ANTIFEEDANT AND GROWTH REGULATORY ACTIVITY OF *Sphagneticola trilobata* (L) Pruski ON TOBACCO CATERPILLAR *Spodoptera litura* (Fab) (Lepidoptera: Noctuidae)

by

RAHUL RAJ M

(2019-11-134)

DEPARTMENT OF AGRICULTURAL ENTOMOLOGY

COLLEGE OF AGRICULTURE

VELLANIKKARA, THRISSUR – 680656

KERALA, INDIA

2021

ANTIFEEDANT AND GROWTH REGULATORY ACTIVITY OF *Sphagneticola trilobata* (L) Pruski ON TOBACCO CATERPILLAR, *Spodoptera litura* (Fab) (Lepidoptera: Noctuidae)

by

RAHUL RAJ M

(2019-11-134)

THESIS

Submitted in partial fulfillment of the requirement for the degree of

Master of Science in Agriculture

Faculty of Agriculture

Kerala Agricultural University

DEPARTMENT OF AGRICULTURAL ENTOMOLOGY

COLLEGE OF AGRICULTURE,

VELLANIKKARA, THRISSUR – 680656

KERALA, INDIA

2021

DECLARATION

I hereby declare that the thesis entitled **"Antifeedant and growth regulatory activity of *Sphagneticola trilobata* (L) Pruski on tobacco caterpillar, *Spodoptera litura* (Fab) (Lepidoptera: Noctuidae)"** is a *bona fide* record of research work done by me during the course of research and the thesis has not previously formed the basis for the award of any degree, diploma, fellowship or other similar title, of any other University or Society.

Place: Vellanikkara
Date: 4/02/2022

Rahul Raj M
(2019-11-134)

CERTIFICATE

Certified that the thesis entitled **"Antifeedant and growth regulatory activity of *Sphagneticola trilobata* (L) Pruski on tobacco caterpillar, *Spodoptera litura* (Fab) (Lepidoptera: Noctuidae)"** is a *bona fide* record of research work done independently by **Mr. Rahul Raj M. (2019-11-134)** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to him.

Dr. Mani Chellappan

(Chairman, Advisory Committee)

Professor and Head

Department of Agricultural Entomology

College of Agriculture, KAU

Vellanikkara

Place: Vellanikkara

Date: 4/02/2022

CERTIFICATE

We, the undersigned members of the advisory committee of **Mr. Rahul Raj M. (2019-11-134),** a candidate for the degree of **Master of Science in Agriculture** with major field in **Agricultural Entomology**, agree that this thesis entitled **"Antifeedant and growth regulatory activity of *Sphagneticola trilobata* (L) Pruski on tobacco caterpillar, *Spodoptera litura* (Fab) (Lepidoptera: Noctuidae)"** may be submitted by **Mr. Rahul Raj M.** in partial fulfilment of the requirement for the degree.

Dr. Mani Chellappan
(Chairman, Advisory Committee)
Professor and Head
Department of Agricultural Entomology
College of Agriculture, KAU
Vellanikkara.

Dr. Berin Pathrose
(Member, Advisory Committee)
Assistant Professor
Department of Agricultural Entomology
College of Agriculture, KAU
Vellanikkara.

Dr. Ranjith M T
(Member, Advisory Committee)
Assistant Professor,
AICRP on ANPVPM
College of Agriculture, KAU
Vellanikkara, Thrissur

Dr. Anitha S
(Member, Advisory Committee)
Professor (Agronomy)
Instructional Farm, KAU
Vellanikkara, Thrissur.

ACKNOWLEDGEMENT

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

With great respect and pleasure, I would like to express the deepest gratitude to my major advisor ***Dr. Mani Chellappan*** *Professor and Head Department of Agricultural Entomology College of Agriculture Vellanikkara, for his invaluable support and guidance throughout this venture. I feel fortunate to have had he as my major advisor. I am highly indebted for her patience and co-operation over these years for the completion of this work.*

I also would like to express my gratitude to my advisory committee members am extremely thankful to ***Dr. Berin Pathrose,*** *Assistant Professor, Department of Agricultural Entomology,* ***Dr.Ranjith M T,*** *Assitant Professor ANPVPM College of Agriculture Vellanikkara and* ***Dr. Anitha. S*** *Professor (Agronomy), Instructional Farm, Vellanikkara, KAU, Thrissur for regular support, valuable suggestions and immense help rendered during the study.*

*Whole hearted thanks to my teachers****, Dr. Haseena Bhaskar****, Dr, Madhu Subramanian, Dr. Deepthy K. B., Dr Sreeja P, Dr. Smitha Revi, Dr. Smitha M S.* *and* ***Dr. Neena Lenin*** *for their expert teaching, moral support and co-operation throughout the period of study.*

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

*I also use this opportunity to thank my batchmates**, Abhishek V, Sreesha M, Sikha Raju and Sreelakshmi K B** whose helping hands, love and affection will never fade from my heart. I take this opportunity to extend my gratitude to my seniors **Pavi chettan**, **Sachin chettan Abinsha ikka**, **Sravanthi chechi**, **Laya chechi**, **Salma itha**, **Anna chechi, Anju chechi, Anu chechi, Anusree chechis, Athira chechi, Jancy chechi, Nimisha chechis, Sharanu chettan Vineetha chechi and Vishak etten,** and juniors **Asish V V, Ansila**, **Sreelakshmi**, **Sushuma**, **Tejaswee and Kavya** for their support in different stages of the study.*

