 80.00, 80.00 and 78.33 per cent on the day of preparation, one MAP (Month After Preparation) and two MAP, 76.67 per cent each on 3rd, 4th and 5th MAP and 71.67 per cent on 6th MAP. Application of formulation A @ 0.5% resulted in mean mortality of 46.67 per cent from day of preparation to 5th MAP. Mean larval mean mortality became 45.00 per cent at the end of six MAP.

Mean mortality values observed with the application of formulation B @ 1% were 75.00 per cent each on the day of preparation and on 1st MAP. Then, mean percentage mortality became 73.33 each on 2nd and 3rd MAP. Thereafter, mean mortality values became 71.67 per cent till the end of observational period. When formulation B was applied @ 0.5%, mean mortality values noticed were 43.33 per cent each at on the day of preparation and one MAP, 43.00 per cent at 2nd and 3rd MAP and became 40.00 to 41.67 per cent during 4th, 5th and 6th MAP.

4.4.4.2. On Henosepilachna septima

The shelf life of *S. indica* seed extracts against *H. septima* evaluated as mortality on test insect upto six MAP at monthly intervals is given in Table 29.

The mortality rates observed with formulation A @ 1% varied slightly over a period of six months, but not with wide variation. The mean mortality rates of grubs were observed to be 80.00, 80.00 and 78.33 per cent each from the day of preparation to second month. Then, the mortality rates became 76.67 per cent each on 3rd and 4th MAP and 71.67 per cent each on 5th and 6th MAP. When this formulation was treated at 0.5 per cent on the day of preparation, 46.67 per cent grubs witnessed mortality from 0th DAP to 4th MAP. Thereafter, mortality rates became 45.00 per cent upto 6th MAP.

With regard to formulation B, application of the formulation @ 1% caused 75.00 per cent mortality of treated insects from zero to one MAP of formulation. Mortality became 73.33 per cent when observed in the next two months. There was further reduction in death rate of grubs to 71.67 per cent in 4th and 5th months of preparation of formulation. Seventy per cent insects died when treated during

Table 29. Shelf life evaluation of formulations from *Samadera indica* seed extracts on grubs of *Henosepilachna septima*

Treatments	***Mean mortality of grubs (%)**						
	0^{th} DAP	1^{st} MAP	2^{nd} MAP	3^{rd} MAP	4^{th} MAP	5^{th} MAP	6^{th} MAP
Formulation A @ 0.5%	46.67	46.67	46.67	46.67	46.67	45.00	45.00
Formulation A @ 1%	80.00	80.00	78.33	76.67	76.67	71.67	71.67
Formulation B @ 0.5%	43.33	43.33	43.00	43.00	41.67	40.00	40.00
Formulation B @ 1%	75.00	75.00	73.33	73.33	71.67	71.67	70.00

DAP – Day After Preparation; MAP – Month After Preparation

the sixth month. It was observed that application of 0.5 per cent of the same formulation resulted in 43.33 per cent mean mortality at day of preparation and one MAP. Thereafter, mortality values observed were 43.00 per cent for next two months, 41.67 per cent in fourth month and 40.00 per cent each in fifth and sixth months of observation.

Shelf life evaluation of formulations indicated that both the formulations A and B remained effective with 40.00 to 80.00 per cent mortality upto six months under normal storage conditions.

4.5. FIELD EVALUATION OF FORMULATION AGAINST LEAF FEEDING PESTS IN SNAKE GOURD

Efficacy of formulation A @ 2% was evaluated under field situation both at vegetative and 50 per cent flowering stage by spraying on leaf feeding pests in snakegourd.

4.5.1. Efficacy of Formulation

4.5.1.1. At Vegetative Stage

The efficacy of formulation at vegetative stage of the crop assessed in terms of pest population and damage on leaves is given in Table 30. There was statistically significant difference among various treatments.

4.5.1.1.1. Pest Population

On 1st day of treatment, it was observed that formulation A @ 2% was equally effective as neemazal @ 0.2%, with mean number of pests being 10.20 and 9.60 each. These were preceded by malathion 50 EC @ 0.1% in which treated plants contained 8.40 pests. In untreated plants, there were a mean number of 17.40 pests.

On 3rd day, formulation A @ 2% and neemazal @ 0.2% were statistically on par with average number of pests being 9.00 and 8.80 respectively. Treatment with malathion 50 EC @ 0.1% reduced pest population to an average of 6.00 insects. In untreated plants, there was an average of 18.60 pests.

Table 30. Effect of formulation from *Samadera indica* seed extract on leaf feeding pests of snake gourd at vegetative stage

Treatments	***Mean population of leaf feeding pests plot^{-1}**					****Mean percentage of infested leaves plot^{-1}**			
	Pre-count	DAT				DAT			
		1	3	5	7	1	3	5	7
Formulation A @ 2%	17.20	10.20 (3.19)^{b}	9.00 (3.00)^{b}	7.60 (2.84)^{b}	6.80 (2.70)^{b}	32.00 (34.44)^{b}	30.00 (33.15)^{b}	25.00 (29.77)^{b}	21.00 (27.18)^{b}
Neemazal @ 0.2%	15.60	9.60 (3.10)^{b}	8.80 (2.97)^{b}	7.40 (2.81)^{b}	4.00 (2.12)^{c}	30.00 (33.18)^{b}	28.00 (31.78)^{bc}	24.00 (29.27)^{b}	17.00 (23.43)^{b}
Malathion 50 EC @ 0.1%	16.20	8.40 (2.89)^{c}	6.00 (2.45)^{c}	0.00 (0.71)^{c}	0.00 (0.71)^{d}	28.00 (31.82)^{b}	23.00 (28.56)^{c}	8.00 (16.00)^{c}	5.00 (12.92)^{c}
Untreated	16.00	17.40 (4.17)^{a}	18.60 (4.31)^{a}	18.80 (4.35)^{a}	19.20 (4.42)^{a}	54.00 (47.33)^{c}	63.00 (49.61)^{a}	70.00 (56.94)^{a}	75.00 (60.28)^{a}
SE (m)		0.06	0.03	0.04	0.04	1.36	1.15	2.26	1.12
CD (0.05)		(0.199)	(0.109)	(0.156)	(0.159)	(4.107)	(4.269)	(6.099)	(5.107)

*Figures in parentheses are square root transformed values; **Figures in parentheses are arc sine transformed values
DAT - Days After Treatment

On 5th DAT, the treatments with neemazal @ 0.2% and formulation A @ 2% were statistically on par with each other with mean pest population of 7.40 and 7.60 respectively. No pest was observed in plants treated with malathion 50 EC @ 0.1%. In untreated plants, there was a mean of 18.80 pests.

Observations at one week after treatment showed that best treatments in the order of effectiveness were malathion 50 EC @ 0.1%, neemazal @ 0.2% and formulation A @ 2%, which differed statistically among each other. Mean pest population observed was 0.00, 4.00 and 6.80 respectively. In control, average pest population was 19.20.

4.5.1.1.2. Damage on Leaves

Observations on 1st DAT indicated that plants sprayed with formulation A @ 2%, neemazal @ 0.2% and malathion 50 EC @ 0.1% had an average of 32.00, 30.00 and 28.00 per cent infested leaves respectively. These treatments exhibited statistical similarity among them. Here, untreated plants had an average of 54.00 per cent infested leaves.

At the end of third day, it was noticed that plants treated with formulation A @ 2% had 30.00 per cent infested leaves. This treatment was statistically similar to neemazal @ 0.2% with 28.00 per cent infested leaves. Application of chemical treatment resulted in 23.00 per cent infested leaves and was on par with neemazal @ 0.2%. In untreated plants, there was an average of 63.00 per cent infested leaves. Observations at 5th DAT indicated that in plants treated with formulation A @ 2%, there was an average of 25.00 per cent infested leaves and it was on par with neemazal (24.00 per cent). Plants treated with malathion 50 EC @ 0.1% had an average of 8.00 per cent infested leaves. In untreated plants, there were 70.00 per cent infested leaves.

At one week after treatment, there were 21.00 and 17.00 per cent infested leaves each in plants treated with formulation A @ 2% and neemazal @ 0.2%. These treatments were statistically on par with each other. Treatment with malathion 50 EC @ 0.1% resulted in 5.00 per cent infested leaves. In untreated plants, the mean percentage of infested leaves was 75.00.

4.5.1.2. At 50 per cent Flowering

Data related to the population of pests and damage on leaves after treatment at 50 per cent flowering stage of the crop exhibited statistically significant variation among them as illustrated in Table 31.

4.5.1.2.1. Pest Population

On first day of treatment, there were 13.20 and 11.40 pests each in plants treated with formulation A @ 2% and neemazal @ 0.2% and effect of these treatments found statistically on par. There was mean population of 10.80 pests in plants treated with malathion 50 EC @ 0.1% and this treatment exhibited statistical similarity with neemazal @ 0.2%. Untreated plants had an average of 26.60 pests.

On 3rd day of treatment, average pest population observed in treatments formulation A @ 2%, neemazal @ 0.2% were 11.40 and 10.80 respectively and were statistically on par. In treatment with chemical control, there was an average of 9.00 pests and this treatment differed statistically among others. Control plants had an average of 27.00 pests.

On 5th day of treatment, the treatments neemazal @ 0.2% and formulation A @ 2% were statistically similar with 10.20 each. There was no pest in treatment with malathion 50 EC @ 0.1%. Plants in control had a mean population of 29.00 pests.

At 7 DAT also, spraying of malathion 50 EC @ 0.1%, neemazal @ 0.2% and formulation A @ 2% exhibited statistically significant difference among them. Here, mean pest population was 0.00, 5.00 and 9.60 respectively. In plants sprayed with distilled water, there was an average of 31.00 pests.

Table 31. Effect of formulation from *Samadera indica* seed extract on leaf feeding pests of snake gourd at 50 per cent flowering stage

Treatments	*Mean population of leaf feeding pests plot^{-1}					**Mean percentage of infested leaves plot^{-1}			
	Pre-count	DAT				DAT			
		1	3	5	7	1	3	5	7
Formulation A @ 2%	24.00	13.20 (3.63)^{b}	11.40 (3.45)^{b}	10.20 (3.26)^{b}	9.60 (3.17)^{b}	48.00 (43.85)^{b}	45.00 (42.13)^{b}	41.00 (39.80)^{b}	26.00 (30.60)^{b}
Neemazal @ 0.2%	22.20	11.40 (3.37)^{bc}	10.80 (3.28)^{b}	10.20 (3.26)^{b}	5.00 (2.33)^{c}	40.00 (39.23)^{c}	38.00 (38.04)^{c}	20.00 (26.43)^{c}	10.00 (17.97)^{c}
Malathion 50 EC @ 0.1%	23.60	10.80 (3.28)^{c}	9.00 (3.00)^{c}	0.00 (0.71)^{c}	0.00 (0.71)^{d}	39.00 (38.63)^{c}	17.00 (24.30)^{d}	14.00 (16.00)^{d}	7.00 (15.13)^{c}
Untreated	25.40	26.60 (4.96)^{a}	27.00 (5.19)^{a}	29.00 (5.43)^{a}	31.00 (5.60)^{a}	54.00 (47.30)^{a}	56.00 (48.46)^{a}	61.00 (48.45)^{a}	65.00 (49.61)^{a}
SE (m)		0.10	0.06	0.09	0.08	0.88	1.47	1.72	1.21
CD (0.05)		(0.305)	(0.241)	(0.280)	(0.408)	(2.846)	(3.246)	(4.707)	(4.579)

*Figures in parentheses are square root transformed values; **Figures in parentheses are arc sine transformed values
DAT - Days After Treatment

4.5.1.2.2. Damage on Leaves

Observations on the first day of treatment indicated that the mean percentage of infested leaves seen in plants treated with formulation A @ 2% was 48.00 This was preceded by treatments with neemazal @ 0.2% and malathion 50 EC @ 0.1% with 40.00 and 39.00 per cent of infested leaves each. There was an average of 54.00 per cent infested leaves in untreated plants.

On 3rd day of treatment, plants treated with formulation A @ 2%, neemazal @ 0.2% and malathion 50 EC @ 0.1% had 45.00, 38.00 and 17.00 per cent of infested leaves and these treatments differed statistically among them. In untreated plants, there was an average of 56.00 per cent infested leaves.

Observations on 5th day of treatment exhibited similar trend as on 3rd day of treatment. The average of infested leaves in treatments with formulation A @ 2%, neemazal @ 0.2% and malathion 50 EC @ 0.1% were 41.00, 20.00 and 14.00 each. Control plants had an average of 61.00 per cent infested leaves.

Observations at one week after spraying indicated there were 26.00 per cent infested leaves in treatment with formulation A @ 2%. This was preceded by neemazal @ 0.2% and malathion 50 EC @ 0.1% with an average of 10.00 and 7.00 per cent leaves each. In untreated plants, an average of 65.00 per cent leaves found infested.

It can be deduced that formulation A @ 2% was as effective as neemazal @ 0.2% as evident with reduction in pest population at vegetative and 50 per cent flowering stage of the crop. With regard to reduction in leaf damage, formulation A @ 2% excelled equally as neemazal @ 0.2% at vegetative stage.

Discussion

5. DISCUSSION

Snake gourd, a cucurbitaceous vegetable that is cultivated by the farmers across Kerala both commercially and on home-stead basis is prone to the attack of several pests and diseases. Some of the insect pests include leaf feeders like pumpkin caterpillar, *Diaphania indica* Saund. and epilachna beetle, *Henosepilachna septima* (Dieke). Proper management of these pests is of utmost importance to contain them and to increase the yield. Though there are several options to manage these pests, many of these are not environmentally viable. In this scenario, botanical pesticides can replace synthetic pesticides as they show less mammalian toxicity, more target specificity and are bio-degradable (Orlikowski and Skrzypczak, 2001).

Effective utilisation of the anti-insect properties of botanicals demands identification and isolation of the bioactive compounds and developing them into appropriate formulations. Hence, the present study carried out in 2018 to 2021 focused on exploring the anti-insect properties of *Samadera indica* Gaetrn. and to develop suitable formulations from it. Development of insecticidal formulations from *S. indica* is a *de novo* venture as per the realm of literature.

5.1. SCREENING OF BOTANICAL PREPARATIONS FOR INSECTICIDAL PROPERTIES

Studies were conducted to assess antifeedant and insecticidal effect of bark and seed extracts in n-hexane, acetone, ethanol and aqueous extracts of *S. indica* on *D. indica* and *H. septima* under *in vitro* conditions. Effect on biology of the pests was also evaluated.

The lethal doses of bark extract from *S. indica* in hexane, acetone, ethanol and aqueous extract against *D. indica* and *H. septima* are given in para 4.1.1.1. and 4.1.2.1. The results revealed that LD_{50} of bark extracts in hexane, acetone, ethanol and aqueous extract were 1.66, 1.03, 1.51 and 2.38% respectively against *D. indica* and 2.17, 1.95, 1.68 and 2.49% respectively against *H. septima*. Meanwhile, LD_{90} values were 6.49, 7.05, 5.24 and 9.17% respectively against

D. indica and 9.53, 6.60, 6.17 and 9.02% against *H. septima*. LD_{50} values of seed extracts were 1.79, 0.59, 1.03 and 1.54% respectively against *D. indica* and 1.63, 0.83, 1.60 and 2.43% against *H. septima*. LD_{90} values were 6.99, 5.17, 7.05 and 7.90% against *D. indica* and 6.82, 5.27, 7.94 and 7.85% against *H. septima* (para 4.1.3.1. and 4.1.4.1.).

The results in para 4.1.1.2. and 4.1.2.2. revealed that *S. indica* bark extracts in ethanol @ 5.24 and 6.17% each exhibited more feeding repellency against *D. indica* and *H. septima*. Mean leaf protection of 60.47 and 65.00 per cent was observed with these extracts at 72 HAT (Hours After Treatment) (Fig. 14). Antifeedant effect of *S. indica* was earlier studied by Anusree *et al*. (2018) wherein they reported that the leaf extract in methanol @ 5% exhibited 45.62 per cent leaf protection against tobacco cutworm, *Spodoptera litura* (F.). Antifeedant effect of bark extracts from other plants towards leaf feeders was also illustrated by Syahputra (2013) who reported that ethanolic extracts from stem bark of woundwort, *Barringtonia sarcostachys* (Blume) Miq. @ 0.80 - 0.24% inhibited feeding in cabbage head caterpillar, *Crocidolomia pavonana* (Fabricius) larvae by 68.79 to 99.10 per cent. Likewise, Martinez *et al*. (2017) also reported that application of ethanolic extract from stem bark of Mexican poppy, *Argemone ochroleuca* Sweet @ 15.00 - 30.00% deterred feeding in 3rd instar larvae of fall armyworm, *Spodoptera frugiperda* (J. E. Smith) by 13.00 to 14.00 per cent. These results are in accordance with this study.

The results in para 4.1.3.2. and 4.1.4.2. showed that acetone extract from *S. indica* seeds @ 5.17 and 5.27% each effectively inhibited feeding capacity in *D. indica* and *H. septima* respectively. Mean leaf protection offered by these extracts against the two pests were 74.41 and 69.23 per cent respectively (Fig. 15). This finding is substantiated by the findings of Rani and Rajasekharreddy (2009) who reported that acetone extract from seeds of *Strychnos nux-vomica* L. showed feeding deterrence in *S. litura* (100 per cent) and castor semilooper, *Achaea janata* (L.) (20 per cent). As per current study, application of ethanolic extract from *S. indica* @ 7.05 and 7.94% also exhibited significant antifeedant

effect against *D. indica* and *H. septima*. This finding is in conformity with that of Tridiptasari *et al*. (2019) who reported that application of seed extracts of drumstick tree, *Moringa oleifera* Lam. in ethanol @ 20% exhibited antifeedant effect on third larval instars of *S. litura*.

The present study on antifeedant properties of *S. indica* bark and seed extracts against *D. indica* and *H. septima* indicated that bark extract in ethanol @ 5.24 and 6.17% each and seed extract in acetone @ 5.17 and 5.27% exhibited feeding deterrence in *D. indica* and *H. septima*. Feeding repellent properties of bark and seed extracts from *S. indica* could be attributed to the presence of bitter principles which act on chemosensillae of insects and make them unpalatable.

The results on insecticidal effect of bark extract in para 4.1.1.3. and 4.1.2.3. showed that treatment with bark extract in ethanol @ 5.24 and 6.17% each resulted in mortality of 93.33 per cent in *D. indica* and 91.67 per cent in *H. septima* at 72 HAT. Insecticidal property of *S. indica* was earlier reported by Anusree *et al*. (2018) who noticed that leaves of this plant in methanol extract 5% showed 73.55 per cent mortality in *S. litura* after three days of exposure. Toxicity of ethanolic extracts from bark against lepidopterans was reported by several researchers. Bark extract of *B. sarcostachys* in ethanol was insecticidal in larvae of *C. pavonana* and LC_{50} was 0.14% (Syahputra, 2013). Ali *et al*. (2014) proved that bark extract of loop-root mangrove, *Rhizophora mucronata* Lam. in ethanol was effective against fourth instar larvae of *Aedes aegypti* (L.) with LC_{50} value of 0.03 ± 0.0076 µg mL^{-1} and LC_{90} value of 0.0915 ± 0.156 µg mL^{-1}. Oigiangbe *et al*. (2007) claimed that aqueous extract from bark of cheese wood, *Alstonia boonei* de Wild. exhibited toxicity against pink stalk borer, *Sesamia calamistis* Hampson, with LC_{50} of 2.80%. This can be correlated with the present observation on insecticidal effect of aqueous extract from bark of *S. indica* wherein application of the extract @ 9.02% resulted in significant mortality (86.67 per cent) of *H. septima* at 72 HAT.

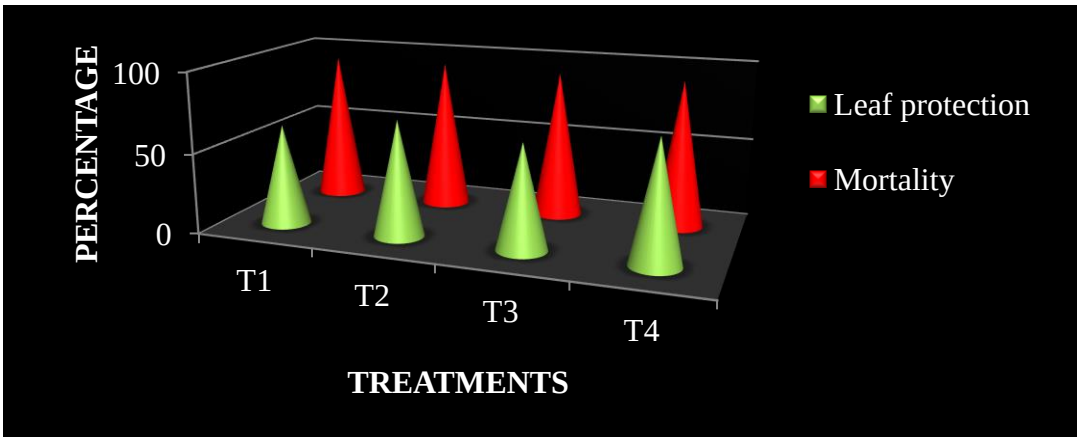
Application of seed extract in acetone @ 5.17 and 5.27% each exhibited 86.67 per cent mortality in *D. indica* and *H. septima* (para 4.1.3.3. and 4.1.4.3.).

This finding is supported by Lenin (2011) who found that contact application of seed extracts of annona @ 5% resulted in 100.00 per cent mortality of *D. indica*. Apart from acetone extract, ethanol extract from seed @ 7.05 and 7.94% also caused significant mortality in *D. indica* and *H. septima* at 72 HAT on topical application of the extract. This finding go hand in hand with that of Abbasipour *et al*. (2010) who observed that seed extract of wild rue, *Peganum harmala* L. in ethanol @ 30 and 40 mg mL^{-1} inflicted mortality of 66 and 100 per cent each in third instar larvae of *Plutella xylostella* (L.) when fed with extract treated leaves of cabbage. Moreira *et al*. (2007) opined that the lethality of botanical pesticides is associated with blockage of electron transportation in respiratory processes of insects, resulting in paralysis, death.

Both bark and seed extracts of *S. indica* presented more lethal effects in *D. indica* and *H. septima* in comparison with antifeedant effect (Fig. 14 and 15). This might be due to the highly toxic nature of bioactive compounds in the extract and it also points out the prospects of developing effective insecticides from bark and seed extracts of *S. indica.*

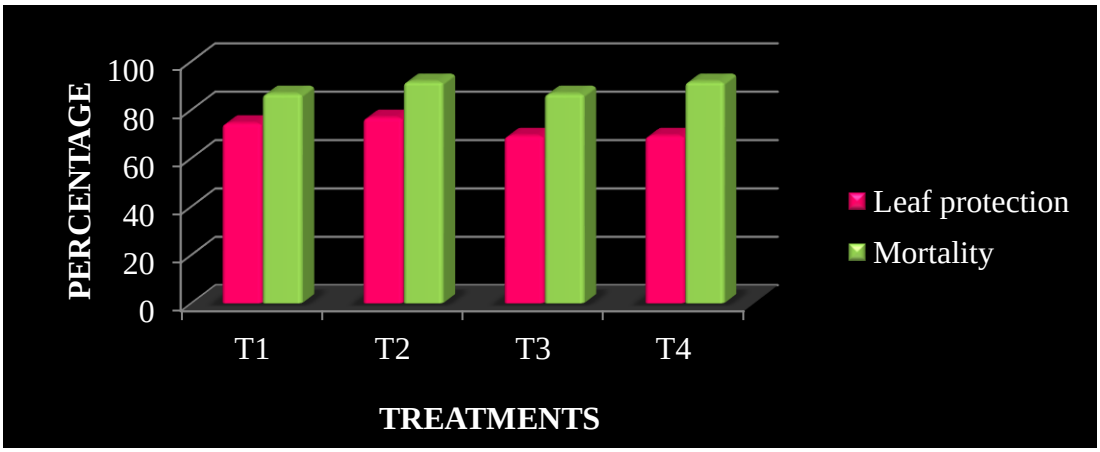
The results in para 4.1.1.4. and 4.1.2.4. showed the effect of bark extracts of *S. indica* on the biology of *D. indica* and *H. septima*. It was observed that ethanolic extract of bark @ 5.24% caused prolongation in larval and pupal period to 15.00 and 5.33 days each in *D. indica*. Meanwhile, there was reduction in pupal weight and adult longevity to 0.19 g and 4.33 days respectively. In control, larval period was 9.67 days, pupal period was 2.67 days, pupal weight was 0.31 g and adult longevity was 7.67 days. In *H. septima* also, application of ethanol extract @ 6.17% resulted lengthening in grub duration (16.33 days), pupal period (8.33 days) and reduction in pupal weight (0.74 g) and adult longevity (11.33 days). While in control, grub period was 10.33 days, pupal period was 4.33 days, pupal weight was 1.34 g and adult longevity was 19.33 days.

In both insects, there was increase in duration of larvae/grubs and pupae. Observations on prolongation of larval duration as noticed in this study is in concurrence with that of Vasudev *et al*. (2015) who reported that there was



T1 – Bark extract @ 5.24% (*D. indica*)
T2 – NSKE 5% and malathion 50 EC @ 2 mLL^{-1} (*D. indica*)
T3 - Bark extract @ 6.17% (*H. septima*)
T4 – NSKE 5% and malathion 50 EC @ 2 mLL^{-1} (*H. septima*)

Fig. 14. Comparison between antifeedant effect and insecticidal effect of *Samadera indica* bark extracts in ethanol against *Diaphania indica* and *Henosepilachna septima* at 72 HAT



T1 – Seed extract @ 5.17% (*D. indica*)
T2 – NSKE 5% and malathion 50 EC @ 2 mLL^{-1} (*D. indica*)
T3 - Seed extract @ 5.27% (*H. septima*)
T4 – NSKE 5% and malathion 50 EC @ 2 mLL^{-1} (*H. septima*)

Fig. 15. Comparison between antifeedant effect and insecticidal effect of *Samadera indica* seed extracts in acetone against *Diaphania indica* and *Henosepilachna septima* at 72 HAT

extension in larval period in melon fly, *Bactrocera cucurbitae* (Coquillett) when acetone extract from bark of gum Arabic tree, *Acacia nilotica* (Linn.) was applied @ 625 ppm. Silva *et al*. (2018) reported that larvae of diamondback moth, *P. xylostella* treated with bark extracts of croton plants, *Croton rhamnifolius* Willd. (42.40 µg mL^{-1}) and *Croton jacobinensis* Baill. (116.21 µg mL^{-1}) in ethanol exhibited prolongation in larval duration. Extended pupal duration observed in this study was in accordance with that of Martinez *et al*. (2017) who also reported an increase in the duration of pupal stage in 3rd instar larvae of *S. frugiperda* with the application of whole plant extract of pale Mexican poppy, *Argemone ochroleuca* Sweet in ethanol @ 15%.

The effect of seed extracts of *S. indica* on the biology of *D. indica* and *H. septima* presented in para 4.1.3.4. and 4.1.4.4. revealed that application of acetone extract from seed @ 5.17% resulted in elongation of larval stage to 17.00 days and pupal stage to 8.67 days in *D. indica*. In the mean time, pupal weight was decreased to 0.25 g and adult longevity was reduced to 6.33 days. In control, larvae and pupae existed for 12.67 and 5.33 days respectively, pupal weight was 0.35 g and adult longevity was 7.67 days. Application of acetone extract of *S. indica* @ 5.27% extended grub stage and pupal stage in *H. septima* to 14.00 and 8.00 days each. Here, pupal weight and adult longevity were reduced to 1.25 g and 12.33 days respectively. In control, duration of grubs and pupae were 11.33 and 4.33 days each, pupal weight was 1.35 g and adult longevity was 19.67 days.

In both insects, there was extension in larval/grub and pupal duration and reduction in pupal weight and adult longevity. Prolonged larval duration noticed in this study was in conformity with that of Atshan *et al*. (2017) who reported that application of aqueous extracts from seeds of Indian soapnut, *Sapindus trifoliatus* L. and red pea eggplant, *Solanum trilobatum* L. (100 mg kg^{-1}) delayed pupation in lepidopteran pests in cabbage. An incongruity to this is the finding by Leatemia and Isman (2004) who reported reduction in larval period of *S. litura* and cabbage looper, *Trichoplusia ni* (Hubner) with the application of ethanolic seed extracts of sweetsop, *Annona squamosa* L. and soursop, *Annona muricata* L. Meanwhile,

there was reduction in pupal weight and adult longevity. Reduced pupal weight observed in this study was in agreement with that of Gokce *et al*. (2010) who reported that methanolic extract from seeds of *H. lupulus* @ 4% reduced pupal weight in oblique banded leaf roller, *Choristoneura rosaceana* (Harris).

Reduced longevity in adults emerged from larvae treated with ethanolic extract from bark of *S. indica* and acetone extract of seeds in agreement with the findings of Khosravi *et al*. (2011). They reported that lesser was the longevity in adults of lesser mulberry pyralid, *Glyphodes pyloalis* Walker (4.53 days as against 9.20 days in control) when larvae were treated with LC_{50} value of methanolic extract from leaves of sweet wormwood, *Artemisia annua* L..

Morphogenetic changes in *D. indica* and *H. septima* observed in this study were pupal discolouration in both *D. indica* and *H. septima*, malformation in the adults of *D. indica* and poor sclerotization in *H. septima* (Plates 14 and 15). This observation is well supported by the findings of Arivoli and Tennyson (2013) wherein there was reduction in size, deformities in head size, body length and darkened colouration on wings with the application of ethyl acetate extract of poison nut, *Strychnos nux-vomica* L. (0.05%) in *S. litura*. Malformation in adult insects due to hormonal imbalance might be responsible for their reduced longevity and absence of fecundity noticed in the present study.

The present study established the bioefficay of *S. indica* bark extract in ethanol and seed extract in acetone on *D. indica* and *H. septima*. This can be correlated with the findings of Maheswari and Govindaiah (2017) who reported that population of second instar larvae of leaf roller (Diaphania pulverulental*is* Hampson) was reduced to 46.87 per cent on fifth day of treatment with 2% of Lantana camara L. extract. Zhou *et al*. (2012) also reported that *D. indica* showed feeding repellency and lethality on application of alcoholic extract from castor leaves. Management of *Epilachna* spp. using leaf extracts of castor, *Ricinus communis* L., Sodom apple, *Calotropis procera* (Aiton W. T. Aiton and Indian thornapple, *Datura metel* L., fruit extracts of wild cherry, *Antidesma*

Plate 14A. Pupal discolouration

Plate 14B. Malformation in adults

Plate 14. Morphogenetic changes in *Diaphania indica*

Plate 15A. Pupal discolouration

Plate 15B. Poor sclerotization in adults

Plate 15. Morphogenetic changes in *Henosepilachna septima*

bunius (Bignay) and seed extracts of *A. squamosa*, neem (*Azadirachta indica* A. Juss), *D. metel* and *R. communis* was exemplified by Islam *et al*. (2011), Belmi *et al*. (2014) and Ara *et al*. (2015).

In general, there was an increase in larval and pupal duration and reduction in pupal weight and adult longevity in *D. indica* and *H. septima* with the application of bark extract of *S. indica* in ethanol @ 5.24 and 6.17% and seed extract in acetone @ 5.17 and 5.27%. The findings by Ntalli and Menkissoglu-Spiroudi (2011) substantiated that extension in larval and pupal period in treated insects is due to the interference of botanical extracts with the production of enzymes responsible for moulting, thus inhibiting growth and development. Reduced pupal weight noticed in treated insects may be due to poor sclerotization of puparium which is resultant of improper hormonal metabolism (Abdel-Aal, 1996). Etebari *et al*. (2007) opined that botanicals intervene with digestive mechanism of insects resulting in storage of less quantity of lipids and glycogens during immature stages. This reduced quantity of nutrients result in various deformities in insect and reduces longevity and egg production. Emergence of adults with malformed wings as in this study may be associated with the inability of the insects to flatten the wings after emergence (Saxena *et al*., 1981). Josephrajkumar *et al*. (1999) opined that poor sclerotization in adults may be due to the difference in ecdysteroid titer, leading to changes in normal morphology. Ray *et al*. (2012) claimed that imbalance between growth stimulating and growth inhibiting hormones in insects is responsible for morphogenetic changes in insects.

A comparison between lethal doses of effective extracts from bark and seeds of *S. indica viz*., ethanol extract and acetone extract pointed out that seed extract in acetone had a more pronounced effect than bark extract, indicating the presence of more potent insecticidal compounds in seed extract. Solvents used for extracting bioactive compounds from plants vary based on the solubility of compounds. Kchaou *et al*. (2013) and Do *et al*. (2014) suggested that ethanol, acetone, methanol and water: methanol (1: 1) are the best combination of solvents as they yield more quantity of bioactive compounds.

5.2. BIOEFFICACY STUDY OF ISOLATED BIOACTIVE COMPOUND

A comparison of lethal doses revealed that seed extract of *S. indica* in acetone exhibited more toxicity towards insects when compared to bark extracts. Accordingly, seed extract in acetone was subjected to column chromatography using gradient elution technique and chromatographic fractions with same R_f (Retention Factor), in combination at 5, 2.5 and 1.25% were assessed against second instar larvae and grubs of *D. indica* and *H. septima* for antifeedant effect and insecticidal effect.

The results in para 4.2.1.1. and 4.2.1.2. showed that chromatographic fractions from acetone extract of seeds @ 5% exhibited better antifeedant properties in *D. indica* and *H. septima*. Mean leaf protection was 75.61 per cent in leaves fed by *D. indica* and 68.18 per cent in those consumed by *H. septima* at 72 HAT. This finding is in accordance with that of Talukder and Howse (2000) who reported that sub-fractions from acetone extract of pithraj tree, *Aphanamixis polystachya* (Wall.) R. Parker seeds exhibited antifeedant effect on adults of red flour beetle, *Tribolium castaneum* (Herbst). Feeding deterrence observed with chromatographic fraction against *H. septima* in this study is in concurrence with that of Rumape (2015) who also observed feeding deterrence (71.00 per cent) in another species of epilachna beetle, *Epilachna varivestis* Mulsant on treatment with methanolic extract of leaves from castor, *Ricinus communis* L.

Results on insecticidal effect of chromatographic fractions from acetone extract containing bioactive compounds on *D. indica* and *H. septima* illustrated in para 4.2.1.2. and 4.2.2.2. revealed that chromatographic fractions from acetone extract @ 5% exhibited 73.33 per cent mortality against second instars of *D. indica* and *H. septima*. The report on efficacy of chromatographic fractions in acetone is the first of its kind. As per the finding by Talukder *et al.* (2015), fractions in petroleum ether extract from rhizomes of Cassumunar ginger, *Zingiber cassumunar* Roxb. were found to be toxic to *T. castaneum* with LD_{50} value of 225.91 μg cm^{-2}. Similarly, Khanavi *et al*. (2017) also reported 100 per cent larvicidal activity in *Anopheles stephensi* with the application of hexane

fraction from ground pine, *Ajuga chamaecistus tomentella* Rech. f. @ 102 ppm. Sudrajat *et al*. (2018) reported termiticidal property with n-hexane and ethyl acetate fractions from stem bark extract of garlic nut, *Scorodocarpus borneensis* Becc.

The efficacy of chromatographic fractions from acetone extract on antifeedant and insecticidal effect showed that fractionation of acetone extract might have resulted in isolation of most of the bioactive compounds from seeds of *S. indica*. These compounds, being bitter principles might have acted on gustatory system of insects or resulted in mortality of insects. Bioactive compounds in *S. indica* might be having more lethal effect rather than repellent effect as indicated in Fig. 16 and 17.

5.3. STRUCTURAL CHARACTERISATION OF THE BIOACTIVE COMPOUND

Structural characterisation of bioactive compounds present in *Samadera* seed extracts excavated the presence of four quassinoids *viz*., samaderin A, samaderin B, samaderin C and cedronin in the extract. These quassinoids are bitter principles which are responsible for anti-insect properties of *Samadera* seed extracts. Govindachari *et al*. (2001) had isolated samaderins A, B and C from bark and seeds of the tree. In addition to samaderins, they had also isolated indaquassin, which is yet another quassinoid. According to them, samaderin C increased pupal duration and resulted in pupal mortality in *S. litura*. Meanwhile, indaquassin exhibited antifeedant effect. The antifeedant and insecticidal property of samaderins A and B and cedronin is reported for the first time in this study.

Quassinoids are degraded triterpenes present in plants belonging to the family Simaroubaceae like *S. indica, Ailanthus altissima* (Mill.) Swingle etc. Antifeedant properties of quassinoids from *S. indica* reported in our study is in agreement with that of Daido *et al*. (1995), in which quassinoids isolated from Indian quassiawood, *Picrasma ailanthoides* (D. Don) Benn. were effective against *P. xylostella* in terms of antifeedant effect and insecticidal activity. Similarly, Masayuki *et al*. (1993) also reported the insecticidal activity of quassin from

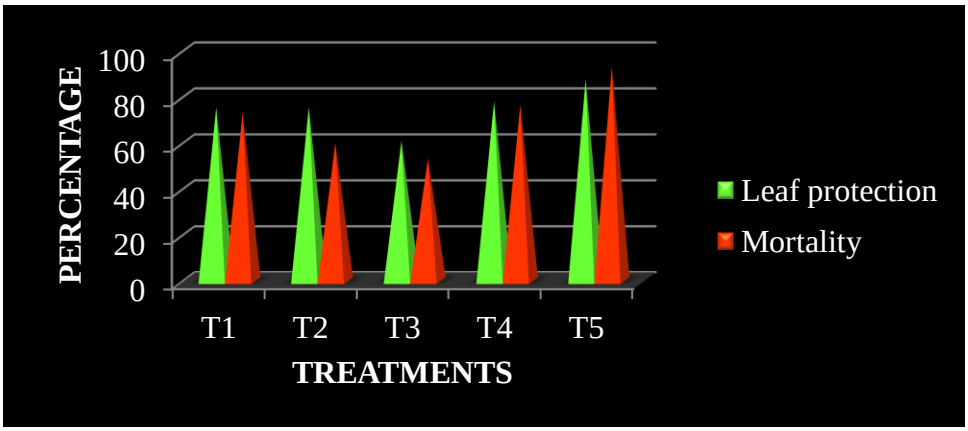
Ailanthus spp. against *P. xylostella*. The findings by Mao *et al.* (2019) also revealed that bruceine D, a quassinoid in Macassar kernel tree, *Brucea javanica* (L.) Merr. showed feeding inhibition in *P. xylostella*, beet armyworm (*Spodoptera exigua* Hübner) and *S. litura*. Likewise, Yang *et al.* (2020) claimed that quassinoids isolated from roots of Malaysian ginseng, *Eurycoma longifolia* Jack also resulted in significant antifeedant effect on larvae of *P. xylostella*. However, present investigation is the first report on bioefficay of quassinoids against *D. indica* and *H. septima*.