*My special thanks to **Sooraj C S, Asish Benny** and **Shri Hari** for helping and supporting me throughout the research work. I wish to extend my thanks to my friends **Suchitra B, Hari Krishnan, Swathy, Vysakh Wilson, Muhammad Anees, Abhin Divakaran, Sanjay Sabu, Gokul Vigneswaran, Akhil Reddy** and all **Anokha 19 boys** for their support and kindness which rendered mental strength to me during the research.*

*I also wish to express my special thanks to **Sibi chettan, Mallika chechi, Nanu chettan, Sanjay Sathyan, Anoop V S, Kasi, Manjunath,** and **Vysakh,** for their companionship and invaluable help throughout these years.*

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

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

RAHUL RAJ M

TABLES OF CONTENTS

LIST OF TABLES

LIST OF PLATES

LIST OF FIGURES

Introduction

1. INTRODUCTION

Synthetic insecticides are being used in crop protection programmes around the world. Abuse of these chemicals resulted in environmental pollution, pest resurgences, pesticide resistance and adverse effects on non-target species in agro-ecosystems. The insecticide-induced resurgence of arthropod pests has long been known to occur due to a decrease in natural enemy population, allowing the pest population to become unmanaged. Excessive pesticide use has the potential to destroy different ecosystems. Also, toxic pesticides threaten the health of many birds, marine plants, and livestock.

As a result, innovative pest control approaches that decrease traditional pesticides are needed today more than ever. Botanical pesticides are safe and effective alternatives to conventional pesticides, and they would help in reducing synthetic insecticide usage. Botanical pesticides have several properties that make them effective against agriculturally important pests, including toxicity, repellency, antifeedancy and insect growth regulatory effects. Chemical interactions between insects and plants are relatively modest. Rather than killing insects directly, most plant defense compounds discourage insect herbivory by inhibiting larval development or discouraging food consumption and oviposition.

Antifeedant compounds are considered to play a significant role in herbivorous insects' food plant selection because insects avoid plants that contain those chemicals (Schoonhoven *et al*., 1998). Secondary metabolites are generated in several ways by terrestrial plants, and some of them are necessary for plant defence against herbivores (Schoonhoven, 1982); these chemical compounds are of natural origin and are safe to the environment (Gebbinck *et al*., 2002).

Sphagneticola trilobata (L.) Pruski, is a herb in the Asteraceae family that naturally grows in coastal regions, barren lands and forests, or as a weed in crops in many countries. This plant is also known as Singapore daisy, Wedelia, trailing or creeping daisy, water zinnia, and rabbit's paw in some countries (Meena *et al*., 2011). Muscle cramps, rheumatism, stubborn burns, swellings, and arthritic swollen joints are all treated with *S. trilobata* in folk medicine (Arvigo and Balik, 1993). Junhirun *et al*.

(2018) reported antifeedant activity of ethyl acetate extract of *S. trilobata* against *Spodoptera litura, Plutella xylostella* and *Spodoptera exigua.* Few extracts and isolated compounds from *S. trilobata*, such as kaurenoic acid and luteolin, have been shown to have anticonvulsant properties (Fucina *et al.*, 2016). Histochemical techniques enabled the identification of constituents of essential oil of *S. trilobata*. It had a high per centage of hydrocarbons, sesquiterpenes, monoterpenes, and oxygenated sesquiterpenes.

Agricultural crops are always under the threat of pest attack. When the ecological and geographical conditions are favourable, the pest population flourishes in a logarithmic manner. *Spodoptera litura* (Fab.) is a serious cosmopolitan polyphagous pest of more than 120 cultivated species of crops. Indiscriminate use of chemical pesticides has caused the development of resistance in this polyphagous pest. A wide host range is a potential evolutionary strategy that favours *S. litura* (Armes *et al.,* 1997). *Spodoptera litura* has been responsible for a 71 per cent reduction in groundnut yields in India's southern states. It also accounts for the reduction of tobacco production by 23–50 per cent.

Keeping all these into account, the present study has been formulated with the following objective

1. To evaluate biological activities of aerial and root extracts of *Sphagneticola trilobata* against *Spodoptera litura*

2. Characterization of constituents of aerial and root extracts of *Sphagneticola trilobata*

Review of Literature

2. REVIEW OF LITERATURE

Botanical pesticides play an important role in insect pest control and crop protection. In the last 20 years, only a few botanical products have made into the markets due to increasingly strict regulatory requirements in many countries. The regulatory environment and public health demands are paving the way for botanicals to be used in industrialized nations for pest management in and around homes and gardens, commercial kitchens and food storage facilities, and on companion animals. Botanicals may also be used in organic food production, both in the field and in the laboratory. Pyrethrum, rotenone, nicotine, sabadilla and quassin were the only botanical pesticides used in the Western Hemisphere until World War II. Because of its high toxicity to fish, rotenones are currently used only on few crops, and natural pyrethrum from chrysanthemum flowers is primarily used as a rapid knockdown agent against crawling and flying insects that affect humans and domestic animals. Currently plants belonging to families of Meliaceae, Rutaceae, Asteraceae, *etc*. have shown promising results in agricultural pest management.

Sphagneticola trilobata (L.) Pruski, is a herbaceous plant belongs to the family, Asteraceae that naturally grows in coastal regions, barren lands and forests, or as weed in crops, in many countries. This plant is also known as Singapore daisy, Wedelia, trailing or creeping daisy, water zinnia, and rabbit's paw in some countries (Meena *et al*., 2011). The tincture of this plant has long been used to treat hematomas and other skin diseases, as well as pain, muscle cramps, rheumatism, chronic headache, fevers and respiratory tract aliments (Maldini *et al*., 2009). *Spodoptera litura,* a widely distributed polyphagous pest, which causes extensive damage to many of the cultivated crops in India. (Armes *et al*., 1997). The management of *S. litura* in an eco-friendly manner is a big task to farmers.