5.4. DEVELOPMENT OF SUITABLE FORMULATIONS OF BIOACTIVE COMPOUNDS

Two formulations of *S. indica* seed extract in acetone (para 4.4) were prepared by mixing seed extract, emulsifier and distilled water in different proportions. There are reports on the development of creams and ointments from S. indica seeds in pharmacological industry. However, the development of emulsions from S. indica for pest management is the first of its kind.

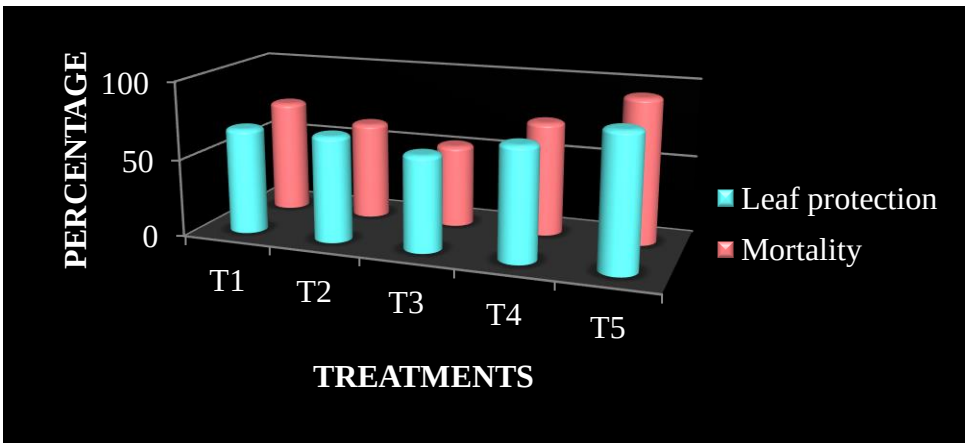
A pesticide formulation consists of an active ingredient, carrier (solvents) and surface-acting agents (emulsifiers) (Libs and Salim, 2017). In this study, formulation A was prepared by mixing acetone extract of *Samadera* seed, tween 80 and distilled water in 15: 5: 80 ratio. Formulation B contained seed extract of *Samadera* in acetone, tween 80 and span 80 (1: 1) and distilled water in 15: 5: 80 ratio.

Emulsifiers used for preparing stable emulsions from S. indica seed extract were tween 80 (polysorbate 20) and span 80 and solvent was distilled water. Selection of tween 80 (HLB value - 16.7) as surfactant/emulsifier in formulation A is supported by the findings of Griffin (1954), who claimed that surfactants with HLB (Hydrophilic Lipophilic Balance) values ranging from 8 to 18 are ideal for developing O/W emulsions. Appropriateness of the emulsifiers tween 80 and span 80 (sorbitan monooleate) (1: 1) in developing a formulation is in line with Bjorkegren *et al.* (2015) who claimed that these emulsifiers being hydrophilic in



T1 – Chromatographic fractions from acetone extract of seeds @ 5%
T2 - Chromatographic fractions from acetone extract of seeds @ 2.5%
T3 - Chromatographic fractions from acetone extract of seeds @ 1.25%
T4 - Neemazal 1% @ 0.2%
T5 - Malathion 50 EC @ 2 mLL^{-1}

Fig. 16. Comparison between antifeedant effect and insecticidal effect of chromatographic fractions from acetone extract of seeds against *Diaphania indica* at 72 HAT



T1 – Chromatographic fractions from acetone extract of seeds @ 5%
T2 - Chromatographic fractions from acetone extract of seeds @ 2.5%
T3 - Chromatographic fractions from acetone extract of seeds @ 1.25%
T4 - Neemazal 1% @ 0.2%
T5 - Malathion 50 EC @ 2 mLL^{-1}

Fig. 17. Comparison between antifeedant effect and insecticidal effect of chromatographic fractions from acetone extract against *Henosepilachna septima* at 72 HAT

nature reduces viscosity of emulsion and forms a homogenous solution. This is further supported by Santos et al. (2020) and Anarakdim et al. (2020) who claimed that mixture of both the emulsifiers in equal proportion had HLB value of 9.7 and exhibited low rate of creaming and no coalescence. Furthermore, both the emulsifiers being food grade are environment-friendly and non-toxic to applicators.

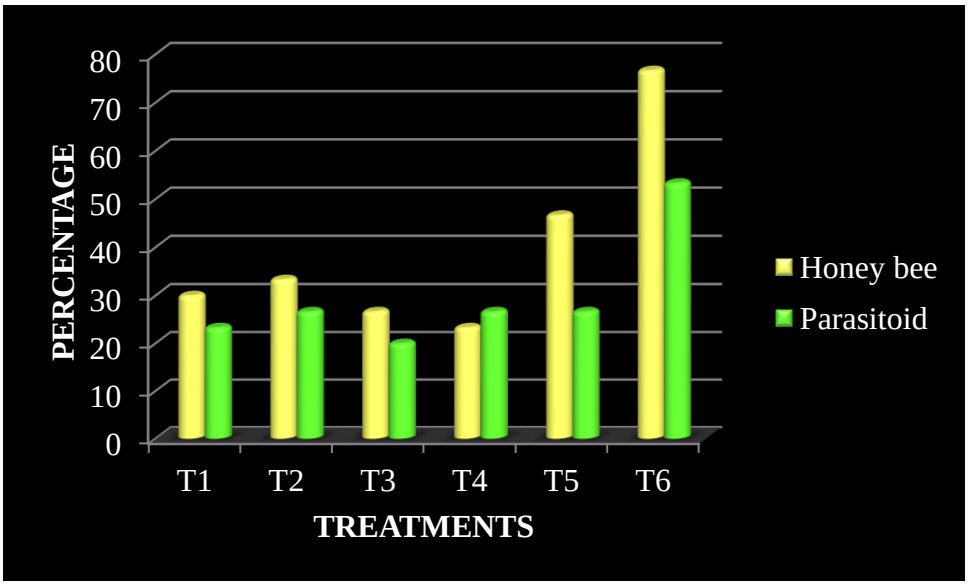
Results in para 4.4.3.1. and 4.4.3.2. indicated that application of formulation A @ 0.5% caused mortality of 46.67 per cent in *D. indica* and *H. septima* respectively at 72 HAT. Mortality was 80.00 per cent each in *D. indica* and *H. septima* with the application of formulation A @ 1%. Meanwhile, application of formulation B @ 0.5% resulted in 43.33 per cent mortality in *D. indica* and *H. septima* respectively. Formulation B @ 1% caused mortality of 71.67 per cent each in *D. indica* and *H. septima*. The results implied more effectiveness to formulation A @ 1% under *in vitro* conditions. Emulsions of botanical insecticides were developed by certain researchers. Formulations containing pyrethrin (1.80%), β-assarone (7.80%) and eugenol (4%) @ 10 µL mL^{-1} brought about mortality in the bugs Dasynus piperis L. (75 per cent) and Diconocoris hewetti (Dist.) (92 per cent) (Wiratno, 2008). Purkait *et al.* (2019) demonstrated that application of emulsions from seed extract of *A. squamosa* 40 EC @ 1% resulted in 80.70 per cent mortality in cabbage aphid, *Brevicoryne brassicae* (L.) after 72 hours of treatment. The percentage mortalities observed in these research works are in accordance with the present study.

Results on safety evaluation of the formulation on beneficial insects presented in para 4.4.2.1. revealed that in honey bees, mortality was 30.00 per cent with the application of formulation A @ 1% and it was 26.67 per cent with formulation B @ 1% at 24 HAT. There are many studies that illustrate the effect of botanical insecticides on pollinators. Application of citronella oil (10 mL L^{-1}), eucalyptus oil (10 mL L^{-1}) and rotenone (5 mL L^{-1}) were toxic to adult worker bees with 42 to 60 per cent mortality. However, neem oil (2 mL L^{-1}) induced toxicity in both larvae and adults of worker bees (Xavier *et al.*, 2015). Similarly,

500 µL of essential oil from oregano and thyme were sufficient to cause toxicity in European honey bee, *Apis mellifera* L. when applied through contact and by topical application (da Silva *et al.*, 2020).

Results presented in para 4.4.2.2. showed that application of formulation A @ 1% caused 23.33 per cent mortality in *Apanteles taragamae* Viereck and it was 26.67 per cent with formulation B @ 1%. There are several research works that reports the toxicity of botanical insecticides to natural enemies. Application of neem + sweet-flag and neem + sweet-flag + pongamia @ 0.12 and 0.18% each resulted in mortality of upto 33.89 per cent in adults of the egg parasitoid, *Trichogramma chilonis* Ishii (Boomathi *et al.*, 2005). Ong *et al.* (2020) also observed mortality in the pupal parasitoid *Pteromalus venustus* Walker with the application of ajwain, cinnamon, clove, cumin, fennel, ginger, nutmeg, oregano and turmeric @ 16 to 47 mg cm^{-2}. An incongruity in above findings was reported by Abdelgader and Hassan (2012) as per whom both azadirachtin and quassin were safe to egg parasitoid, *Trichogramma cacoeciae* March. Among the two test insects, formulation A @ 2% exhibited more safety towards parasitoids (Fig. 18).

Data presented in para 4.4.4.1. and 4.4.4.2. showed the shelf life of both the formulations A and B at lab dose in terms of mortality of the pests. There was mortality of 71.67 to 80.00 per cent each in *D. indica* and *H. septima* with the application of formulation A @ 1%. Formulation B @ 1% resulted in mean percentage mortality of 71.67 to 75.00 per cent in *D. indica* and from 70.00 to 75.00 per cent in *H. septima*. Both the formulations were shelf stable under normal room temperature for six months as the effect of both formulations was retained throughout the observational period. Similar was the finding by Lina *et al.* (2018) who reported that emulsifiable concentrates developed using extracts of Vogel tephrosia, *Tephrosia vogelii* Hook. f. and spiked pepper, *Piper aduncum* L. (1: 5) exhibited LC_{95} of 0.19, 0.34 and 0.21 per cent each against *C. pavonana* after storing the formulation at a temperature below 4°C, at room temperature and at 40°C.



T1 – Formulation A @ 1%
T2 – Formulation A @ 2%
T3 – Formulation B @ 1%
T4 – Formulation B @ 2%
T5 - Neemazal 1% @ 0.2%
T6 - Malathion 50 EC @ 2 mLL^{-1}

Fig. 18. Comparison between safety of formulations from *Samadera indica* seeds on beneficial insects

5.5. FIELD EVALUATION OF FORMULATION AGAINST LEAF FEEDING PESTS IN SNAKE GOURD

Field experiment carried out to test the efficacy of the botanical in comparison with biopesticide and chemical at Instructional Farm, Vellayani in snake gourd (variety Kaumudi) revealed that formulation A @ 2% was effective against leaf feeding pests at vegetative stage and 50 per cent flowering stage. Application of formulation A @ 2% resulted in 64.58 and 69.03 per cent reduction in pest population at vegetative and 50 per cent flowering stage respectively at 72 HAT (Fig. 19). With regard to leaf damage, formulation A @ 2% excelled equally as neemazal 1% @ 0.2% at vegetative stage (para 4.5.1.1. and 4.5.1.2.). The percentage of infested leaves observed in these treatments was 21.00 and 17.00 respectively, as against 75.00 per cent in untreated plants.

The efficacy of botanical insecticide formulations on leaf feeders was explored by several researchers. Dadang *et al*. (2009) reported that the application of emulsions developed from *A. squamosa* and *Annona odorata* Linden @ 0.1% decreased the population of *C. pavonana* and *P. xylostella*. Erler *et al*. (2010) opined that greeneem oil and water extracts of *Origanum onites* L. and *Pimpinella anisum* L. exhibited larvicidal activity against cedar leaf moth, *Acleris undulana* Walsingham. Treatment of turfgrass with 2% black pepper (*Piper nigrum* L.) effectively reduced the population of second and third instars of European chafer, *Rhizotrogus majalis* (Razoumowsky) (Scott *et al*., 2005).

There are several reports on efficacy of botanical emulsions on other pests. Emulsions developed from pongam oil exhibited mortality ranging from 96.00 to 97.00 per cent in *M. persicae* on 12th day of application (Pavela, 2009). Lucia *et al*. (2020) observed that application of *A. squamosa* 40 EC @ 1% in field reduced population of aphids (67.30 to 72.30 per cent) and whiteflies (67.50 to 75.60 per cent) within 5 to 14 days of treatment. Application of 0.75% of the botanical formulation containing neem (*Azadirachta indica* A. Juss) oil and D-limonene resulted in 62.40 per cent reduction in population of coffee berry borer, *Hypothenemus hampei* (Ferrari) (Brito *et al*., 2021).

The present study revealed that bark extract of *S. indica* in ethanol and seed extract in acetone exhibited feeding deterrence, insecticidal properties and caused adverse effects on biology of *D. indica* and *H. septima* with more efficacy attributed to seeds. Chromatographic fractions from acetone extract of seeds @ 5% showed antifeedant action and insecticidal effect on test insects. Bioactive compounds present in seeds were identified and structurally characterised as samaderins A, B, C and cedronin. Formulation A was developed by mixing acetone extract of *Samadera* seed, tween 80 and distilled water in 15: 5: 80 ratio, while formulation B was prepared by mixing seed extract of *Samadera* in acetone, tween 80 and span 80 (1: 1) and distilled water in 15: 5: 80 ratio. Both these formulations @ 1% each were effective against *D. indica* and *H. septima* under *in vitro* conditions. Both the formulations were also stable for a period of six months under room temperature with no microbial contamination and field studies proved the efficiency of formulation A @ 2% on test insects in terms of reduced pest population and lesser leaf damage. The results obtained in this study clearly indicate the possibility of replacing chemical pesticides with a potent, but safer botanical insecticide from *S. indica*, which are rich sources of quassinoids.

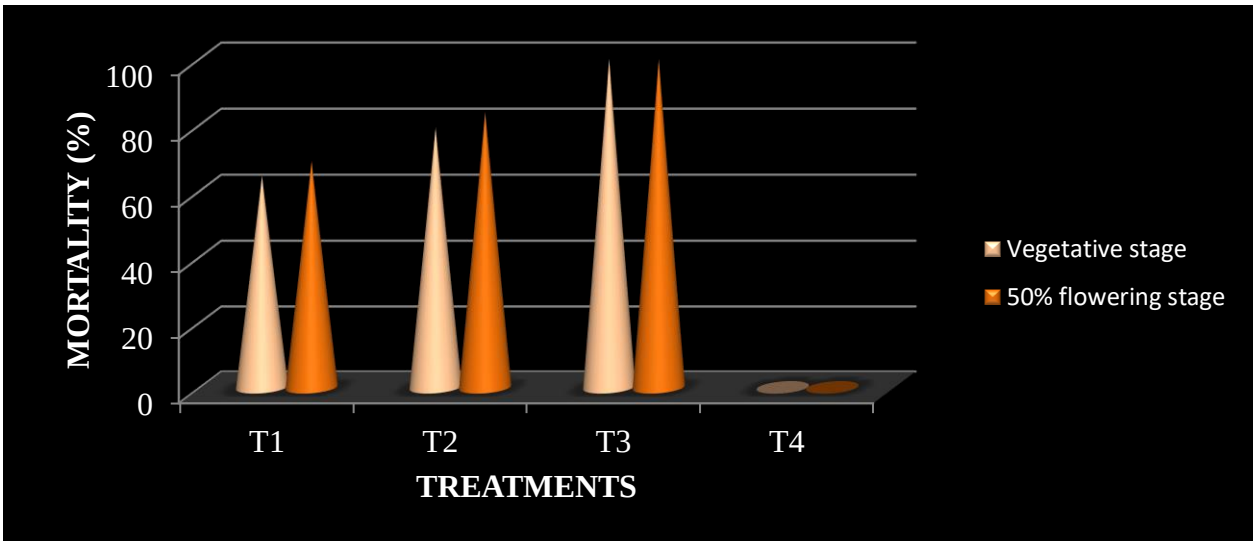


Fig. 19. Comparative reduction in population of leaf feeding pests in snake gourd at both stages of spraying

T1 – Formulation A @ 2%	T2 - Neemazal 1% @ 0.2%
T3 - Malathion 50 EC @ 2 mLL^{-1}	T4 - Control

Summary

6. SUMMARY

The present study on "Development of formulations from *Samadera indica* Gaetrn. for the management of leaf feeding pests in snake gourd (*Trichosanthes anguina* L.)" was carried out during 2018 to 2021 in the Department of Agricultural Entomology, College of Agriculture, Vellayani and at Council of Scientific and Industrial Research – National Institute for Interdisciplinary Science and Technology (CSIR-NIIST), Thiruvananthapuram. Objectives of the study were exploration of anti-insect properties of bark and seed extracts from *S. indica*, identification of bioactive compounds in the effective extract, development of suitable formulations from the extract and field evaluation of the same on pumpkin caterpillar *Diaphania indica* Saund. and epilachna beetle *Henosepilachna septima* (Dieke). The salient findings of the study are summarised below.

Bark and seed extracts of *S. indica* in various solvents *viz*., hexane, acetone and ethanol and aqueous extract were prepared by cold extraction method. These extracts in varying concentrations ranging from 0.5 to 5% were subjected to probit-dose analysis and LD_{50} and LD_{90} for *D. indica* and *H. septima* were assessed. LD_{50} of bark extract in hexane, acetone, ethanol and aqueous extract were 1.66, 1.03, 1.51 and 2.38% respectively against *D. indica* and 2.17, 1.95, 1.68 and 2.49% respectively against *H. septima*. Meanwhile, LD_{90} values were 6.49, 7.05, 5.24 and 9.17% respectively against *D. indica* and 9.53, 6.60, 6.17 and 9.02% against *H. septima*. With regard to seed extract, LD_{50} values were 1.79, 0.59, 1.03 and 1.54% respectively against *D. indica* and 1.63, 0.83, 1.60 and 2.43% against *H. septima*. LD_{90} values were 6.99, 5.17, 7.05 and 7.90% against *D. indica* and 6.82, 5.27, 7.94 and 7.85% against *H. septima*.

Antifeedant effect of the extracts at LD_{50} and LD_{90} was assessed by no-choice method and the quantity of leaf consumed by treated larvae/grubs at 24, 48 and 72 HAT (Hours After Treatment) was recorded. Among bark extracts, ethanol extract @ 5.24% exhibited maximum antifeedant effect with mean leaf protection of 60.47 per cent against *D. indica*. In the case of *H. septima*, ethanol extract @

6.17% resulted in 65.00 per cent leaf protection. Insecticidal effect and effect of the extracts on insect biology was studied by treating larvae/grubs topically with LD_{50} and LD_{90} of the extracts. Treatment with bark extract in ethanol @ 5.24% resulted in mortality of 93.33 per cent in *D. indica* and application of ethanol extract @ 6.17% brought about 91.67 per cent mortality in *H. septima* at 72 HAT. Considering effect on insect biology, ethanolic extract of bark @ 5.24% caused prolongation in larval and pupal period to 15.00 and 5.33 days each in *D. indica*. Meanwhile, there was reduction in pupal weight and adult longevity to 0.19 g and 4.33 days respectively. In control, larval period was 9.67 days, pupal period was 2.67 days, pupal weight was 0.31 g and adult longevity was 7.67 days. In *H. septima* also, application of ethanol extract @ 6.17% resulted lengthening in grub duration (16.33 days), pupal period (8.33 days) and reduction in pupal weight (0.74 g) and adult longevity (11.33 days). While in control, grub period was 10.33 days, pupal period was 4.33 days, pupal weight was 1.34 g and adult longevity was 19.33 days.