2.1. Origin, distribution and economic importance of *Sphagneticola trilobata*

Sphagneticola trilobata (L.) Pruski (also known as *Wedelia trilobata)* is a creeping perennial herb native to South America. It has now spread throughout the world's tropical and subtropical regions. It has recently been named one of the IUCN's

top 100 invasive species. *S. trilobata* was introduced to Southern China as a groundcover in the 1970s due to its rapid growth and resilience. It quickly escaped cultivation and became invasive.

W. trilobata, a plant with anti-inflammatory, anti-microbial, analgesic, anti-oxidant, hepatoprotective, and anti-diabetic effects, has emerged as a promising source of medication. Flavanoids, triterpenoids, luteolin, arachidonic acid, and other phyto-constituents have been extracted and identified from various parts of *W. trilobata* (Prasanna *et al.,* 2019).

Invasive effect of *S. trilobata* studied from forty plots (5 m×5m) invaded by *this plant* in eight cities across Hainan Island, China. The results showed that when the cover of *S. trilobata* exceeds 10 per cent, there was a significant reduction could be observed in growth and developement of other plant communities in that area (Qi *et al.,* 2014).

Allelopathic effect of *Wedelia trilobata* was studied by Nie *et al*. (2004), and found that the peroxidase activities of germinating rice seeds were decreased by 45.48, 76.13, 69.60, and 65.83 per cent, respectively by aqueous extracts of root, stem, leaf, and entire plant of *W. trilobata*. In addition, the extracts strongly inhibited the activities of the enzyme amylase, increased membrane permeability, and inhibited respiration rate.

Allelopathic influence of residues of *S. trilobata* on the germination and growth of weeds in vegetable fields was studied by Hernández *et al*. (2020). The results demonstrated that residues from *S. trilobata* suppressed weed germination by 31.6 to 72 per cent, depending on the dose administered. Dicotyledonous germination was suppressed by all residual dosages of this species; however, monocotyledons were solely affected by the higher concentrations.

Simarmata *et al.* (2016) evaluated the manure potential of *W. trilobata* in cauliflower field. Mineral fertilizers combined with *W. trilobata* compost increased the fresh weight of shoots and height of cauliflower which were 185.44 g plant $^{-1}$ and 39.4 cm plant^{-1}, respectively.

Ethyl acetate extract of *S. trilobata* was tested for its antioxidant and anticancer properties against MCF-7 breast cancer cells. With an LC_{50} value of 127.43 g/mL, the extract showed DPPH (di phenyl picrylhydrazyl) free radical scavenging activity. The cytotoxic investigation was carried out at concentrations ranging from 1 to 200 g/mL, with the LC_{50} value of 58.143 g/mL indicating the highest inhibitory action against MCF-7 (Mardina *et al*., 2009).

2.2. Chemistry of *Sphagneticola trilobata*

Methanol extract of *Wedelia trilobata* was analyzed through gas chromatography and the major bio active compounds detected were sesquiterpene lactones, trilobolide-6-O-isobutyrates A and B (Huang *et al*., 2006).

The eudesmanolide sesquiterpene skeleton of trilobolide-6-O-isobutyrate ($C_{23}H_{32}O_{9)}$ discovered from the flowers of *W. trilobata* was formed by the fusion of two cyclohexane rings and a lactone ring. Wedelides A and B, two novel sesquiterpene lactones, were recovered from the leaves of *W. trilobata* via bioassay-guided fractionation, together with the known trilobolides 6-O-isobutyrate and 6-O-methacrylate (Nithin *et al*., 2011).

Essential oil extracted from different parts of *S. trilobata* was studied using capillary gas chromatography–flame ionization detector. Pinene (78.6-83.3 %), Phyllandrene (1.3-4.1%), Sabinene (1.4-1.9%), limonene (1.2-1.9%), camphene (0.7-2.0%), Germacrene D (0.1-1.4%), and Amorphene (0.05-1.3%) were the major constituents of the essential oil. There were no significant differences in the oil content of *S. trilobata* gathered in different seasons, except that during the rainy season, α-pinene was somewhat lower concentration, while sabinene, α-cadinene, and 10-nor-calamenen-10-one were present at slightly greater quantity than in other seasons (Verma *et al*., 2014).

Hydro-alcoholic extract of leaves of *Sphagneticola trilobata* were screened for the identification of its essential constituents by using analytical thin-layer chromatography. The leaves were dehydrated in oven at 40°C, and then crushed and macerated in ethanol (70%) for 72h. The extract was filtered before being concentrated

under decreased pressure at 45°C in a rotary evaporator, yielded crude hydro alcoholic extract with a concentration of 140 mg/ml. The presence of phenolic chemicals, anthracene derivatives, mono, sesqui and diterpenes were detected in a phytochemical screening of the crude hydro alcoholic extract. Total phenol and flavanoid content were 21.7 ± 0.009 mg EqGA/g and 0.23 ± 0.005 mg EqC/g, respectively. (Leite *et al*., 2019).

Hepatoprotective, antimicrobial, anti-hemorrhagic and antiepileptic properties have all been documented for wedelolactone, an important bioactive component of *W. trilobata*. Balekar *et al*. (2014) estimated the concentration of wedalolactone from the ethanolic extract of *W. trilobata* using high-performance thin-layer chromatographic method. The retention factor (RF) for standard wedelolactone was 0.56, which was similar to the value obtained from plant extract. The content of wedelolactone present in the extract was found to be 0.084 per cent w/w.

A study was conducted to analyze the chemical composition of flowers of *W. trilobata*. Powdered plant materials were extracted in methanol and the constituents were isolated using chromatography on a silica gel column, and physicochemical constants and spectral analyses were used to identify the components. Grandiflorenic acid, trilobolide-6-O-isobutyrate, 1-Acetoxy-4, 9-dihydroxy-6-isobutyroxy-prostatolid and 16, 17-dihydroxy-ent-kauran-19-oic acid were the five chemicals identified (Meilan *et al.,* 2009).

2.3. Bio activity of *Sphagneticola trilobata* and other members of family Asteraceae

Junhirun *et al*. (2018) evaluated the contact toxicity and antifeedant activity different extracts of *Sphagneticola trilobata* against third instar larvae of three lepidopteran pests, *Spodoptera litura* (Noctuidae), *Plutella xylostella* (Yponomeutidae) and *Spodoptera exigua* (Noctuidae). Four solvents hexane, dichloromethane, ethyl acetate and methanol were used for extraction of plant materials. Ethyl acetate extract was found to be a good feeding deterrent as well as contact toxicant against all the three species of lepidopteran larvae evaluated. It had shown a FI_{50} of 0.27–2.34 mg/ml and LD_{50} of 0.88–4.2 µg/larvae at a concentration of 0 to 20mg per ml. Hexane extract was the least toxic with LD_{50} of 35.94 µg/larvae against *S. litura* larvae. They also evaluated the impact of de- toxifying enzymes in each larva, In *S. litura* and *P. xylostella,* the ethyl

acetate extract inhibited carboxyl esterase activity, while in *S. exigua*, the enzyme was induced.

The antifeedant activity of *Wedelia chinensis* was tested against rice leaf folder, *Cnaphalocrosis medinalis.* Extracts of aerial, root and flowers of *W. chinensis* were made in methanol and tested against third instar larvae of rice leaf folder by leaf dipping method. Antifeedant rates of different extracts of aerial, root and flowers after 24h of treatment were 80, 60 and 100 per cent respectively at 10 per cent concentration. On all instar larvae, the toxic effects of extracts from stems and roots were not strong, but the toxic effects of extracts from flowers on younger larvae were higher (Qinglong *et al.,* 2012).

Caiyun *et al*. (2006) tested the antifeedant activity of methanol extract different parts of *W. chinensis* against *Ostrinia furnacalis.* Among different extract, methanol extract of flowers of *Wedelia chinensis* showed maximum antifeefdant activity against *Ostrinia furnacalis.*The values of AFC_{50} (medium antifeeding concentration) at 24h on the 3rd instar larvae of *Ostrinia furnacalis* was 3408.31µg/ml.

Methylene chloride extracts of stem of *Wedelia biflora* were shown to have antifeedant property against cotton boll weevil *Anthonomus grandis*. Five compounds *viz*., stigma sterol, Grandifloric acid, kauradienoic acid, 2,4-ethyl coprastanone and stigma-7, 3- difloric acid. These compounds also exhibited antifungal activity against *Pythium ultimum* and *Rhizoctonia solani*. Kauradienoic acid exhibited maximum antifeedant activity against cotton boll weevil (Miles *et al.,* 1990).

Methanol extract from *Wedelia chinensis* was tested for its repellent and poisoning properties against *Aphis medicaginis*. The extract had a strong bioactivity against *A. medicaginis*. According to the results of laboratory trials, in selected and non-selected tests, repelling rates against *A. medicaginis* were 90.1 and 65.3 per cent, respectively. Spraying *A. medicaginis* with 2.5g/L of methanolic extract of *W. chinensis* resulted in 93.7 per cent death on the fifth day. In the first, third, and fifth days, the LC_{50} were 1.4 g/L, 1.2 g/L, and 0.9 g/L, respectively (Yanping *et al*., 2003).

A study was conducted to test the repellent effect of volatiles of twenty non host plants against females of brown plant hopper *Nilpartvata lugens*. Volatiles from *Eucalyptus exsetrta*, *Lantana camara*, *Ageratum conezoides*, *Wedelia chinensis*, *Kaya senagalensis, et*c., were tested using a Y-tube olfacto meter. Volatiles of *W. chinensis*, *K. senagalensis* and *E. exsetrta* showed significant repellent activity of 87.5, 83.3 and 72 per cent respectively against females of *N. lugens* (Zhang *et al*., 2014).

Wedelia trilobata and *Mellisa officianalis* essential oil extracts were tested for bioactivity against the stored grain pest *Tribolium casataneum*. The effect of these oils on larval mortality, total protein, aspartate amino transferase (AST), alanine amino transferase (ALT), and alpha amylase activity were investigated in last instar larvae of *Tribolium castaneum*. The LC_{50} value of *W. trilobata* essential oil was 6.2 per cent. Both extracts increased the total protein content and decreased Asparatate amino transferase (AST), alanine amino transferase (ALT) and alpha amylase activity. α-pinnen, α-phyllandrene and lemonene were the main compounds identified from essential oil of *W. trilobata* using GC/MS analysis (Khater *et al.,* 2015).

Asif *et al*., 2014 evaluated the potential of leaf extract of *W. chinensis* in defective hatching and mortality in root knot nematode *Meloidgyne incognitia*. Aqueous extract of *W. chinensis* showed deleterious effect on the larval hatching of *M. incognita* after 5 days. The hatching gradually decreased with increasing concentration of the extract. Complete mortality was observed at 1 per cent concentration.