In the case of seed extracts of *S. indica*, acetone extract @ 5.17% resulted in maximum leaf protection of 74.41 per cent against *D. indica*. Application of acetone extract @ 5.27% to leaves exhibited 69.23 per cent leaf protection in *H. septima*. Seed extract in acetone @ 5.17 and 5.27% each exhibited 86.67 per cent mortality in *D. indica* and *H. septima*. Application of acetone extract from *S. indica* seed @ 5.17% resulted in elongation of larval stage to 17.00 days and pupal stage to 8.67 days in *D. indica*. In the mean time, pupal weight was decreased to 0.25 g and adult longevity was reduced to 6.33 days. In control, larvae and pupae existed for 12.67 and 5.33 days respectively, pupal weight was 0.35 g and adult longevity was 7.67 days. Application of acetone extract of *S. indica* seed @ 5.27% extended grub stage and pupal stage in *H. septima* to 14.00 and 8.00 days each. Here, pupal weight and adult longevity were reduced to 1.25 g and 12.33 days respectively. In control, duration of grubs and pupae were11.33 and 4.33 days each, pupal weight was 1.35 g and adult longevity was 19.67 days. Morphogenetic changes were also observed in larvae and grubs

treated with both bark extract of *S. indica* in ethanol and seed extract in acetone. There was pupal discolouration in both *D. indica* and *H. septima*. Malformation was noticed in the adults of *D. indica* and in *H. septima*, the adults were poorly sclerotized.

A comparison of lethal doses has revealed that acetone extract from seeds of *S. indica* exhibited more toxicity towards insects when compared to bark extracts. Accordingly, seed extract in acetone was subjected to column chromatography using gradient elution technique and a total of 83 fractions were collected. These fractions were clubbed to a total of five fractions based on Retention Factor (R_f). Further, those fractions with same R_f, in combination at 5, 2.5 and 1.25 per cent each were tested for bioefficacy on larvae/grubs of *D. indica* and *H. septima*. Among these, chromatographic fractions @ 5% exhibited more antifeedant effect with mean leaf protection of 75.61 per cent in *D. indica* and 68.18 per cent in *H. septima* at 72 HAT. Insecticidal effect was also higher at this concentration with mortality of 73.33 per cent each in *D. indica* and *H. septima*.

Chromatographic fractions with bioactive compound/compounds were compared for their similarities with the marker compounds (quassinoids) available in CSIR-NIIST, which were identified using NMR (Nuclear Magnetic Resonance) technique. The presence of samaderins A, B, C and cedronin were detected in seed extract and the molecular structures of these compounds were elucidated to confirm their identity. The antifeedant and insecticidal property of samaderins A and B and cedronin is reported for the first time in this study.

Two formulations from *S. indica* were prepared using its seed extract in acetone as it possessed more antifeedant properties in comparison to bark extract. The formulations were prepared using seed extract, emulsifier and distilled water by 'temperature of inversion phase' technique. Formulation A contained acetone extract of *S. indica* seed, tween 80 and distilled water in 15: 5: 80 ratio. In formulation B, the components were seed extract of *S. indica* in acetone, tween 80 and span 80 (1: 1) and distilled water in 15: 5: 80 ratio. Stability of both the

formulations was ascertained by performing physical tests as prescribed by BIS (Bureau of Indian Standards). Colour, appearance, EC (Electrical Conductivity) and pH of the formulations were observed and filter paper test, cold test, test for heat stability and bloom test were performed for a period of one week. Both the formulations remained yellow to light yellow in colour, free-flowing, spread out rapidly in filter paper, maintained acidic pH, retained stability under hot and cold conditions and formed white bulging emulsion with no oil droplets.

Bioefficacy studies on formulations under *in vitro* conditions exposed that formulation A @ 0.5% caused mortality of 18.33 to 46.67 per cent in *D. indica* and 20.00 to 46.67 per cent in *H. septima* from 24 to 72 HAT. Mortality was 41.67 to 80.00 per cent each in *D. indica* and *H. septima* with the application of formulation A @ 1%. Meanwhile, application of formulation B @ 0.5% resulted in 15.00 to 43.33 per cent mortality in *D. indica* and 18.33 to 43.33 per cent in *H. septima* from 24 to 72 HAT. Formulation B @ 1% caused mortality of 33.33 to 71.67 per cent in *D. indica* and 41.67 to 71.67 per cent in *H. septima*.

Safety evaluation of the formulation on beneficial insects revealed that in honey bees, mortality was 30.00 per cent with the application of formulation A @ 1% and it was 26.67 per cent with formulation B @ 1% at 24 HAT. Meanwhile, formulation A @ 1% caused 23.33 per cent mortality in *Apanteles taragamae* Viereck and it was 26.67 per cent with formulation B @ 1%.

Shelf life of the formulation was assessed for six months under room temperature. Both the formulations remained stable under normal room temperature for six months. Formulation A @ 1% recorded 71.67 to 80.00 per cent mortality in *D. indica* and *H. septima* from the day of preparation till sixth month of storage. Meanwhile, formulation B @ 1% resulted in mortality of 71.67 to 75.00 per cent in *D. indica* and 70.00 to 75.00 per cent in *H. septima*.

Field experiment was carried out to test the efficacy of the botanical in comparison with biopesticide and chemical at Instructional Farm, Vellayani in snake gourd (variety Kaumudi). Formulation A @ 2% was evaluated for its

efficacy against leaf feeding pests at vegetative stage and 50 per cent flowering stage. Observations were taken on pest population and percentage of damaged leaves. It can be deduced that formulation A @ 2% was as effective as neemazal 1% @ 0.2% as evident with reduction in pest population at vegetative and 50 per cent flowering stage of the crop. With regard to reduction in leaf damage, formulation A @ 2% excelled equally as neemazal 1% @ 0.2% at vegetative stage.

The salient findings of the study were

Bark extract of *S. indica* in ethanol @ 5.24 and 6.17% exhibited feeding deterrence, insecticidal properties and caused adverse effects on biology of *D. indica* and *H. septima*. Likewise, seed extract in acetone @ 5.17% and 5.27% exhibited anti-insect properties in *D. indica* and *H. septima*. Seed extract of *S. indica* in acetone exhibited more toxicity towards insects when compared to bark extracts as revealed by lower values of lethal doses against both *D. indica* and *H. septima*. Chromatographic fractions from acetone extract of *S. indica* seeds @ 5% showed antifeedant action and insecticidal effect on test insects. Bioactive compounds present in seeds were identified and structurally characterised as samaderins A, B, C and cedronin. Formulations were prepared from *S. indica* using seed extracts in acetone. Formulation A was developed by mixing acetone extract of *S. indica* seed, tween 80 and distilled water in 15: 5: 80 ratio, while formulation B was prepared by mixing seed extract of *S. indica* in acetone, tween 80 and span 80 (1: 1) and distilled water in 15: 5: 80 ratio. Formulations A and B @ 1% each were effective against *D. indica* and *H. septima* under *in vitro* conditions. Both the formulations A and B from *S. indica* seed extract @ 0.5 and 1% were stable for a period of six months under room temperature. *In vivo* studies proved the efficiency of formulation A @ 2% containing acetone extract of *S. indica*, tween 80 and span 80 on *D. indica* and *H. septima* in terms of reduced pest population and lesser leaf damage.

This study could standardize the protocol for developing insecticidal formulations from seeds of *S. indica*, which is the first attempt of its kind. The

results obtained in this study clearly indicate the possibility of replacing chemical pesticides with a potent, but safer botanical insecticide from *S. indica*, which are rich sources of quassinoids. The study also disclosed that exploration of indigenous plants with anti-insect properties will definitely facilitate safe and sustainable agricultural production in this era of 'safe-to-eat' vegetables. Synergistic interaction between insecticide from *S. indica* and other biopesticides can be evaluated. Molecular docking can be done to find out the mode of action of insecticide developed from *S. indica* seed extract.

References

7. REFERENCES

Aarthi, N. and Murugan, K. 2012. Effect of *Vetiveria zizanioides* L. root extracts on the malarial vector, *Anopheles stephensi* Liston. *Asian Pac. J. Trop. Dis.* 2(2): 154-158.

Abbasipour, H., Mahmoudvand, M., Rastegar, F. and Basij, M. 2010. Insecticidal activity of *Peganum harmala* seed extract against the diamondback moth, *Plutella xylostella*. *Bull. Insectol.* 63(2): 259-263.

Abbott, W. S. 1925. A method of computing the effectiveness of an insecticide. *J. Econ. Entomol*. 18(2): 265-267.

Abdel-Aal, A. E. 1996. Biological, histological and physiological effects of some insect growth regulators on the greasy cutworm, *Agrotis ipsilon* (Lepidoptera: Noctuidae). M.Sc. Thesis, Faculty of Science, Cairo Univeristy, Egypt, 115p.

Abdelgader, H. and Hassan, S. A. 2012. *Effects of botanical insecticides on the egg parasitoid Trichogramma cacoeciae Marchal (Hym. Trichogrammatidae).* In: Third International Scientific Symposium, Jahorina, pp. 445-450.

Abdullah, F. and Subramanian, P. 2008. The feeding response of *Epilachna indica* (Coleoptera: Coccinellidae: Epilachninae) towards extracts of *Azadirachta indica*. *J. Entomol*. 5(2): 77-90.

Abtew, A., Subramanian, S., Cheseto, X., Kreiter, S., Garzia, G. T. and Martin, T. 2015. Repellency of plant extracts against the legume flower thrips *Megalurothrips sjostedti* (Thysanoptera: Thripidae). *Insects* 6(3): 608-625.

Ahbirami, R., Zuharah, W.F., Thiagaletchumi, M., Subramaniam, S. and Sundarasekar, J. 2014. Larvicidal efficacy of different plant parts of railway creeper, *Ipomoea*

cairica extract against dengue vector mosquitoes, *Aedes albopictus* (Diptera: Culicidae) and *Aedes aegypti* (Diptera: Culicidae). *J. Insect Sci.* 14(180): 1-6.

Ali, M. S., Ravikumar, S., Beula, J. M., Anuradha, V. and Yogananth, N. 2014. Insecticidal compounds from Rhizophoraceae mangrove plants for the management of dengue vector *Aedes aegypti*. *J. Vector borne Dis.* 51(2): 106.

Aljedani, D. M. 2018. Assessment of effectiveness of imidacloprid and azadirachtin on the black watermelon bug. *Int. J. Zool. Res.* 14(2): 61-70.

Allawzi, M. A., Allaboun, H. and Qazaq, A. S. 2016. Formulation, emulsion, and thermal stability of emulsifiable malathion concentrate using ethanol as a solvent. *Inter. J. Appl. Eng. Res.* 12(1): 221-231.

Al-Rahimy, S. K. I. and AL-Essa, R. A. 2019. Effect of the extracts of peppermint (*Mentha spicata* L.) in morphological deformations of mosquitoes *Culex molestus* Forskal (1775) (Diptera: Culicidae). In: *AIP Conference Proceedings*, AIP Publishing, USA, pp. 1-8.

Anarkdim, K., Gutiérrez, G., Cambiella, A., Senhadji-Kebiche, O. and Matos, M. 2020. The effect of emulsifiers on the emulsion stability and extraction efficiency of Cr (VI) using emulsion liquid membranes (ELMs) formulated with a green solvent. *Membranes* 10(4): 76-89.

Anjali, C. H., Sharma, Y., Mukherjee, A. and Chandrasekaran, N. 2012. *Pest Manag. Sci*. 68(1): 158–163.

Anusree, S. S., Nisha, M. S. and Sheela, M. S. 2016. Insecticidal activity of *Quisqualis indica* L. flower extract on *Spodoptera litura* Fabricius. *Int. J. Appl. Pure Sci. Agric*. 2(10): 98-103.

Anusree, S. S., Nisha, M. S. and Sheela, M. S. 2018. Evaluation of *Quisqualis indica* and *Samadera indica* Gaertn. as botanical pesticides against *Spodoptera litura* (F.) in Polyhouse. *Indian J. Entomol.* 80(2): 428-431.

Ara, R., Bachchu, M. A. A., Kulsum, M. O. and Sarker, Z. I. 2015. Larvicidal efficacy of some indigenous plant extracts against Epilachna beetle, *Epilachna vigintioctopunctata* (Fab.) (Coleoptera: Coccinellidae). *Bangladesh J. Agric. Res.* 40(3): 451-463.

Arivoli, S. and Samuel, T. 2011. Bioefficacy of *Citrullus colocynthis* (L.) Schrad (Cucurbitaceae) whole plant extracts against *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus* (Diptera: Culicidae). *Inter. J. Curr. Res.* 3(4): 296-304.

Arivoli, S. and Tennyson, S. 2013. Antifeedant activity, developmental indices and morphogenetic variations of plant extracts against *Spodoptera litura* (Fab) (Lepidoptera: Noctuidae). *J. Entomol. Zool. Stud.* 1(4): 87-96.

Arora, M., Sharma, J., Singh, A. and Negi, R. S. 2011. Larvicidal property of aqueous extracts of *Withania somnifera* on *Tribolium castenum*. *Indian J. Fundamental Appl. Life Sci.* 1(2): 32-36.

Arora, S., Mogha, N., Bhardwaj, T. and Srivastava, C. 2017. Antifeedant and insecticidal activity of plant extracts against *Spodoptera litura* (Fab.) and *Lipaphis erysimi*. *Proc. Nat. Acad. Sci., India Section B: Biological Sci.* 87(4): 1229-1236.

Asiry, K. A. 2015. Aphidicidal activity of diferent aqueous extracts of bitter apple *Citrullus colocynthis* (L.) against the bird cherry-oat aphid, *Rhopalosiphum padi* (L.) (Homoptera: Aphididae) under laboratory conditions. *J. Anim. Plant. Sci*. 25(2): 456–462.

Aswin, T., Bhaskar, H. and Subramanian, M. 2016. Efficacy of novel acaricide molecules and botanicals against rice leaf mite *Oligonychus oryzae* (Hirst) (Prostigmata: Tetranychidae). *J. Trop. Agric*. 53(2): 187-190.

Ateyyat, M., Abdel-Wali, M. and Al-Antary, T. 2012. Toxicity of five medicinal plant oils to woolly apple aphid, *Eriosoma lanigerum* (Homoptera: Aphididae). *Aust. J. Basic Appl. Sci.* 6(1): 66–72.

Atshan, K. A., Tirupati Rao, Y. R. K. V. and Raghu Ram, M. 2017. Effect of seed extracts as insecticides against lepidopteran pests on cauliflower. *Asian J. Agric. Res.* 11(1): 78-85.

Aviles, R., Gonzalez, N., Ramos, N. and Sotomayor, E. 2001. Efecto del NeemAzal en hojas de pimiento infestadas con huevos de *Thryps palmi* Karny (Thysanoptera: Thripidae) *Boletínde Sanidad Vegetal. Plagas.* 27(2): 193–197.

Bakar, A. A., Sulaiman, S., Omar, B. and Ali, R. M. 2012. Evaluation of *Melaleuca cajuputi* (Family: Myrtaceae) essential oil in aerosol spray cans against dengue vectors in low cost housing flats. *J Arthropod-borne Dis.* 6(1): 28.

Bakavathiappan, G., Baskaran, S., Pavaraj, M. and Jeyaparvathi, S. 2012. Effect of *Calotropis procera* leaf extract on *Spodoptera litura* (Fab.). *J. Biopestic.* 5(1): 135-138.

Baskar, K., Sasikumar, S., Muthu, C., Kingsley, S. and Ignacimuthu, S. 2011. Bioefficacy of *Aristolochia tagala* Cham. against *Spodoptera litura* Fab. (Lepidoptera: Noctuidae). *Saudi J Biol. Sci.* 18: 23–27.

Belmi, R. M., Giron, J. and Tansengco, M. L. 2014. *Antidesma bunius* (Bignay) fruit extract as an organic pesticide against *Epilachna* spp. *J. Asian Sci. Res.* 4(7): 320.

Bentley, M. D., Leonard, D. E., Stoddard, W. F. and Zalkow, L. H. 1984. Pyrrolizidine alkaloids as larval feeding deterrents for spruce bud worm, *Choristoneura fumiferana* (Lepidoptera: Tortricidae). *Ann. Entomol. Soc. Am.* 77(4): 393-397.

Bessette, S. M. 2007. Synergistic and residual pesticidal compositions containing plant essential oils with enzyme inhibitors. United States patent US 7,241,806. Available: http://www.freepatentsonline.com/7241806 html [08/10/2021].

Bhavyashree, K. 2019. Oil based formulation of *Andrographis paniculata* (Burm. f.) Nees against sucking pests of chilli. M.Sc. (Ag) thesis, Kerala Agricultural University, Thrissur, 50p.

Bhushan, S., Gupta, S., Sohal, S.K. and Arora, S. 2016. Assessment of insecticidal action of 3-Isothiocyanato-1-propene on the growth and development of *Spodoptera litura* (Fab.) (Lepidoptera: Noctuidae). *J. Entomol. Zool. Stud.* 4(5): 1068-1073.

Bhutto, Z. A., Magsi, F. H., Soomro, A. A., Chandio, M. A., Channa, N. A., Lashari, S. H., Mangi, S. and Junejo, A. A. 2017. Integrated pest management of okra insect pests. *Inter. J. Fauna Biol. Stud.* 4(3): 39-42.

Binseena, S. R., Anitha, N., Paul, A., Amritha, V. S. and Anith, K. N. 2018. Management of rice weevil, *Sitophilus oryzae* using essential volatile oils. *Entomon* 43(4): 277-280.

BIS (Bureau of Indian standards) Specification: IS: 12015-1987.

Bjorkegren, S., Karimi, R. F., Martinelli, A., Jayakumar, N. S. and Hashim, M. A. 2015. A new emulsion liquid membrane based on a palm oil for the extraction of heavy metals. *Membranes* 5(1):168–179.

Bolandnazar, A., Ghadamyari, M., Memarzadeh, M. and Sandi, J, J. 2018. Repellent and lethal effect of essential oils of rosemary, peppermint and eucalyptus and thyme extract, formulated as nano and microemulsions, on *Bemisia tabaci* (Homoptera: Aleyrodidae) under greenhouse condition. *J. Entomol. Soc. Iran* 38(1): 81-97.

Boomathi, N., Sivasubramanian, P., Raguraman, S. and Premalatha, K. 2005. Safety of botanical and microbial pesticide mixtures to *Trichogramma chilonis* Ishii (Hymenoptera: Trichogrammatidae). *J. Biol. Control* 19(1): 23-29.

Boopathi, T. K. A., Pathak, N. D. and Bemkaireima, L. 2009. Field efficacy of botanicals and common insecticides against blister beetles, *Mylabris*

pustulata and *Epicauta* sp. in green gram. *J. Eco-friendly Agric.* 4(1): 194–195.