Wang *et al*. (2009) investigated the antifeedant activity of methanol extract of *Wedelia chinensis* against third instar larvae of *Spodoptera litura*. No-choice method of bioassay was carried out using different dilutions of aerial part extract of *W. chinensis*. Antifeedant activity was gradually increased from lower concentrations to higher concentrations and the, highest antifeedant activity of 90 per cent was observed at 10 per cent concentration of extract.

Insecticidal activities of French marigold *Tagetus patula* was evaluated against two pests, *Lygus hesperus* (Hemiptera; Miridae) and *Bemisia tabaci* by diet incorporation method. Different concentrations aqueous and methanol extract of aerial parts of *T. patula* were mixed with the corresponding diets of both insects and results

were recorded. Both methanol and aqueous extract recorded the significant insecticidal activity against both insects at a concentration ranging from 0 to 1 per cent. Compared to aqueous extract, methanol extract showed higher activity. A significant reduction in oviposition of each insect was observed for methanol and aqueous extracts (Fabrick *et al.,* 2020).

Methanol and hexane extract of *Ageratum conizoides* (Asteraceae) were evaluated against diamondback moth, *Plutella xylostella* by choice and no choice bioassays. In the choice method, more than half per centage inhibition of feeding of leaf disc was observed at 0.5 per cent concentration of extract; however, a total suppression of feeding was observed at 2 per cent and higher concentration. In no-choice bioassays, larval feeding continuously reduced with increase in concentration of extract. An antifeedant index (AFI) of 100 was observed at 2 per cent concentration of hexane extract. While AFI values for methanol and aqueous extracts at 5 per cent concentration were 78.4 and 41.5, respectively (Vats *et al*., 2019).

Insect growth regulatory activity of methanol extract of *Parthenium argentatum a*nd two isolated compounds Argentatin A and Argentatin B were evaluated against third instar larvae of *Spodoptera frugiperda*. No-choice diet feeding bioassay was done with Argentatin A, Aregentatin B and methanol extract showed a significant bioactivity with GC_{50} of 17.8, 39 and 6.4 at 50 ppm, respectively. The developmental time of surviving larvae were increased in a concentration dependent manner with a relative growth index of 0.4, 0.6 and 0.26 at 25, 25 and 5 ppm respectively. Significant delay in pupation and adult emergence was also observed in treated larvae (Cespedes *et al.,* 2001).

Toxicity of essential oil of *Wedelia prostrata* was tested against fourth instar larvae of *Spodoptera litura*. LC_{50} and LC_{90} values of 167.46 and 322.12 µg/ml were obtained after 24h of exposure. Main constituents of essential oil were detected using GC-MS technique. Monoterpene, camphene (9.6%) and the sesquiterpenes γ-elemene (7.6%), α-humulene (6.9%), and (*E, E*)-α-farnesene (7.3 %) were the active compounds present in the essential oil. Bioactivity of pure compounds were also tested against *S. litura*; camphene showed the lowest LC_{50} of 6.28 µg/ml, followed by γ-elemene,

(LC_{50} = 10.64 µg/ml), α-humulene (LC_{50} = 12.89 µg/ml), and (*E, E*)-α-farnesene (LC_{50} = 16.77 µg/ml) (Benelli *et al*., 2018).

Three Asteraceae plants *Mantis alcaduriaei*, *Rhaponticum acaule* and *Scorzonera undulate* were extracted in different organic solvents. All the extracts were tested for contact toxicity, growth inhibition and antifeedant activity against larvae and adults and of *Tribolium confusum*. Multiple choice bioassays were used to assess different growth regulatory activities. 1 per cent concentration of various extracts were incorporated into wheat discs and bio assays were carried out. *M. alcaduriaei* extracted in methanol and ethyl acetate, inhibited the growth of *T. confusum* larvae. Methanolic extracts of *M. alcaduriaei* and *R. acaule*, petroleum ether and chloroform extract of *R. acaule*, and ethyl acetate extracts of *M. alcaduriaei* and *Scorzonera undulata* showed good antifeedant properties. Petroleum ether and methanol extract of *M. alcaduriaei* had the highest death rate of 83 and 78 per cent, respectively (Bousadda *et al*., 2008).

Methanol, hexane, chloroform, ethyl acetate and aqueous extracts of flowers of *Tithonia diversifolia* (Asteraceae) were tested against *Corcyra cephalonica* and evaluated the mortality of different stages such as egg, larva and adult. Different concentrations ranging from 0 to 2 per cent were made in respective solvents. *C. cephalonica* eggs were placed in boxes containing treated rice. Methanol extract of flowers exhibited 97.5 ±1.44 per cent mortality of eggs at 2 per cent concentration of the extract. At the lowest dosage of 0.4 per cent, ethyl acetate and hexane extracts were toxic to the eggs, with mortality rates of 46.25 and 45.0 per cent respectively. At the maximum concentration of the methanol extract exhibited the highest larval mortality with an LC_{50} of 0.594 per cent followed by ethyl acetate and aqueous extracts. All the extracts suppressed the emergence of adults at the highest dose of treatment (Roopa *et al.,* 2021).

Essential oil and powder from aerial parts of *W. trilobata* were tested against maize weevil, *Sitophilus zeamais* for their bioactivities. Contact toxicity of dry powder was tested by mixing grains with powder of concentration from 0 to 80g per kg of grains. An LC_{50} of 582.86 g/kg grain was recorded for 72h of treatment. *W. trilobata*

essential oil exhibited an LC_{50} of 1,146.19 µl/l after 24h of treatment. Powder with dose of 80g per kg of grains killed 7.50 per cent of weevils after 12 days of exposure; it also suppressed the progeny of *S. zeamais.* Fumigation with essential oil at doses of 150, 200 and 250 µl/l air exhibited 2.50, 5.00 and 12.50 per cent of adult mortalities after 24 h of exposure (Wanna *et al.,* 2021).