Brito, W. A. D., Siquieroli, A. C. S., Andalo, V., Duarte, J. G., Sousa, R. M. F. D., Felisbino, J. K. R. P. and Silva, G. C. D. 2021. Botanical insecticide formulation with neem oil and D-limonene for coffee borer control. *Pesquisa Agropecuária Brasileira* 56(1): 1-9.

Caballero, C., Lopez-Olguin, J., Ruiz, M. Ortego, F. and Castañera, P. 2008. Antifeedant activity and effects of terpenoids on detoxification enzymes of the beet armyworm, *Spodoptera exigua* (Hübner). *Spanish J. Agr. Res*. 6(1): 177-184.

Calcagno, M. P., Coll, J., Lloria, J., Faini, F. and Alonso-Amelot, M. E. 2002. Evaluation of synergism in the feeding deterrence of some furanocoumarins on *Spodoptera littoralis*. *J Chem. Ecol.* 28(1): 175-191.

Camaroti, J. R. S. L., de Almeida, W. A., do Rego Belmonte, B., de Oliveira, A. P. S., de Albuquerque Lima, T., Ferreira, M. R. A., Paiva, P. M. G., Soares, L. A. L., Pontual, E. V. and Napoleão, T. H. 2018. *Sitophilus zeamais* adults have survival and nutrition affected by *Schinus terebinthifolius* leaf extract and its lectin (SteLL). *Ind. Crops Products* 116(1): 81-89.

Campolo, O., Cherif, A., Ricupero, M., Siscaro, G., Grissa-Lebdi, K., Russo, A., Cucci, L. M., di Pietro, P., Satriano, C., Desneux, N., Biondi, A., Zappala, L. and Palmeri, V. 2017. Citrus peel essential oil nanoformulations to control the tomato borer, *Tuta absoluta*: chemical properties and biological activity. *Sci. Rep.* 7(1):13036.

Cespedes, C. L., Salazar, J. R., Martinez, M. and Aranda, E. 2005. Insect growth regulatory effects of some extracts and sterols from *Myrtillocactus geometrizans* (Cactaceae) against *Spodoptera frugiperda* and *Tenebrio molitor*. *Phytochem.* 66(20): 2481-2493.

Chandrashekara, K. A., Rajendra, H., Anil, K. and Reddy, C. G. 2014. Preparation, analysis and bioefficacy of water soluble botanical pesticide formulation from neem and sweet flag. *Inter. J. Chem. Tech. Res.* 6(14): 5494-5502.

Chattree, P. V., Mishra, M. S. and Srivastava, A. N. 2016. Residual effect of neem leaf extract on the mortality of *Lipaphis erysimi* and its larvae. *J. Environ. Sci. Toxicol. Food Technol.* 10(10): 115-119.

Chowdhury, N. Y., Islam, W. and Khalequzzaman, M. 2009. Insecticidal activities of stem bark extracts from *Vitex negundo* L. against *Tribolium castaneum* (Herbst). *J. Bio. Sci.* 17(1): 63-70.

Coelho, A. A., Paula, J. E. D. and Espíndola, L. S. 2006. Insecticidal activity of cerrado plant extracts on *Rhodnius milesi* Carcavallo, Rocha, Galvao & Jurberg (Hemiptera: Reduviidae), under laboratory conditions. *Neotrop. Entomol.* 35(1): 133-138.

Cruz, G. S., Wanderley-Teixeira, V., Oliveira, J. V., Lopes, F. S. C., Barbosa, D. R. S., Breda, M. O., Dutra, K. A., Guedes, C. A., Navarro, D. M. A. F. and Teixeira, A. A. C. 2016. Sublethal effects of essential oils from *Eucalyptus staigeriana* (Myrtales: Myrtaceae), *Ocimum gratissimum* (Lamiales: Laminaceae), and *Foeniculum vulgare* (Apiales: Apiaceae) on the biology of *Spodoptera frugiperda* (Lepidoptera: Noctuidae). *J. Economic Entomol.* 109(2): 660-666.

Czerniewicz, P., Chrzanowski, G., Sprawka, I. and Sytykiewicz, H. 2018. Aphicidal activity of selected Asteraceae essential oils and their effect on enzyme activities of the green peach aphid, *Myzus persicae* (Sulzer). *Pesticide Biochem. Physiol.* 145(1): 84–92.

da Silva, I. M., Zanuncio, J. C., Brügger, B. P., Soares, M. A., Zanuncio, A. J. V., Wilcken, C. F., de Souza, T. W., Serrão, J. E. and Sediyama, C. S. 2020.

Selectivity of the botanical compounds to the pollinators *Apis mellifera* and *Trigona hyalinata* (Hymenoptera: Apidae). *Sci. Rep.* 10(1): 1-86.

Dadang, E. D. F., Fitriasari, E, D. and Prijono, D. 2009. Effectiveness of two botanical insecticide formulations to two major cabbage insect pests on field application. *J. Inter. Soc. Southeast Asian Agric. Sci.* 15(1): 42-51.

Daido, M., Ohno, N., Imamura, K., Fukamiya, N., Hatakoshi, M., Yamazaki, H., Tagahara, K., Lee, K. H. and Okano, M. 1995. Antifeedant and insecticidal activity of quassinoids against the diamondback moth (*Plutella xylostella*) and structure-activity relationships. *Biosci., Biotechnol., Biochem.* 59(6): 974-979.

de Carvalho, G. H. F., de Andrade, M. A., de Araújo, C. N., Santos, M. L., de Castro, N. A., Charneau, S., Monnerat, R., de Santana, J. M. and Bastos, I. M. D. 2019. Larvicidal and pupicidal activities of eco-friendly phenolic lipid products from *Anacardium occidentale* nutshell against arbovirus vectors. *Environ. Sci. Pollut. Res.* 26(6): 5514-5523.

de Oliveira, J. L., Campos, E. V., Camara, M. C., Vechia, D. J. F., de Matos, S. T. S., de Andrade, D. J., Goncalves, K. C., Nascimento, J. D., Polanczyk, R. A., de Araujo, D. R. and Fraceto, L. F. 2019. Hydrogels containing botanical repellents encapsulated in zein nanoparticles for crop protection. *ACS Appl. Nano Materials* 3(1): 207-217.

Desneux, N. R., Denoyelle and Kaiser, L. 2006. A multi-step bioassay to assess the effect of deltamethrin on the parasitic wasp *Aphidius ervi*. *Chemosphere* 65: 1697-1706.

Do, Q. D., Angkawijaya, A. E. and Tran-Nguyen, P. L. 2014. Effect of extraction solvent on total phenol content, total flavonoid content and antioxidant activity of *Limnophila aromatic*. *J. Food Drug Anal.* 22(3):296-302.

Elanchezhiyan, K., Gokulakrishnan, J., Deepa, J. and Selvakumar, B. 2015. Phyto-toxicity of Indian medicinal plants Tinospora crispa (Menispermaceae) and Psidium guajava (Myrtaceae) against tobacco cutworm, Helicoverpa armigera (Hub.) (Lepidoptera: Noctuidae). Int. J. Curr. Agr Sci. 4(1): 1-6.

El-Bokl, M. M. 2016. Toxicity and bioefficacy of selected plant extracts against the mosquito vector *Culex pipiens* L. (Diptera: Culicidae). *Environ.* 6(1): 7.

Erler, F., Cetin, H., Saribasak, H. and Serttas, A. 2010. Laboratory and field evaluations of some botanical pesticides against the cedar leaf moth, *Acleris undulana*. *J. Pest Sci.* 83(3): 265-272.

Etebari, K., Bizhannia, A. R., Sorati, R. and Matindoost, L. 2007. Biochemical changes in haemolymph of silkworm larvae due to pyriproxyfen residue. *Pestic. Biochem. Phys.* 88(1): 14–19.

Ezemuoka, L. C., Nwankwo, E. N. and Ogbonna, C. U. 2019. Toxicity of the aqueous leaf and Stem-bark extracts of *Annona muricata* to the 4th instar larvae of *Aedes aegypti*. *J Entomol. Zool. Stud.* 7(4): 1047-1052.

Fatima, K., Bashar, K., Rahman, K. M. Z. and Howlader, A. J. 2011. Oviposition deterrent activity of some indigenous plant leaf extracts on mosquito *Culex quinquefasciatus* Say (Diptera: Culicidae). *Bangladesh J. Life Sci.* 23(1): 25-31.

Fernandes, C. P., de Almeida, F. B., Silveira, A. N., Gonzalez, M. S., Mello, C. B., Feder, D., Apolinário, R., Santos, M. G., Carvalho, J. C. T., Tietbohl, L. A. C. and Rocha, L. 2014. Development of an insecticidal nanoemulsion with *Manilkara subsericea* (Sapotaceae) extract. *J. Nanobiotechnol.* 12(1): 1-9.

Fernandes, C. P., Mascarenhas, M. P., Zibetti, F. M., Lima, B. G., Oliveira, R. P. R. F. and Falcao, D. Q. 2013. HLB value,an important parameter for the development of essential oil phytopharmaceuticals. *Braz. J. Pharmacog*. 23(1): 108-114.

Gaur, S. K. and Kumar, K. 2020. Toxicity and insect growth regulatory effects of root extract from the medicinal plant, *Withania somnifera* (Linnaeus) against red flour beetle, *Tribolium castaneum* (Coleoptera: Tenebrionidae). *Arch. Phytopathol. Plant Prot.* 53(1): 856-875.

Ge, Y., Liu, P., Yang, R., Zhang, L., Chen, H., Camara, I., Liu, Y. and Shi, W. 2015. Insecticidal constituents and activity of alkaloids from *Cynanchum mongolicum*. *Molecules* 20(9): 17483-17492.

Germinara, G. S., Distefano, M. G., Acutis, L. D., Pati, S., Delfne, S., Cristofaro, A. D. and Rotundo, G. 2017. Bioactivities of *Lavandula angustifolia* essential oil against the stored grain pest *Sitophilus granaries*. *Bull. Insectol.* 70(1): 129–138.

Ghaly, N. S., Mina, S. A., Abdel-Aziz, N. F. and Sammour, E. A. 2014. Insecticidal activity of the main flavonoids from the leaves of *Kalanchoe beharensis* and *Kalanchoe longiflora*. *J. Nat. Prod*. *7*(1): 196-202.

Gokse, A., Stelinski, L. L., Whalon, M. E. and Gut, L. J. 2010. Toxicity and antifeedant activity of selected plant extracts against larval oblique banded leafroller, *Choristoneura rosaceana* (Harris). *The Open Entomol. J.* 4(1): 18-24.

Gomathi, S. and Rathinam, K. S. 2017. Insecticidal activity of *Saraca asoca* and *Terminalia arjuna* bark extracts against *Sitophilus oryzae*. *World J. Sci. Res.* 2(1): 60-63.

Govindachari, T. R., Kumari, G. K., Gopalakrishnan, G., Suresh, G., Wesley, S. D. and Sreelatha, T. 2001. Insect antifeedant and growth regulating activities of quassinoids from *Samadera indica*. *Fitoterapia* 72(5): 568-571.

Griffin, W. C. 1954. Calculation of HLB values of non-ionic surfactants. *J. Soc. Cosmet. Chem.* 5(1): 249–256.

Gutierrez, P. M., Antepuesto, A. N., Eugenio, B. A. L. and Santos, M. F. L. 2014. Larvicidal activity of selected plant extracts against the dengue vector *Aedes aegypti* mosquito. *Inter. Res. J Biol. Sci.* 3(4): 23-32.

Han, M. K., Kim, S. I. and Ahn, Y. J. 2006. Insecticidal and antifeedant activities of medicinal plant extracts against *Attagenus unicolor japonicus* (Coleoptera: Dermestidae). *J. Stored Products Res.* 42(1): 15-22.

Hematpoor, A., Liew, S. Y., Azirun, M. S. and Awang, K. 2017. Insecticidal activity and the mechanism of action of three phenylpropanoids isolated from the roots of *Piper sarmentosum* Roxb. *Sci. Rep. 7*(1): 1-13.

Ilahi, I., Yousafzai, A. M., Haq, T. U., Ali, H., Rahim, A., Sajad, M. A., Khan, A. N., Ahmad, A., Ullah, S., Zaman, S. and Bibi, A. 2019. Oviposition deterrence and adult emergence inhibition activities of *Cymbopogon nardus* against *Culex quinquefasciatus* with study on non-target organisms. *Appl. Ecol. Environ. Res*. 17(1): 4915-4931.

Inocente, E. A., Nguyen, B., Manwill, P. K., Benatrehina, A., Kweka, E., Wu, S., Cheng, X., Rakotondraibe, L. H. and Piermarini, P. M. 2019. Insecticidal and antifeedant activities of Malagasy medicinal plant (*Cinnamosma* sp.) extracts and drimane-type sesquiterpenes against *Aedes aegypti* mosquitoes. *Insects*. 10(11): 373.

Ishikawa, Y. and Kanke, T. 2000. Role of gramine in the feeding deterrence of barley against the migratory locust, *Locusta migratoria* (Orthoptera: Acrididae). *J. Appl. Entomol. Zool.* 35: 251-256.

Islam, K., Islam, M. S. and Ferdousi, Z. 2011. Control of *Epilachna vigintioctopuntata* Fab. (Coleoptera: Coccinellidae) using some indigenous plant extracts. *J. Life Earth Sci*. 6(1): 75-80.

Jahan, F., Abbasipour, H. and Hasanshahi, G. 2016. Fumigant toxicity and nymph production deterrence effect of three essential oils against two aphid species in the laboratory condition. *J. Essential Oil Bearing Plants* 19(3): 706-711.

Jayasekara, T. K., Stevenson, P. C., Hall, D. R. and Belmain, S. R. 2005. Effect of volatile constituents from *Securidaca longepedunculata* on insect pests of stored grain. *J. Chemical Ecol.* 31(2): 303-313.

Jbilou, R., Ennabili, A. and Sayah, F. 2006. Insecticidal activity of four medicinal plant extracts against *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae). *African J. Biotechnol.* 5(10).

Jeyabalan, D., Arul, N. and Thangamathi, P. 2003. Studies on effects of *Pelargonium citrosa* leaf extracts on malarial vector, *Anopheles stephensi* Liston. *Bioresour. Technol.* 89(2): 185-189.

Josephrajkumar, A., Subrahmanyam, B. and Srinivasan. 1999. Plumbagin and azadirachtin deplete haemolymph ecdysteroid levels and alter the activity profiles of two lysosomal enzymes in the fat body of Helicoverpa armigera (Lepidoptera: Noctuidae). Eur. J. Entomol. 96(1): 347-353.

Juarez, Z. N., Fortuna, A. M., Sanchez-Arreola, E., Lopez-Olguín, J. F., Bach, H. and Hernandez, L. R. 2014. Antifeedant and phagostimulant activity of extracts and pure compounds from *Hymenoxys robusta* on *Spodoptera exigua* (Lepidoptera: Noctuidae) larvae. *Nat. Product Commun*. 9(7): 895-898.

Kala, S., Sogan, N. and Patanjali, P. K. 2017. Multiple activity based bio-botanical water dispersible granules formulation and their efficacy for pest management in agriculture. Inter. J. Advanced Res. 5(1): 2846-2851.

Kang, S. H., Kim, M. K., Noh, D. J., Yoon, C. and Kim, G. H. 2009. Spray adulticidal effects of plant oils against house mosquito, *Culex pipiens pallens* (Diptera: Culicidae). *J. Pestic. Sci.* 34(2): 100–106.

Kaoneka, B. and Mollel, M. 2012. Antifeedant activity of twenty six plant extracts and pure compounds from the root bark of *Toddalia asiatica* (L) (Rutaceae) against the anomalous emperor moth *Nudaurelia belina* (Saturnidae). *Huria: J. Open Univ. Tanzania* 12(1): 102-109.

Kashiwagi, T. B. Wu, K., Iyota, X. H., Chen, S., Tebayashi and Kim, C. 2007. Antifeedants against *Locusta migratoria* from the Japanese Cedar, *Cryptomeria japonica*. *Biosci. Biotechnol. Biochem.* 71(4): 966-970.

KAU (Kerala Agricultural University). 2016. *Package of Practices Recommendations: Crops* (16th Ed.). Kerala Agricultural University, Thrissur, 310 p.

Kaushik, R. and Saini, P. 2008. Larvicidal activity of leaf extract of *Millingtonia hortensis* (Family: Bignoniaceae) against *Anopheles stephensi*, *Culex quinquefasciatus* and *Aedes aegypti*. *J. Vector Borne Dis.* 45(1): 66.

Kchaou, W., Abbès, F., Blecker, C., Attia, H. and Besbes, S. 2013. **Effects of extraction solvents on phenolic contents and antioxidant activities of Tunisian date varieties (***Phoenix dactylifera L***.)**. *Ind. Crops Products* 45(1): 262-269.

Keita, S. M., Vincent, C., Schmit, J. P., Arnason, J. T. and Belanger, A. 2001. Efficacy of essential oil of *Ocimum basilicum* L. and *O. gratissimum* L. applied as an insecticidal fumigant and powder to control *Callosobruchus maculatus* (Fab.)[Coleoptera: Bruchidae]. *J. Stored Products Res.* 37(4): 339-349.

Khan, S., Taning, C. N. T., Bonneure, E., Mangelinckx, S., Smagghe, G., Ahmad, R., Fatima, N., Asif, M. and Shah, M. M. 2019. Correction: Bioactivity-guided isolation of rosmarinic acid as the principle bioactive compound from the butanol extract of *Isodon rugosus* against the pea aphid, *Acyrthosiphon pisum*. *PloS one* 14(10): 25-30.

Khan, S., Taning, C. N. T., Bonneure, E., Mangelinckx, S., Smagghe, G. and Shah, M. M. 2017. Insecticidal activity of plant-derived extracts against different economically important pest insects. *Phytoparasitica* 45(1): 113-124.

Khanam, L. M., Talukder, D. and Hye, M. A. 2006. Toxic and repellent action of sugarcane bagasse-based lignin against some stored grain insect pests. *University J. Zool.* 25(1): 27-30.

Khanavi, M., Najafi, B., Sadati, S. N., Abai, M. R. and Vatandoost, H. 2017. Chemical constitute and larvicidal activity of fractions of *Ajuga chamaecistus tomentella* plant against malaria vector *Anopheles stephensi*. *J. Arthropod-borne Dis.* 11(1): 116.

Khoshnoud, H., Ghiyasi, M., Amirnia, R., Fard, S. S., Tajbakhsh, M., Salehzadeh, H. and Alahyary, S. 2008. The potential of using insecticidal properties of medicinal plants against insect pests. *Pakistan J. Biol. Sci.* 11(10): 1380-1384.

Khoshraftar, Z., Safekordi, A. A., Shamel, A., and Zaefizadeh, M. 2019. Synthesis of natural nanopesticides with the origin of *Eucalyptus globulus* extract for pest control. *Green Chem. Lett. Rev.* 12(3): 286-298.

Khosravi, R., Sendi, J. J., Ghadamyari, M. and Yezdani, E. 2011. Effect of sweet wormwood Artemisia annua crude leaf extracts on some biological and physiological characteristics of the lesser mulberry pyralid, *Glyphodes pyloalis*. *J. Insect Sci.* 11(1): 156.

Kim, S. I. and Ahne, Y. J. 2017. Larvicidal activity of lignans and alkaloid identified in *Zanthoxylum piperitum* bark toward insecticide-susceptible and wild *Culex pipiens pallens* and *Aedes aegypti*. *Parasit. Vectors* 10(1): 221.