2.4. Bio activity of plant extracts against *Spodoptera litura*

Hexane extract of *Inula racemosa* (Asteraceae) was used to test its bioactivity against third instar larvae of *S. litura* (Fabricius). The extract had both the larvicidal and growth inhibitory effects. A moderate antifeedant effect was recorded at 1500 ppm of the extract, with a maximum feeding deterrence of 24.85 per cent. A food supplemented with 1500–2000 ppm of the extract caused significantly higher mortality in *S. litura* larvae. In comparison to control, the highest concentration (2500 ppm) extended the development of *S. litura* by 21.06 days. At doses of 1500–2500 ppm, adult emergence was severely reduced due to toxic effects of *I. racemosa*. *S. litura* larvae and pupae showed morphological abnormalities as a result of the extract's sub lethal effects (Kaur *et al.,* 2019).

Methanol extract of *Thevetia nerifolia* leaf extract was tested against *Spodoptera litura* for its insect growth regulatory activity. Different concentrations of leaf extract were made using serial dilution method and applied directly to third instar larvae of *S. litura* using potters tower, after application larvae were reared in artificial diet. At a dose of 2.5 per cent, methanol extract of leaves resulted in 53.8 per cent larval death, 29.6 per cent pupation, and 22.3 per cent adult emergence. In order to find a superior IGR activity, the extract was sub fractionated with different polar solvents, and the chloroform extract was shown to be the most active in terms of larval mortality (27.5-61.5%), pupation (28.4-60.2%), and adult emergence (28.4-60.2%). The extract's GI_{50} was found to be 3.02 per cent. The glycosides contained in the extract were shown to be active (Ray *et al.,*2013).

Aerial parts of *Synedrella nodiflora* were extracted in chloroform, petroleum ether, benzene, water and methanol and tested against fourth instar larvae of *S. litura* to determine its insecticidal activity. Fresh castor leaves dipped in different concentrations

of extract (0.01 to 0.08%) were given to fourth instar larvae of *S. litura*. Among all the five extract, methanol extract was the most toxic with an LD_{50} value of 0.003 per cent and water extract was the least toxic with and LD_{50} of 0.061 per cent. Benzene, chloroform and petroleum ether showed LD_{50} value of 0.018, 0.028 and 0.030 per cent respectively. Phyto chemical analysis of methanol extract revealed the presence of steroids, reducing sugars, phenolic compounds, saponins and tannins (Rathi *et al*., 2005).

Aerial parts of *Conyza dioscoridis* were extracted in chloroform and its insecticidal activity was tested against the fourth instar larvae of *S. litura* by diet incorporation method. The cumulative mortality rate during the pupal and adult stages were 76.6 and 83.3 per cent respectively. Five main components were identified by column chromatography of bioactive chloroform extract including three triterpenes: amyrin-3-acetate, lupeol-3-acetate, amyrenone, dotriacontane hydrocarbon, and 5,4-dihydroxy-6,7-dimethoxyflavone. At concentrations of 0.3, 0.5, and 0.5 per cent, amyrenone, lupeol acetate, and 5, 4-dihydroxy-6, 7-dimethoxyflavone inhibited 50, 60, and 73.3 per cent of *S. littoralis* 4^{th} instar larvae respectively. (Matloub *et al.,* 2021).

Chloroform, hexane and ethyl acetate extracts of leaves of *Hygrophila auriculata* and *Blumea mollis* were tested against *S. litura* to evaluate its growth inhibitory activities. Different concentration ranging from 0 to 5 per cent were made in carrier solvent acetone. Fresh castor leaves dipped in each concentration were given to *S. litura* larvae for 24h and transferred them to boxes containing fresh un-treated castor leaves. Observations on larval mortality, pupal mortality and duration larval and pupal period were made. Among the different extracts, ethyl acetate extract of *H. auriculata* and *B. mollis* exhibited the highest pupal mortality of 70 and 16 per cent respectively. The larval duration was extended to 14.08 days, when compared 8.02 days in control in ethyl acetate extract of *H. auriculata*. While comparing the activity of both the extracts, *H. auriculata* had recorded the longer pupal duration in all doses wheras the duration of the pupae was dose dependent in *B. mollis* (Baskar *et al*., 2011).

Materials and Methods

3. MATERIALS AND METHODS

The study on the "Antifeedant and growth regulatory activity of *Sphagneticola trilobata* (L) Pruski on *Spodoptera litura* (Fab))" was carried out in the Department of Agricultural Entomology, College of Agriculture, Vellanikkara, Thrissur, Kerala from 2020 to 2021. The facilities at Pesticide Residue Testing Laboratory were utilized for this purpose. Antifeedant and growth regulatory activity of different extracts of *Sphagneticola trilobata* were tested against *S. litura*. Chemical characterization of active fractions was also done. The materials used and methods followed in the laboratory are presented in this chapter.