Kim, S., Shin, O., Song, C., Cho, K. and Ahne, Y. 2001. Insecticidal activities of aromatic plant extracts against four agricultural insects. *Agric. Chem. Biotechnol*. 44(1): 23-26.

Kostic, M. Z., Popovic, D., Brkic, S., Milanovic, I., Sivcev and Stankovic. S. 2008. Larvicidal and antifeedant activity of some plant-derived compounds to *Lymantria dispar* L. (Lepidoptera: Limantriidae). *Bioresour. Technol.* 99: 7897-7901.

Kovarikova, K. and Pavela, R., 2019. United forces of botanical oils: efficacy of neem and karanja oil against Colorado potato beetle under laboratory conditions. *Plants* 8(12): 608.

Krishnan, R. and Murugan, K. 2015. Insecticidal potentiality of flavonoids from cell suspension culture of *Marchantia linearis* Lehm. & Lindenb against *Spodoptera litura* F. *Int. J. Appl. Biol. Pharma. Technol.* 6(2): 23-32.

Kumar, J. A., Rekha, T., Devi, S. S., Kannan, M., Jaswanth, A. and Gopal, V., 2010. Insecticidal activity of ethanolic extract of leaves of *Annona squamosa*. *J. Chem. Pharma. Res.* 2(5): 177-180.

Kumar, S., Umrao, R. S., Kumar, A., Patel, V. K., Debnath, R. and Kumar, A. 2020. Evaluation of the efficacy of insecticides and bio-pesticides against *Helicoverpa armigera* (Hubner) on tomato. *J. Entomol. Zool. Stud*. 8(3): 555-558.

Kutas, J. and Nadasy, M. 2005. Antifeedant effects of several plant extracts on Colorado potato beetle larvae. *Acta Phytopathologica et Entomologica Hungarica* 40(4): 355-365.

Leatemia, J. A. and Isman, M. B. 2004. Insecticidal activity of crude seed extracts of *Annona* spp., *Lansium domesticum* and *Sandoricum koetjape* against lepidopteran larvae. *Phytoparasitica* 32(1): 30-37.

Lenin, N., 2011. Infestation of the pumpkin caterpillar, *Diaphania indica* Saunders in cucurbits and its management. M.Sc. (Ag) thesis, Kerala Agricultural University, Thrissur, 120p.

Libs, E. and Salim, E. 2017. Formulation of essential oil pesticides technology and their application. *Agri. Res. Tech*. 9(2): 42-60.

Lina, E. C., Tama, D. P., Nelly, N. and Djamaan, A. 2020. Design of nanoemulsion of *Tephrosia vogelii* extract as botanical insecticide to control cabbage pest. October.

In: *IOP Conference Series: Earth and Environmental Science*. IOP Publishers, UK, pp. 1-8.

Lina, E. C., Yulianti, N., Ernis, G., Arneti, A. and Nelly, N. 2018. Storage temperature of botanical insecticide mixture formulations and its activity against *Crocidolomia pavonana* (F.) (Lepidoptera: Crambidae). *Agrivita, J. Agric. Sci.* 40(3): 498-505.

Lingathurai, S., Vendan, S.E., Paulraj, M.G. and Ignacimuthu, S. 2011. Antifeedant and larvicidal activities of *Acalypha fruticosa* Forssk.(Euphorbiaceae) against *Plutella xylostella* L. (Lepidoptera: Yponomeutidae) larvae. *J. King Saud Univ. - Sci. 23*(1): 11-16.

Loko, L. Y., Alagbe, O., Dannon, E. A., Datinon, B., Orobiyi, A., Thomas-Odjo, A., Dansi, A. and Tamo, M. 2017. Repellent effect and insecticidal activities of *Bridelia ferruginea*, *Blighia sapida* and *Khaya senegalensis* leaves powders and extracts against *Dinoderus porcellus* in infested dried yam chips. *Psyche* 81(3): 1-18.

Lucia, A., Girard, C., Fanucce, M., Coviella, C., Rubio, R. G., Ortega, F. and Guzman, E. 2020. Development of an environmentally friendly larvicidal formulation based on essential oil compound blend to control *Aedes aegypti* larvae: Correlations between physicochemical properties and insecticidal activity. *ACS Sustain. Chem. Eng.* 8(29): 10995-11006.

Madanat, H., Al Antary, T. M. and Zarqa, M. 2016. Toxicity of six ethanol plant extracts against the green peach aphid *Myzus persicae* Sulzer (Homoptera: Aphididae). *Fresen. Environ. Bull.* 25(3): 706-718.

Magierowicz, K., Gorska-Drabik, E., and Golan, K. 2020. Effects of plant extracts and essential oils on the behavior of *Acrobasis advenella* (Zinck.) caterpillars and females. *J. Plant Dis. Prot*. 127(1): 63-71.

Magsi, F. H., Syed, T. S., Memon, A. U. R., Bhutto, Z. A., Chang, B. H. and Chandio, M. A. 2017. Effect of different tobacco extracts on the population build up of sucking complex in okra crop. *Inter. J. Fauna Biol. Stud.* 4(2): 120-123.

Maheswari, M. and Govindaiah, G. 2017. Evaluation of different plant extracts for management of mealy bugs and leaf rollers in the mulberry field. *Screening* 11(1): 12.

Mao, G., Tian, Y., Sun, Z., Ou, J. and Xu, H. 2019. Bruceine D isolated from *Brucea javanica* (L.) Merr. as a systemic feeding deterrent for three major lepidopteran pests. *J. Agric. Food Chem.* 67(15): 4232-4239.

Martinez, A. M., Aguado-Pedraza, A. J., Vinuela, E., Rodríguez-Enriquez, C. L., Lobit, P., Gomez, B. and Pineda, S. 2017. Effects of ethanolic extracts of *Argemone ochroleuca* (Papaveraceae) on the food consumption and development of *Spodoptera frugiperda* (Lepidoptera: Noctuidae). *Fla. Entomologist*. 100(2): 339-345.

Marzanghi, J., Hassan, M., Alizadeh, S. and Moghaddam, P. N. 2013. Investigation on the insecticidal efficacy of novel pellet formulation against *Callosobruchus maculatus* f. (Col.: Bruchidae) in three different heights and compared with phosphin. *World Appl. Sci. J.* 28(12): 1913-1917.

Masayuki, D, N., Fukamiya, M., Okano, K., Tagahara, M., Hatakoshi. and Yamazaki, H. 1993. Antifeedant and insecticidal Activity of quassinoids against diamondback moth (*Plutella xylostella*), *Biosci. Biotech. Biochem*. 57(2): 244-246.

Min, B. I. R. H. A. N. D. L. and Yueguan, Z. L. H. M. F. 2009. Toxicity of 2% *Piper Sarmentosum* Roxb. microemulsion to several pathogens and insects. *Trop. Agric. Eng*. 83(2): 1- 6.

Misni, N., Othman, H. and Sulaiman, S. 2011. The effect of *Piper aduncum* Linn. (Family: Piperaceae) essential oil as aerosol spray against *Aedes aegypti* (L.) and *Aedes albopictus* Skuse. *Trop. Biomedicine* 28(2): 249-258.

Mollah, J. U. and Islam, W. 2002. Efficacy of akanda *Calotropis procera* (Ait.) extracts against the pulse beetle, *Callosobruchus maculates* F. (Coleoptera: Bruchidae). *Bangladesh J. Entomol.* 12(1): 115-120.

Morehead, J. A. 2016. Efficacy of organic insecticides and repellents against brown marmorated stink bug in vegetables. Doctoral dissertation, Virginia Technology, Virginia, 210p.

Moreira, D., Picanço, M. C., Cláudio de Almeida, L. B., Guedes, R. N. C., Ribeiro de Campos, M., Silva, G. A. and Martins, J. C. 2007. Plant compounds insecticide activity against coleopteran pests of stored products. *Pesquisa Agropecuária Brasileira.* 7(1): 909–915.

Moreno, S. C., Carvalho, G. A., Picanco, M. C., Morais, E. G. and Pereira, R. M. 2012. Bioactivity of compounds from *Acmella oleracea* against *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) and selectivity to two non-target species. *Pest Manag. Sci.* 68(3): 386-393.

Motazedian, N., Aleosfoor, M., Davoodi, A. and Bandani, A. R. 2014. Insecticidal activity of five medicinal plant essential oils against the cabbage aphid, *Brevicoryne brassicae*. *J. Crop Prot.* 3(2): 137–146.

Mustafa, I., and Gaur, G. 2009. Aphidicidial activity of seven essential oils against the cabbage aphid, *Brevicoryne brassicae* L. (*Hemiptera*: *Aphididae*). *Mun. Entomol. Zool*. 4(1): 424-431.

Muthukrishnan, J. and Puspalatha, E. 2001. Effects of plant extracts on fecundity and fertility of mosquitoes. *J. Appl. Entomol*. 125(1): 31–35.

N'Guessan, K. F, Kouassi, A. F. and Atindehou, K. 2006. Study on the effect of the neem, *Azadiractha indica* juss (Meliaceae) on *Sahlbergella singularis* (Hemiptera: Miridae), an important pest of cocoa. In: *Proceedings of 15th Internatonal Cocoa Research Conference,* 9-10 October 2006, San Jose, Costa Rica pp. 1287-1296.

Nath, R. K., Begum, K. H. and Ahmed, P. 2020. Integrated pest management of okra (Abelmoschus esculentus L.). Int. J. Curr. Microbiol. App. Sci. 9(1): 1725-1729.

Ntalli N. G. and Menkissoglu-Spiroudi, U. 2011. Pesticides of Botanical Origin: a Promising Tool in Plant Protection. Pesticides—formulations, effects, fate. (Rijeka: InTech Europe). 10.5772/13776. pp. 1-23.

Oigiangbe, O. N., Igbinosa, I. B. and Tamo, M. 2007. Insecticidal activity of the medicinal plant, *Alstonia boonei* De Wild, against *Sesamia calamistis* Hampson. *J. Zhejiang Univ. Sci. B* 8(10): 752-755.

Ojiako, F.O., Dialoke, S. A., Ihejirika, G.O., Ahuchaogu, C. E. and Ohiri, C.P. 2015. Comparative performance of pyrethrum [*Chrysanthemum cinerariifolium* Treviranus (Vis.)] extract and cypermethrin on some field insect pests of Groundnut (*Arachis hypogaea* L.) in Southeastern Nigeria. *J. Exp. Agric. Int.* 7(2): 96-106.

Omar, D., Asib, N., Choupanian, M. and Kamarudin, N. 2016. Nanoemulsion formulation of botanical insecticides for effective insect control. In: *Conference on Nanoemulsion formulation of botanical insecticides for effective insect control*, Malaysia, pp. 1-4.

Omar, S. M., Marcotte, P., Fields, P. E., Sanchez, L., Poveda, R., Mata, A., Jimenez, T., Durst, J., Zhang, S., MacKinnon, D., Leaman, J. T., Arnason and Philogene, B. J. R. 2007. Antifeedant activities of terpenoids isolated from tropical Rutales. *J. Stored Prod. Res*. 43(1): 92-96.

Ong, M., Chomistek, N., Dayment, H., Goerzen, W. and Baines, D. 2020. Insecticidal activity of plant powders against the parasitoid, *Pteromalus venustus* and its host, the alfalfa leaf cutting bee. *Insects* 11(6): 359.

Orlikowski, L. B. and Skrzypczak, C. 2001. Biopreparations from grapefruit progress in the biological control of plant diseases. *Horticultura* 23(1**)**: 261-269.

Overgaard, H. J., Sirisopa, P., Mikolo, B., Malterud, K. E., Wangensteen, H., Zou, Y. F., Paulsen, B. S., Massamba, D., Duchon, S., Corbel, V. and Chandre, F. 2014. Insecticidal activities of bark, leaf and seed extracts of *Zanthoxylum heitzii* against the African malaria vector *Anopheles gambiae*. *Molecules*. 19(12): 21276-21290.

Packiam, S. M., Anbalagan, V., Ignacimuthu, S., and Vendan, S. E. 2012. Formulation of a novel phytopesticide PONNEEM and its potentiality to control generalist herbivorous lepidopteran insect pests, *Spodoptera litura* (Fabricius) and *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae). *Asian Pac. J. Trop. Dis.* 2(1): 720-723.

Panse, V. G. and Sukhatme, P. V. 1967. *Statistical Method for Agricultural Workers*. (2nd Ed.). Indian Council of Agricultural Research. New Delhi, 381 p.

Pavela, R. 2009. Effectiveness of some botanical insecticides against *Spodoptera littoralis* Boisduval (Lepidoptera: Noctudiae), *Myzus persicae* Sulzer (Hemiptera: Aphididae) and *Tetranychus urticae* Koch (Acari: Tetranychidae). *Plant Prot. Sci*. 45(4): 161-167.

Pavela, R., 2010. Antifeedant activity of plant extracts on *Leptinotarsa decemlineata* Say. and *Spodoptera littoralis* Bois. larvae. *Ind. Crops Products*. 32(3): 213-219.

Pavela, R., Benelli, G., Pavoni, L., Bonacucina, G., Cespi, M., Cianfaglione, K., Bajalan, I., Morshedloo, M. R., Lupidi, G., Romano, D. and Canale, A. 2019. Microemulsions for delivery of Apiaceae essential oils—towards highly effective and eco-friendly mosquito larvicides? *Ind. Crops Products*. 129: 631-640.

Pavunraj, M., Subramanian, K., Muthu, C., Seenivasan, S. P., Duraipandiyan, V., Pakiam, S. M., Ignacimuthu, S. 2006. Bioefficay of Excoecaria agallocha (L.) leaf extract against the armyworm Spodoptera litura (Fab.) (Lepidoptera: Noctuidae). Entomon 31(1): 37-40.

Permatasari, A. and Sumanto, D. 2019. The Effectiveness of *Derris elliptica* (Wall.) Benth Root Extract Against Temephos-resistant *Aedes aegypti* Larvae. In *IOP Conference Series: Earth and Environmental Science* IOP Publishers, UK, pp.1-8.

Pinto, E., Moreira, E., Braz, J. and Santos, C, D. 2013. The control and protection of cotton plants using natural insecticides against the colonization by *Aphis gossypii* Glover (Hemiptera: Aphididae). *Acta Scientiarum Agron.* 35(2): 169-174.

Prajapati, V. A. K. Tripathi, S. P., Khanuja, S. and Kumar, S. 2003. Anti-insect screening of medicinal plants from Kukrail forest, Lucknow, India. *Pharmaceut. Biol*. 41: 166-170.

Puripattanavong, J., Songkram, C., Lomlim, L. and Amnuaikit, T. 2013. Development of concentrated emulsion containing *Nicotiana tabacum* extract for use as pesticide. *J. Appl. Pharma. Sci.* 3(11): 16.

Purkait, A., Biswas, S., Saha, S., Hazra, D. K., Roy, K., Biswas, P. K., Ghosh, S. K. and Kole, R. K. 2019. Formulation of plant based insecticides, their bio-efficacy evaluation and chemical characterization. *Crop Prot.* 125(1): 104907.

Purkait, A., Mukherjee, A., Hazra, D. K., Roy, K., Biswas, P. K. and Kole, R. K. 2021. Encapsulation, release and insecticidal activity of *Pongamia pinnata* (L.) seed oil. *Heliyon*. 7(3): 06557.

Qi, B., Gordon, G. and Gimme, W. 2001. Effects of neem-fed prey on the predacious insects *Harmonia conformis* (Boisduval) (Coleoptera: Coccinellidae) and *Mallada signatus* (Schneider) (Neuroptera: Chrysopidae). *Biol. Control*. 22(1): 185-190.

Rahuman, A. A., Bagavan, C., Kamaraj, E., Saravanan, A., Zahir, A. A. and Elango, G. 2009. Efficacy of larvicidal botanical extracts against *Culex quinquefasciatus* Say (Diptera: Culicidae). *Parasitol. Res.* 104(6): 1365-1372.

Rajashekar, Y. and Tonsing, N. 2014. The herbal insecticides from root stock of *Alocasia indica* as potential sources for control of stored-product insect pests. *Entomol., Ornithol. Herpetol.* 3(3): 1-4.

Rajashekar, Y., Gunasekaran, N. and Shivanandappa, T. 2010. Insecticidal activity of the root extract of *Decalepis hamiltonii* against stored-product insect pests and its application in grain protection. *J. Food Sci. Technol.* 47(3): 310-314.

Ramya, S., Rajasekaran, C., Sundararajan, G., Alaguchamy, N. and Jayakumararaj, R. 2008. Antifeedant activity of leaf aqueous extracts of selected medicinal plants on VI instar larva of *Helicoverpa armigera* (Hübner), *Ethnobot. Leafl.* 12(1): 938-943.

Rani, P. U. and Rajasekharreddy, P. 2009. Toxic and antifeedant activities of *Sterculia foetida* (L.) seed crude extract against *Spodoptera litura* (F.) and *Achaea janata* (L.). *J. Biopestic.* 2(2): 161-164.

Ravaomanarivo, L. H. R., Razafindraleva, H. A., Raharimalala, F. N., Rasoahantaveloniaina, B., Ravelonandro, P. H. and Mavingui, P. 2014. Efficacy of seed extracts of *Annona squamosa* and *Annona muricata* (Annonaceae) for the control of *Aedes albopictus* and *Culex quinquefasciatus* (Culicidae). *Asian Pac. J. Trop. Biomedicine* 4(10): 798-806.

Ray, D. P., Dutta, D., Srivastava, S., Kumar, B. and Saha, S. 2012. Insect growth regulatory activity of *Thevetia nerifolia* Juss. against *Spodoptera litura* (Fab.). *J. Appl. Bot. Food Qual.* 85(1): 212-215.

Reddy, B. K., Balaji, M., Reddy, P. U., Sailaja, G., Vaidyanath, K. and Narasimha, G. 2009. Antifeedant and antimicrobial activity of *Tylophora indica*. *Afr. J. Biochem. Res.* 3(12): 393-397.

Remi-Esan, I. A. and Bankole, O. O. 2020. Insecticidal activity of stem bark extract of *Lophira alata* (Ekki) against cowpea bruchid (*Callosobruchus maculatus*). *Inter. J. Trend in Sci. Res. Dev.* 4(4): 776-781.

Rizvi, S. A. H., Xie, F., Ling, S. and Zeng, X. 2019. Development and evaluation of emulsifiable concentrate formulation containing *Sophora alopecuroides* L. extract for the novel management of Asian citrus psyllid. *Environ. Sci. Pollut. Res.* 26(21): 21871-21881.

Rumape, O. 2015. Isolate bioactivity of castor leaf (*Ricinus communis* L.) as antifeedant on beetle *Epilachna varivestis* Mulsant. *Inter. J. Chem. Tech. Res.* 7(5): 2244-2250.

Sadek, M. M. 2003. Antifeedant and toxic activity of *Adhatoda vasica* leaf extract against *Spodoptera littoralis* (Lep., Noctuidae). *J. Appl. Entomol.* 127(7): 396-404.

Sahayaraj, K., Selvaraj, P. and Raju, G. 2003. Evaluation of biopesticidal property of *Christella parasitica* and *Ipomea cornea* on *Achaea janata*. *Appl. Zoologic. Res.* 14(1): 48-50.