3.1. Mass rearing of host insect, *Spodoptera litura*

Egg masses of *S. litura* were collected from Banana Research Station, Kannara, Thrissur. After hatching, neonates were transferred to plastic boxes (25cm long, 15cm wide, and 7cm high). In a single box, twenty larvae were kept. Fresh castor leaves were provided for feeding, and the boxes were cleaned on a daily basis. When the larvae entered the non-feeding stage after gut purging, they were shifted to clean boxes (25cm long, 15cm wide, and 7cm high) for pupation. After pupation, pupae were transferred to large boxes. When the adults emerged, they were placed in oviposition cages for oviposition. Honey and vitamin E capsule mix was given to the adults to increase fertility. Fresh castor leaves with their petiole covered with moist cotton were provided for oviposition inside the cages.

3.2. Preparation of *Sphagneticola trilobata* extracts

Aerial and root extract are prepared as per Fig. 1

Dried, powdered *S. trilobata* plant materials (100g) were steeped in (300ml) hexane and mixed properly by placing in a rotary shaker. After 24h, the mixture was filtered through a Whatman® No.42 filter paper and concentrated in *vacuo* in a rotary evaporator at low temperature. This process was performed thrice to get crude hexane extract. The plant materials, after extraction with hexane, were subjected to re extraction with methanol (300ml). The same procedure of extraction was followed similar to hexane extract. After the removal of solvents by rotary evaporator, crude methanol

extract was obtained. Separate extraction was conducted for both aerial and root parts of *S. trilobata.*