Sampson, B. J., Tabanca, N., Kirimer, N. E., Demirci, B., Baser, K. H. C., Khan, I. A., Spiers, J. M. and Wedge, D. E. 2005. Insecticidal activity of 23 essential oils and their major compounds against adult *Lipaphis pseudobrassicae* (Davis) (Aphididae: Homoptera). *Pest Manag. Sci.* 61(11): 1122-1128.

Samuel, A. J. S. J., Radhamani, S., Gopinath, R., Kalusalingam, A., Gnana K. A. V. and Husain, H. A. 2009. *In vitro* screening of anti–lice activity of *Pongamia pinnata* leaves. *Korean J. Parasitol.* 47(1): 377–380.

Sandoval-Mojica, A.F. and Capinera, J. L. 2011. Antifeedant effect of commercial chemicals and plant extracts against *Schistocerca americana* (Orthoptera: Acrididae) and *Diaprepes abbreviatus* (Coleoptera: Curculionidae). *Pest Manag. Sci.* 67(7): 860-868.

Santos, J., Calero, N., Trujillo-Cayado, L. A., Martín-Piñero, M. J. and Muñoz, J. 2020. Processing and formulation optimization of mandarin essential oil-loaded emulsions developed by microfluidization. *Materials*. 13(16): 1-11.

Saranya, M., R., Mohanraj, S. and Dhanakkod, B. 2013. Larvicidal, pupicidal activities and morphological deformities of *Spathodea campanulata* aqueous leaf extract against the dengue vector *Aedes aegypti*. *Eur. J. Exp. Biol*. 3(1): 205–213.

Saric, A., Kalafati, M., Rusak, G., Kovacevic, G., Franjevic, D. and Gutzeit, H. O. 2007. Postembryonic development of *Drosophila melanogaster* Meigen, 1830 under the influence of quercetin. *Entomol. News* 118(1): 235-240.

Saxena, R. C., Waldbauer, G. P., Liquido, N. J. and Puma, B. C. 1981. Effect of neem oil on the leaf-folder, Cnaphlocrosis medinalis. Proceedings of First International Neem Conference, Rottach-Egern, pp. 189-203.

Scott, I. M., Gagnon, N., Lesage, L., Philogene, B. J. R. and Arnason, J. T. 2005. Efficacy of botanical insecticides from *Piper* species (Piperaceae) extracts for control of European chafer (Coleoptera: Scarabaeidae). *J Econ. Entomol*. 98(3): 845-855.

Selvamuthukumaran, T. and Arivudainambi, S. 2010. Dose dependant differential anti insect activity of lactone glycoside, a potent plant derived molecule. *J. Biopestic.* 3(1): 259.

Shahjahan, M. and Amin, M. R. 2000. Evaluation of some plant extracts against rice weevil *Sitophilus oryzae* L. *J. Asiat. Soc. Bangladesh* 26(1): 213-222.

Sharma, D. and Abrol, D. P. 2005. Contact toxicity of some insecticides to honeybee *Apis mellifera* (L.) and *Apis cerana* (F.). *J. Asia-Pac. Entomol*. 8(1): 113-115.

Sharma, R., Kumari, A., Singh, N. S., Singh, M. K., Dubey, S., Iqbal, N. and Patanjali, P. K. 2019. Development and stability enhancement of neem oil based microemulsion formulation using botanical synergist. *J. Mol. Liquids* 296(1): 112012.

Shi, W., Yan, R. and Huang, L. 2021. Preparation and insecticidal performance of sustained release cinnamon essential oil microemulsion. *J Sci. Food Agric.* 52(4):74-85.

Silva, C. G., Oliveira, J. and da Camara, C. A. 2018. Insecticidal activity of the ethanolic extract from *Croton* species against *Plutella xylostella* L. (Lepidoptera: Plutellidae). *Revista Facultad Nacional de Agronomía Medellín*. 71(2): 8543-8551.

Sisay, B., Tefera, T., Wakgari, M., Ayalew, G. and Mendesil, E. 2019. The efficacy of selected synthetic insecticides and botanicals against fall armyworm, *Spodoptera frugiperda* in maize. *Insects.* 10(2): 45.

Sudrajat, S., Kustiawan, W., Mardji D. and Kusuma I. W. 2018. Chemical composition and termicidal activity of *Scorodocarpus borneensis* Becc. bark extracts. *J. Adv. Sci. Res*. 9(1): 24-30.

Summarwar, S. and Pandey, J. 2015. Antifeedant activity of leaf extracts of *Catharanthus roseus* and *Ocimum sanctum* against fourth instar larvae of *Spodoptera litura*. *Inter. J. Pure Appl. Zool.* 3(3): 259-262.

Sundaran, C. P. and Faizal, M. H. 2018. Laboratory evaluation of cashew nut shell liquid against chilli aphid *Aphis gossypii* Glover (Homoptera: Aphididae). *Entomon* 43(3): 219-222.

Susaimanickam, M., Veeramuthu, A., Savrimuthu, I. and Subramanian, E. 2012. Formulation of a novel phytopesticide PONNEEM and its potentiality to control generalist herbivorous Lepidopteran insect pests, *Spodoptera litura* (Fabricius) and *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae). *Asia Pac. J. Trop. Dis.* 59(3): 720–723.

Suvanthini, S., Mikunthan, G., Thurairatnam, S. and Pakeerathan, K. 2012. Evaluation of *Withania somnifera* extracts on a storage pest Rice weevil, *Sitophilus oryzae*. *Ann. Res. Rev. Biol*. 3(11): 5050-5053.

Syahputra, E. 2013. Insecticidal activities of *Barringtonia sarcostachys* bark extract against cabbage head caterpillar *Crocidolomia pavonana* (F.). *J. Inter. Soc. Southeast Asian Agric. Sci.* 19(2): 8-17.

Talukder, D., Haque, A. M., Zaman, S., Islam, M. S., Islam, M. N. and Khatun, A. 2015. Insecticidal activity of different fractions of petroleum ether extract of *Zingiber cassumunar* rhizome against *Tribolium castaneum*. *Bangladesh J. Sci. Ind. Res.* 50(2): 143-152.

Talukder, F. A. and Howse, P. E. 2000. Isolation of secondary plant compounds from *Aphanamixis polystachya* as feeding deterrents against adult *Tribolium castaneum* (Coleoptera: Tenebrionidae) *J. Plant Dis. Prot.* 25(4): 498-504.

Tang, X. and Hou, T. 2008. Development of chamaejasmin microemulsion and its biological activity against *Aphis craccivora* and *Culex pipiens pallens*. *Flavour Fragrance J.* 23(4): 258-262.

Thomas, T. G., Rao, S. and Lal, S. 2004. Mosquito larvicidal properties of essential oil of an indigenous plant, *Ipomoea carica* Linn. *Jpn. J. Infect. Dis.* 57: 176–177.

Tian, W. J., Zhuang, T. Y., Zhuang, C. X. and Liang, M. F. 2010. A report on the field control efficacy of *Derris hancei* root powder mixture against the red imported fire ant. *J. Environ. Entomol.* 32(3): 415-418.

Tran, D. H., Le, K. P., Tran, H. D. T. and Ueno, T. 2016. Control efficacy of pongam (*Pongamia pinnata* L.) leaf extract against the turnip aphid *Lipaphis pseudobrassicae* (Davis) (Hemiptera: Aphididae). *J. Fac. Agric. Kyushu Univ*. 61 (1): 141-145.

Tridiptasari, A., Leksono, A. S. and Siswanto, D. 2019. Antifeedant effect of *Moringa oleifera* (L.) leaf and seed extract on growth and feeding activity of *Spodoptera litura* (Fab.) (Lepidoptera: Noctuidae). *J Exp. Life Sci.* 9(1): 25-31.

Tukiran. 2013. Bioinsecticide test of crude stem bark extracts of some meliaceous plants against *Spodoptera litura. Global J Biol., Agric. Health Sci.* 2(3): 28-31.

Udo, I. O. 2011. Potentials of *Zanthoxylum xanthoxyloides* (Lam.) for the control of stored product insect pests. *J Stored Products Postharvest Res.* 2(3): 40-44.

Ulrichs, C., Mewis, I., Adhikary, S., Bhattacharyya, A. and Goswami, A. 2008. Antifeedant activity and toxicity of leaf extracts from *Porteresia coarctata* Takeoka and their effects on the physiology of *Spodoptera litura* (F.). *J. Pest Sci*. 81(2): 79-84.

Vasudev, A., Kaur, J., Punj, I., Gill, P. K. and Sohal, S. K. 2015. Evaluation of methanol and acetone bark extracts from *Acacia nilotica* (Linn.) as a source of growth inhibitors against *Bactrocera cucurbitae* (Diptera: Coquillett). *J. Entomol. Zool. Stud*. 3(4): 260-266.

Ventura, M. U. and Ito, M. 2000. Antifeedant activity of *Melia azedarach* (L.) extracts to *Diabrotica speciosa* (Genn.) (Coleoptera: Chrysomelidae Wang *et al.* (2015) reported that methanol extract from stem bark of *Zanthoxylum schinifolium* Siebold & Zucc. exhibited repellency against red flour beetle,

Tribolium castaneum (Herbst) as evident with the FDI value of 41.12 per cent. beetles. *Braz. Archiv. Biol. Technol.* 43(2): 215-219.

Wang, C. F., You, C. X., Yang, K., Guo, S. S., Geng, Z. F., Fan, L., Du, S. S., Deng, Z. W. and Wang, Y.Y. 2015. Antifeedant activities of methanol extracts of four *Zanthoxylum* species and benzophenanthridines from stem bark of *Zanthoxylum schinifolium* against *Tribolium castaneum*. *Ind. Crops Products*. 74(1): 407-411.

Wanna, R. and Kwang-Ngoen, P. 2019. Efficiency of Indian borage essential oil against cowpea bruchids. *Inter. J. Geomate*. 16(56): 129-134.

Wetwitayaklung, P., Limmatvapirat, C., Phaechamud, T. and Keokitichai, S. 2007. Kinetics of acetylcholineesterase inhibition of *Quisqualis indica* Linn. flower extract. *Silpakorn Univ. Sci. Technol. J.* 1(2): 20-28.

Wheeler, D. A. and Isman, M. B. 2001. Antifeedant and toxic activity of *Trichilia americana* extract against the larvae of *Spodoptera litura*. *Entomologia Experimentalis et Applicata*. 98(1): 9-16.

Wiratno, W. 2008. Effectiveness and Safety of Botanical Pesticides Applied in Black Pepper (*Piper Nigrum*) Plantations. PhD (Ag) thesis, Wageningen University and Research, Wageningen, 144p.

Wiwattanapatapee, R., Sae-Yun, A., Petcharat, J., Ovatlarnporn, C. and Itharat, A. 2009. Development and evaluation of granule and emulsifiable concentrate formulations containing *Derris elliptica* extract for crop pest control. *J. Agric. Food Chem*. 57(23): 11234-11241.

Xavier, V. M., Picanco, M. C., Chediak, M., Junior, P. A. S., Ramos, R. S. and Martins, J. C. 2015. Acute toxicity and sublethal effects of botanical insecticides to honey bees. *J. Insect Sci.* 15(1): 137-142.

Yang, W. Q., Shao, X. H., Deng, F., Hu, L. J., Xiong, Y., Huang, X. J., Fan, C. L., Jiang, R. W., Ye, W. C. and Wang, Y. 2020. Unprecedented quassinoids from *Eurycoma longifolia*: biogenetic evidence and antifeedant effects. *J. Nat. Products* 83(5): 1674-1683.

Yogesh, W. H., Deepali, G. M. and Sachin, P. P. 2013. Plant Weeds *Parthenium hysterophorus* and *Ageratina adenophora* for Fall Armyworm Control. *Int. J. Life Sci.* 1(1): 71-75.

Zapata, N., Budia, F., Vinuela, E. and Medina, P. 2009. Antifeedant and growth inhibitory effects of extracts and drimanes of *Drimys winteri* stem bark against *Spodoptera littoralis* (Lep., Noctuidae). *Ind. Crops Products*. 30(1): 119-125.

Zhong, B., Lv, C. and Qin, W. 2017. Effectiveness of the botanical insecticide azadirachtin against *Tirathaba rufivena* (Lepidoptera: Pyralidae). *Fla. Entomol.* 100(2): 215-218.

Zhou, F., Ai-Xiang, G. U., Gui-Sheng, Z., Xue-Hao, C., Xiao-Hua, Q., Nan-Nan, H., Jia, R. and Jiu-Zhi, S. 2012. Effect of castor leaf alcohol extract on *Diaphania indica*. *Chin. J. Eco-Agric*. 20(5): 619-624.

Zuleta-Castro, C., Rios, D., Hoyos, R. and Orozco-Sánchez, F. 2017. First formulation of a botanical active substance extracted from neem cell culture for controlling the armyworm. *Agron. Sustain. Dev.* 37(5): 40.

Abstract

DEVELOPMENT OF FORMULATIONS FROM *Samadera indica* Gaetrn. FOR THE MANAGEMENT OF LEAF FEEDING PESTS IN SNAKE GOURD (*Trichosanthes anguina* L.)

by

REMYA, S.
(2018-21-042)

Abstract of the thesis

Submitted in partial fulfilment of the requirement for the degree of

DOCTOR OF PHILOSOPHY IN AGRICULTURE

Faculty of Agriculture
Kerala Agricultural University

DEPARTMENT OF AGRICULTURAL ENTOMOLOGY
COLLEGE OF AGRICULTURE
VELLAYANI, THIRUVANANTHAPURAM-695522
KERALA, INDIA

2022

ABSTRACT

The study entitled 'Development of formulations from *Samadera indica* Gaetrn. for the management of leaf feeding pests in snake gourd (*Trichosanthes anguina* L.)' was carried out in the Department of Agricultural Entomology, College of Agriculture, Vellayani and at Council of Scientific and Industrial Research – National Institute for Interdisciplinary Science and Technology (CSIR-NIIST), Thiruvananthapuram during 2018 to 2021. Objectives of the study were exploration of anti-insect properties of bark and seeds of *S. indica*, identification of bioactive compounds in the effective extract, development of suitable formulations and field evaluation of the same against pumpkin caterpillar, *Diaphania indica* Saund and epilachna beetle, *Henosepilachna septima* (Dieke).

Estimation of lethal doses *viz.*, LD_{50} and LD_{90} of bark and seed extracts by probit dose analysis showed that LD_{50} of bark extract in hexane, acetone, ethanol extract and aqueous extract were 1.66, 1.03, 1.51 and 2.38% respectively against *D. indica* and 2.17, 1.95, 1.68 and 2.49% respectively against *H. septima*. Meanwhile, LD_{90} values were 6.49, 7.05, 5.24 and 7.90% respectively against *D. indica* and 6.82, 6.60, 6.17 and 7.85% against *H. septima*. In the case of seed extract, LD_{50} values were 1.79, 0.59, 1.03 and 1.54% respectively against *D. indica* and 1.63, 0.83, 1.60 and 2.43% against *H. septima*. LD_{90} values were 6.99, 5.17, 7.05 and 9.17% against *D. indica* and 9.53, 5.27, 7.94 and 9.02% against *H. septima*.

Results of *in vitro* studies on antifeedant effect of *S. indica* bark extracts against *D. indica* showed that ethanol extract @ 5.24% exhibited the highest leaf protection of 62.79 per cent at 72 Hours After Treatment (HAT). In the case of *H. septima*, ethanol extract @ 6.17% resulted in 65.00 per cent leaf protection. Regarding insecticidal effect, ethanol extract @ 5.24% resulted in 93.33 per cent mortality of second instar larvae of *D. indica*. Meanwhile, in grubs of *H. septima*, ethanol extract @ 6.17% inflicted 91.67 per cent mortality. Insecticidal effect of both the extracts was found to be statistically on par with chemical check

(Malathion 50 EC 0.1%). With regard to insect biology, there was increase in larval duration (15.00 days), pupal duration (5.33 days), reduction in pupal weight (0.19 g) and reduction in adult longevity (4.33 days) in *D. indica*, whereas in control, larval and pupal period were 9.67 and 2.67 days each, pupal weight was 0.31 g and longevity of adults was 7.67 days. Similar trend was observed in *H. septima*.

Among various extracts from *S. indica* seeds, acetone extract @ 5.17% resulted in the highest leaf protection of 74.41 per cent in *D. indica* and acetone extract @ 5.27% exhibited 71.79 per cent leaf protection in *H. septima*. With respect to insecticidal effect, acetone extract @ 5.17 and 5.27% each caused 86.67 per cent mortality in *D. indica* and *H. septima* respectively. Considering biology of the insects, there was prolongation in larval period to 17.00 days, pupal period to 8.67 days, reduction in pupal weight to 0.25 g and reduction in adult longevity to 6.33 days in *D. indica* as against larval period of 12.67 days, pupal period of 5.33 days, pupal weight of 0.35 g and adult longevity of 7.67 days in control. Similar was the trend with *H. septima* also. A comparison between LD_{50} and LD_{90} of bark and seed extracts pointed out that seed extract contained more potent compounds with insecticidal properties. Hence, seed extract was taken for further studies.

Chromatographic fractions from acetone extract of seeds @ 5% exhibited mean leaf protection of 91.83 per cent in *D. indica* and 85.71 per cent in *H. septima*. Meanwhile, there was mortality of 73.33 per cent in both the pests. Analysis of chromatographic fractions revealed the presence of quassinoids *viz*., samaderin A, samaderin B, samaderin C and cedronin in the seed extract. Molecular structures were elucidated to confirm the identity of the compounds.

Two formulations of *S. indica* seed extract were prepared by mixing seed extract, emulsifier and distilled water in different proportions. Formulation A contained acetone extract of *S. indica* seed, tween 80 and distilled water in 15: 5:

80 ratio. Formulation B was prepared by mixing seed extract of *S. indica* in acetone, tween 80 and span 80 (1: 1) and distilled water in 15: 5: 80 ratio.

Both the formulations A and B were equally effective against *D. indica* and *H. septima* under *in vitro* conditions, with 80.00 per cent mortality each at 72 HAT. Safety evaluation on beneficial insects indicated that formulation A @ 1% resulted in 30.00 per cent mortality in honey bee after 24 HAT, while in *A. taragamae*, it was 23.33 per cent. Furthermore, both the formulations were stable under normal room temperature for six months. Formulation A @ 1% recorded 71.67 to 80.00 per cent mortality in *D. indica* and *H. septima* from the day of preparation till sixth month of storage. Meanwhile, formulation B @ 1% resulted mean percentage mortality of 70.00 and 75.00 per cent in *D. indica* and *H. septima*. Considering the insecticidal effect under *in vitro* conditions and environmental feasibility, formulation A was chosen for *in vivo* studies.

Field experiment was carried out to test the efficacy of the botanical in comparison with biopesticide and chemical at Instructional Farm, Vellayani in snake gourd (variety Kaumudi). Formulation A @ 2% was evaluated for its efficacy against *D. indica* and *H. septima* at vegetative stage and 50 per cent flowering stage. Observations were taken on pest population and percentage of damaged leaves. It can be deduced that formulation A @ 2% was as effective as neemazal @ 0.2% as evident with reduction in pest population at vegetative and 50 per cent flowering stage of the crop. With regard to reduction in leaf damage, formulation A @ 2% excelled equally as neemazal @ 0.2% at vegetative stage.

The study revealed that both bark and seed extracts of *S. indica* exhibited feeding deterrence, insecticidal properties and caused adverse effects in the biology of *D. indica* and *H. septima.* Anti-insect properties are more prevalent in the seeds and they contained the bioactive compounds samaderins A, B, C and cedronin. Formulations containing acetone extract of *S. indica* seeds, tween 80 and distilled water (15: 5: 80) @ 1 and 2% each were effective against both the pests under *in vitro* and *in vivo* conditions respectively and were stable for a period of six months under room temperature. Hence, it can be concluded that

formulations from *S. indica* can be considered as safer botanical insecticides in this era of organic farming.

സംഗ്രഹം

'പടവലത്തിലെ ഇലതീനിക്കീടങ്ങളെ നിയന്ത്രിക്കുവാനായി കരിങ്ങോട്ടയിൽ നിന്നുള്ള രൂപികകളെ (formulations) വികസിപ്പിക്കൽ' എന്ന തലക്കെട്ടോടുകൂടി 2018 മുതൽ 2021 വരെയുള്ള കാലയളവിൽ വെള്ളായണി കാർഷിക കോളജിലെ കീടശാസ്ത്ര വിഭാഗത്തിലും തിരുവനന്തപുരത്തുള്ള 'കൌൺസിൽ ഓഫ് സയന്റിഫിക് ആൻഡ് ഇന്ഡസ്ട്രിയൽ റിസേർച്' – 'നാഷണൽ ഇൻസ്റ്റിറ്റ്യൂട്ട് ഓഫ് ഇന്റെർഡിസിപ്ളിനറി സയൻസ് ആൻഡ് ടെക്നോളജി'യിലുമായി നടത്തുകയുണ്ടായി. കരിങ്ങോട്ടയുടെ പുറംതൊലിയുടെയും വിത്തിന്റെയും പ്രാണി-വിരുദ്ധ (anti-insect) സവിശേഷതകൾ കണ്ടു പിടിക്കുക, മികച്ച സത്തിലെ സംയുക്തങ്ങളെ കണ്ടു പിടിക്കുക, ഉചിതമായ രൂപികകൾ നിർമിച്ച് മത്തൻ പുഴുവിനും (*ഡയഫാനിയ ഇൻഡിക്ക*) ആമവണ്ടിനും (*ഹീനോസ്എപ്പിലാക്ന സെഫ്രിമ*) എതിരെ പ്രകൃതിയിൽ പരീക്ഷിക്കുക എന്നിവയാണ് ഈ പഠനത്തിന്റെ ലക്ഷ്യങ്ങൾ.

കരിങ്ങോട്ടയിലെ പുറംതൊലിയുടെയും വിത്തിന്റെയും സത്തിൽ മത്തൻ പുഴുക്കൾക്കും ആമവണ്ടിനും 50 ശതമാനം മാരകമായ വീര്യവും 90 ശതമാനം മാരകമായ വീര്യവും പ്രോബിറ്റ് – ഡോസ് വിശകലനം വഴി കണ്ടെത്തി. പുറംതൊലിയുടെ സത്ത് ഹെക്സേൻ, അസെറ്റോൺ, എഥനോൾ, വെള്ളം എന്നിവ ഉപയോഗിച്ച് വേർതിരിച്ചെടുത്തപ്പോൾ 1.66, 1.03, 1.51, 2.38% വീതം സത്ത് മത്തൻ പുഴുവിനു 50 ശതമാനവും 2.17, 1.95, 1.68, 2.49% വീതം ആമവണ്ടിനു 50 ശതമാനവും മാരകമായിട്ടുള്ളതായി കണ്ടെത്തി. വിത്തിന്റെ സത്ത് ഉപയോഗിച്ചപ്പോൾ 1.79, 0.59, 1.03, 1.54% എന്നീ വീര്യത്തിലുള്ളവ മത്തൻ പുഴുവിനും 1.63, 0.83, 1.60, 2.43% എന്നീ വീര്യത്തിലുള്ളവ ആമവണ്ടിനും 50% മാരകമായി കണ്ടെത്തി. മത്തൻ പുഴുവിന് 6.99, 5.17, 7.05, 9.17% എന്നീ വീര്യത്തിലുള്ളവയും ആമവണ്ടിനു 9.53, 5.27, 7.94, 9.02% എന്നീ വീര്യത്തിലുള്ളവയും 90% മാരകമായി കണ്ടെത്തി.

കരിങ്ങോട്ടയുടെ പുറംതൊലിയിൽ നിന്നെടുത്ത സത്ത് കീടങ്ങൾ ആഹാരം കഴിക്കുന്നതിനെ എങ്ങനെ ബാധിക്കുന്നു (antifeedant effect) എന്നറിയുവാനായി ലബോറട്ടറിയിൽ ഒരു പരീക്ഷണം നടത്തി. ഇതിൽ എഥനോൾ ഉപയോഗിച്ച് വേർതിരിച്ച സത്ത് 5.24% ഇലകളിൽ തളിച്ചപ്പോൾ 72 മണിക്കൂറിനു ശേഷം മത്തൻ പുഴുവിൽ നിന്നും മികച്ച സംരക്ഷണം (62.79%) ലഭിക്കുന്നതായി കണ്ടെത്തി. എഥനോളിൽ നിന്ന് വേർതിരിച്ച സത്ത് 6.17% ആമവണ്ടിനെതിരെ 65% സംരക്ഷണം നൽകുന്നതായി കണ്ടെത്തി. എഥനോളിൽ നിന്ന് വേർതിരിച്ചെടുത്ത സത്ത് 5.24% മത്തൻ പുഴുക്കളിൽ തളിച്ചപ്പോൾ മരണനിരക്ക് 93.33 ശതമാനം ആയി കണ്ടെത്തി. ആമവണ്ടിന്റെ കുഞ്ഞുങ്ങളിൽ ഇത് 6.17% വീര്യത്തിൽ തളിച്ചപ്പോൾ 91.67 ശതമാനം ആയിരുന്നു മരണനിരക്ക്. ഈ രണ്ടു സത്തുകളുടെയും

കീടനാശിനി സവിശേഷത പരീക്ഷണത്തിനുപയോഗിച്ച രാസകീടനാശിനിയുമായി (മാലത്തയോണ് 50 EC) തുല്യതയിലാണ് എന്നു കണ്ടെത്തി. പുറംതൊലിയുടെ സത്ത് ഉപയോഗിച്ചപ്പോൾ കീടങ്ങളുടെ ജീവശാസ്ത്രത്തിൽ പ്രകടമായ മാറ്റങ്ങൾ ഉണ്ടായി. സാധാരണ മത്തൻ പുഴുക്കൾ 9.67 ദിവസങ്ങൾ കൊണ്ട് പുഴുദശയും 2.67 ദിവസങ്ങൾ കൊണ്ട് സമാധിദശയും പൂർത്തിയാക്കുമ്പോൾ സത്തിന്റെ പ്രയോഗത്തിന് വിധേയമായ പുഴുക്കൾ പുഴുദശ പൂർത്തിയാക്കാൻ 15 ദിവസങ്ങളും സമാധിദശ പൂർത്തിയാക്കാൻ 5.33 ദിവസങ്ങൾ എടുക്കുന്നതായും കണ്ടു. അതുപോലെ, സത്ത് ഉപയോഗിച്ചപ്പോൾ പുഴുക്കളിൽ സമാധിയുടെ തൂക്കം 0.19 ഗ്രാം ആയി കുറയുന്നതായും പൂർണവളർച്ചയെത്തിയവ 4.33 ദിവസങ്ങൾ മാത്രം നിലനിൽക്കുന്നതായും കണ്ടെത്തി. എന്നാൽ, സാധാരണ പുഴുക്കളിൽ സമാധിക്ക് 0.31 ഗ്രാം തൂക്കം ഉള്ളതായും പൂർണവളർച്ച എത്തിയവക്ക് 7.67 ദിവസം ജീവിതദൈർഘ്യം ഉള്ളതായും കണ്ടെത്തി. പുറംതൊലിയുടെ സത്ത് ഉപയോഗിച്ചപ്പോൾ മത്തൻ വണ്ടിലും ഇതേ മാറ്റങ്ങൾ തന്നെ പ്രകടമായി.

കരിങ്ങോട്ടയുടെ വിത്തിന്റെ അസെറ്റോനിൽ വേർതിരിച്ചെടുത്ത സത്ത് 5.17% വീര്യത്തിൽ ഇലകളിൽ തളിച്ചപ്പോൾ മത്തൻപുഴുവിനെതിരെ 74.41 ശതമാനം സംരക്ഷണവും 5.27% വീര്യത്തിൽ തളിച്ചപ്പോൾ 71.79 ശതമാനം സംരക്ഷണവും കാണുവാൻ സാധിച്ചു. വിത്തിന്റെ അസെറ്റോണിൽ വേർതിരിച്ചെടുത്ത സത്ത് 5.17% വീര്യത്തിൽ മത്തന്പുഴുവിലും 5.27% വീര്യത്തിൽ ആമവണ്ടിലും തളിച്ചപ്പോൾ രണ്ടു കീടങ്ങളിലെയും മരണനിരക്ക് 86.67 ശതമാനം വീതമായിരുന്നു. മാത്രമല്ല, മത്തന്പുഴുവിൽ പുഴുദശ 17 ദിവസവും സമാധിദശ 8.67 ദിവസവും നീണ്ടുനിന്നു. ഇവയിൽ സമാധിയുടെ തൂക്കം 0.25 ഗ്രാം ആയി കുറയുകയും പൂർണവളർച്ചയെത്തിയ കീടം 6.33 ദിവസം മാത്രം ജീവിക്കുന്നതായും കണ്ടെത്തി. സാധാരണ മത്തന്പുഴുക്കളിൽ പുഴുദശ 12.67 ദിവസവും സമാധിദശ 5.33 ദിവസവും ആയിരുന്നു. കൂടാതെ സമാധിയുടെ തൂക്കം 0.35 ഗ്രാമും പൂർണവളർച്ചയെത്തിയവയുടെ ജീവിതകാലം 7.67 ദിവസവും ആയിരുന്നു. പുറംതൊലിയിൽ നിന്നും വിത്തിൽ നിന്നും എടുത്ത സത്തുകൾ തമ്മിൽ താരതമ്യം ചെയ്യുമ്പോൾ വിഷവീര്യത്തിന്റെ അടിസ്ഥാനത്തിൽ കൂടുതൽ മാരകമായത് വിത്തിൽ നിന്നുള്ള സത്ത് ആയിരുന്നു. അതിനാൽത്തന്നെ, തുടർപഠനങ്ങൾക്കായി വിത്തിൽ നിന്നുള്ള സത്തായിരുന്നു ഉപയോഗിച്ചത്.

അസെടോണിൽ നിന്ന് വേർതിരിച്ചെടുത്ത വിത്തിന്റെ സത്ത് 'ക്രോമാടോഗ്രഫി' എന്ന പ്രക്രിയക്ക് വിധേയമാക്കിയ ശേഷം അതിൽ നിന്ന് ലഭിച്ച ഘടകങ്ങൾ 5% വീര്യത്തിൽ ഇലകളിൽ തളിച്ചപ്പോൾ മത്തൻ പുഴുവിനെതിരെ 91.83 ശതമാനവും ആമവണ്ടിനെതിരെ 85.71 ശതമാനവും സംരക്ഷണം ഉള്ളതായി കണ്ടു. അതേസമയം, രണ്ടു കീടങ്ങളിലും മരണനിരക്ക് 73.33 ശതമാനം ആയിരുന്നു. ഈ ഘടകങ്ങളെ വിശകലനം ചെയ്തപ്പോൾ അവയിൽ 'കുക്കുർബിറ്റാസിനോയിഡുകൾ'

സമാഡിറിൻ എ, സമാഡിറിൻ ബി, സമാഡിറിൻ സി, സെട്രോണിൻ) എന്നീ സംയുക്തങ്ങൾ ഉണ്ടെന്നു കണ്ടെത്തി. ഇവയിലെ തന്മാത്രകളുടെ ഘടന നിർണയിക്കുക വഴി ഇവയുടെ സാന്നിധ്യം സ്ഥിരീകരിച്ചു.

കരിങ്ങോട്ടയുടെ വിത്തിൽ നിന്നെടുത്ത സത്ത്, 'എമല്ലിഫയർ', വാറ്റിയ വെള്ളം (distilled water) എന്നിവ വിവിധ അനുപാതങ്ങളിൽ കൂട്ടിച്ചേർത്ത് രണ്ടു രൂപികകൾ (formulations) ഉണ്ടാക്കി. അസെടോണിൽ വേർതിരിച്ചെടുത്ത സത്ത്, 'ടവീൻ' 80, വാറ്റിയ വെള്ളം എന്നിവ 15: 5: 80 അനുപാതത്തിൽ കൂട്ടിക്കലർത്തിയാണ് രൂപിക 'എ' തയാറാക്കിയത്. മേൽപ്പറഞ്ഞ അനുപാതത്തിൽത്തന്നെ അസെടോണിൽ വേർതിരിച്ചെടുത്ത സത്ത്, 1: 1 അനുപാതത്തിൽ 'ടവീൻ' 80 യും 'സ്പാൻ' 80 യും, വാറ്റിയ വെള്ളം എന്നിവ ചേർത്താണ് രൂപിക 'ബി' തയ്യാറാക്കിയത്.

ലബോറട്ടറിയിൽ നടത്തിയ പരീക്ഷണത്തിൽ രണ്ടു രൂപികകളും ഉപയോഗിച്ചു 72 മണിക്കൂറിനു ശേഷം മത്തന്പുഴുവിലും ആമവണ്ടിലും 80 ശതമാനം മരണനിരക്ക് കാണിച്ചു. അതിനാൽത്തന്നെ ഈ രണ്ടു രൂപികകളും രണ്ടു കീടങ്ങൾക്കുമെതിരെ ഒരുപോലെ ഫലപ്രദമാണെന്ന് തെളിഞ്ഞു. രൂപികകൾ മിത്രകീടങ്ങൾക്കെതിരെ സുരക്ഷിതമാണോ എന്നറിയാൻ ലബോറട്ടറിയിൽ നടത്തിയ പരീക്ഷണത്തിൽ രൂപിക 'എ' 1% വീര്യത്തിൽ ഉപയോഗിച്ച് 24 മണിക്കൂറിനു ശേഷം തേനീച്ചയിൽ 30 ശതമാനവും 'അപ്പാന്ടീലസ് താരഗമെ' എന്ന പരാദത്തിൽ 23.33 ശതമാനവും മരണനിരക്ക് കാണിച്ചു. രണ്ടു രൂപികകളും സാധാരണ താപനിലയിൽ ആറു മാസം വരെ സ്ഥിരത നിലനിർത്തുന്നതായി കണ്ടെത്തി. രൂപിക 'എ' 1% വീര്യത്തിൽ ഉപയോഗിച്ചപ്പോൾ മത്തന്പുഴുവിലും ആമവണ്ടിലും ആറുമാസം വരെ 71.67 മുതൽ 80 ശതമാനം വരെയായിരുന്നു മരണനിരക്ക്. അതേസമയം, രൂപിക 'ബി' 1% വീര്യത്തിൽ ഉപയോഗിച്ചപ്പോൾ മത്തന്പുഴുവിൽ 70 ശതമാനവും ആമവണ്ടിൽ 75 ശതമാനവും ആയിരുന്നു മരണനിരക്ക്. രൂപിക 'എ'യുടെ മെച്ചപ്പെട്ട കീടനാശിനി സവിശേഷതയും പാരിസ്ഥിതിക സാധ്യതയും കണക്കിലെടുത്ത് ഈ രൂപികയെ നിലം പരീക്ഷണത്തിനു (field experiment) വിധേയമാക്കി.

കരിങ്ങോട്ടയിൽ നിന്നുള്ള കീടനാശിനിയുടെ കാര്യക്ഷമത ജൈവകീടനാശിനിയും രാസകീടനാശിനിയുമായി താരതമ്യം ചെയ്യുന്നതിനായി വെള്ളായണി ഇന്സ്ട്രക്ഷണൽ ഫാർമിൽ കൌമുദി എന്നയിനം പടവലത്തിൽ ഒരു പരീക്ഷണം നടത്തുകയുണ്ടായി. ചെടി പൂവിടുന്നതിന് മുൻപും 50% പൂവിട്ടതിനു ശേഷവും രൂപിക 'എ' 2% വീര്യത്തിൽ മത്തന്പുഴുവിനും ആമവണ്ടിനുമെതിരായി പരീക്ഷിച്ചു. കീടങ്ങളുടെ എണ്ണം നിരീക്ഷിക്കുകയും കീടങ്ങൾ ആക്രമിച്ച ഇലകളുടെ ശതമാനം കണക്കാക്കുകയും ചെയ്തു. ചെടിയുടെ രണ്ടു വളർച്ചാഘട്ടങ്ങളിലും രൂപിക 'എ' 2% വീര്യത്തിൽ ഉപയോഗിച്ചപ്പോൾ 'നീമസാൽ'

0.2 ശതമാനം തളിക്കുന്നതിനു സമാനമായി കീടങ്ങളുടെ എണ്ണം കുറയുന്നതായി കണ്ടു. അതുപോലെതന്നെ, രണ്ടു കീടനാശിനികളും ഒരുപോലെ ഇലയിലെ കീടാക്രമണം കുറയ്ക്കുന്നതായി കണ്ടെത്തി.

കരിങ്ങോട്ടയുടെ പുറംതൊലിയുടെയും വിത്തിന്റെയും സത്ത് മത്തന്പുഴുവിലും ആമവണ്ടിലും ആഹാരം കഴിക്കുന്നത് തടസപ്പെടുത്തുകയും, കീടനാശിനി സ്വഭാവം കാണിക്കുന്നതായും ഈ കീടങ്ങളുടെ ജീവശാസ്ത്രത്തിൽ മാറ്റം വരുത്തുന്നതായും ഈ പഠനത്തിലൂടെ തെളിയിക്കപ്പെട്ടു. കരിങ്ങോട്ടയുടെ വിത്തിലാണ് പ്രാണി-വിരുദ്ധ സവിശേഷതകൾ കൂടുതലായി കാണപ്പെട്ടത്. കൂടാതെ, ഇവയിൽ സമാഡിറിൻസ് 'എ', 'ബി', 'സി', സെട്രോണിൻ എന്നീ സംയുക്തങ്ങൾ അടങ്ങിയിരിക്കുന്നു. അസെടോണിൽ വേർതിരിച്ചെടുത്ത കരിങ്ങോട്ടയുടെ വിത്തിന്റെ സത്ത്, ടീൻ 80, വാറ്റിയ വെള്ളം (15: 5: 80) 1% വീര്യത്തിൽ ലാബിലും 2% വീര്യത്തിൽ പ്രകൃതിയിലും രണ്ടു കീടങ്ങൾക്കുമെതിരെ ഫലപ്രദമാണെന്ന് കണ്ടെത്തി. കൂടാതെ, സാധാരണ താപനിലയിൽ ആറുമാസത്തോളം സ്ഥിരത നിലനിർത്തുന്നതായും കണ്ടെത്തി. ചുരുക്കത്തിൽ, ജൈവകൃഷിയുടെ ഈ കാലഘട്ടത്തിൽ ചെടിയിൽ നിന്നുള്ള സുരക്ഷിത കീടനാശിനികളായി കരിങ്ങോട്ടയിൽ നിന്നുള്ള രൂപികകളെ പരിഗണിക്കാവുന്നതാണ്.