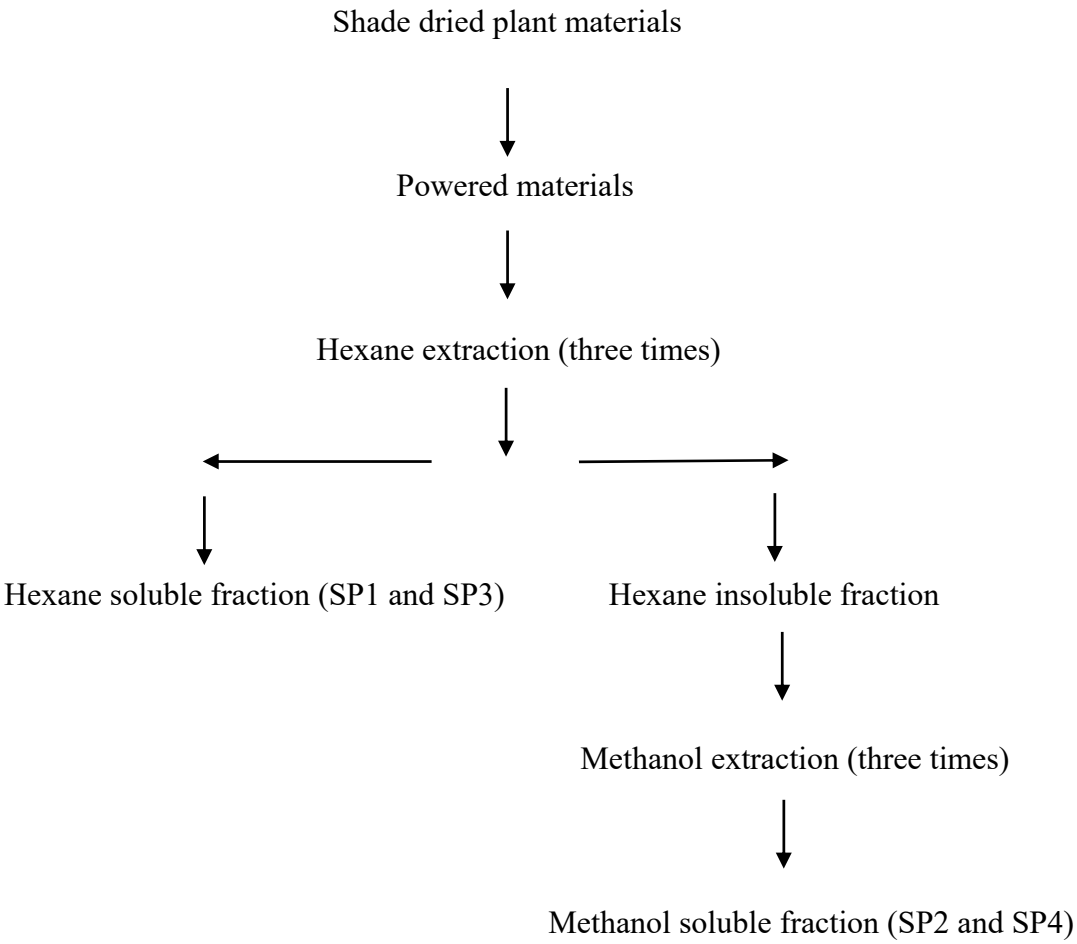


Fig 1. Extraction of aerial and root extracts from *Sphagneticola trilobata*

Plate 1a: Egg mass of *Spodoptera litura*

Plate 1b: Larvae of *Spodoptera litura*

Plate 1c: Pupae of *Spodoptera litura*

Plate 1d*:* Adult of *Spodoptera litura*

Plate 1. Life stages of *Spodoptera litura*

Plate 2a. *Sphagneticola trilobata*

Plate 2b. Powdered materials

Plate 2. *Sphagneticola trilobata* plant materials

Plate 3a. Plant parts extracted in hexane

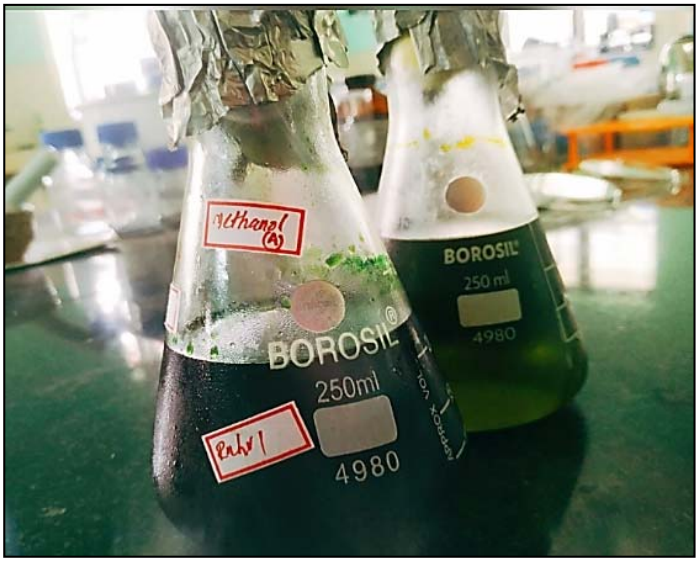


Plate 3b. Plant parts extracted in methanol

Plate 3. Extraction of *Sphagneticola trilobata* plant materials

Plate 4a. SP1 extract

Plate 4b. SP3 extract

Plate 4c. SP2 extract

Plate 4d. SP4 extract

Plate 4. Extracts obtained from S*phagneticola trilobata*

3.3. Bioassay of extracts

Aerial and root extracts of *S. trilobata* were evaluated for their antifeedant and growth regulatory activity against *S. litura.*

3.3.1. Antifeedant activity

The antifeedant activity of aerial and root extracts was evaluated by choice and no choice bioassay against 7-day old larvae of *S. litura*. Observations on leaf area consumption were made after 24 and 48h of treatment. Stock solutions of extracts were prepared in carrier solvent acetone. Different concentrations were made from stock solution by serial dilution technique.

3.3.1.1. No-choice method

Castor leaf discs with a diameter of 4 cm were punched out from washed and air-dried castor leaves. Different concentrations of aerial and root extracts (0.005, 0.01, 0.03, 0.05 and 0.1%) were made in carrier solvent. The punched-out castor leaf discs were thoroughly dipped in each concentration and air-dried for one hour. Glass Petri plates of 9cm diameter were used for the experiment. Single treated leaf disc was placed at the centre of Petri plate on which single pre-starved 7-day old larva of *S. litura* was released. Leaf disc treated with acetone was kept as control. Twelve replications of each treatment were made. The leaf area consumed after 24h of treatment was measured by using a mobile application (Easy Leaf Area Free). Another set of experiment was kept, and leaf area was measured after 48h of feeding.

3.3.1.2. Choice method

The procedure was similar to no-choice method of bio assay. In addition, an untreated leaf disc was placed along with the treated leaf disc in the Petri plate. Single pre-starved 7-day old third instar larva of *S. litura* was released at the centre of the two leaf discs. The leaf area consumed after 24 and 48h of treatment was measured using the mobile application (Easy Leaf Area Free).

3.3.2. Insect growth regulatory activity (IGR)

Insect growth regulatory activity was tested using diet incorporation method. Artificial diet (kidney bean based) was made according to the procedure given by Mani and Rao (1998).

Table 1. Composition and preparation of artificial diet of *Spodoptera litura*

Ingredient	Quantity
Kidney bean	65g
Wheat bran	65g
Yeast extract powder	25g
Agar powder	12g
Caesin	3.0g
Ascorbic acid	4.0g
Sorbic acid	0.9g
Methyl para hydroxy benzoate	0.4g
Cholesterol	0.3g
Streptomycin sulphate	0.1g
Multi vitamin	1No
Formaldehyde	2.0 ml
Distilled water	600 ml

Different concentrations of plant extracts were mixed into the artificial diet, according to Akhtar and Isman (2004). Diet containing different concentrations of aerial and root extract was prepared as follows. For a diet containing 0.01 per cent concentration of extract, 25.856mg of extract was dissolved in 5ml acetone and pipetted to the dry portion of the diet in a Petri plate. After the evaporation of acetone, it was mixed with 140 ml of double distilled water in a blender. Agar powder (3.4g) was dissolved in 60ml double distilled water and boiled. After boiling, agar was added to the diet and mixed well in a blender. Diet was poured to Petri plates and allowed to cool in a refrigerator.

Six prestarved (3-4h.) larvae of *S. litura* were introduced into plastic boxes containing treated diet or control. Four replicates were kept for each concentration. Observations were made on larval weight after five days, larval duration, pupal weight, pupal duration, abnormal pupa, larval-pupal and pupal-adult intermediates and adult emergence.

3.3.3. Statistical analysis of data

3.3.3.1. No-choice method

Per cent feeding and per cent antifeedancy was calculated as per Singh and Pant (1980).

$$\text{Per cent feeding} = \frac{\text{Initial area given for feeding - Area left after feeding}}{\text{Initial area given}} \times 100$$

$$\text{Per cent antifeedancy} = \frac{\text{\% protection in treatment - \% protection in control}}{\text{100 - \% protection in control}}$$

3.3.3.2. Choice method

Per cent feeding deterrence was calculated as per Isman *et al.*, (1990)

$$\text{Feeding deterrency (\%)} = \frac{C - T}{C + T} \times 100$$

C = area consumed in control

T = area consumed in treatment

3.3.3.3. Insect growth regulatory activity (IGR)

Per cent reduction in larval/pupal weight =

$$\frac{\text{Weight gain in (C)} - \text{Weight gain in (T)}}{\text{Weight gain in (C)}} \times 100$$

All the data were subjected to ANOVA, after angular transformation (wherever necessary), in completely randomized design (CRD); the means were separated out by performing Tukey's test at five per cent level of significance. The analysis was carried out in GRAPES software developed by Kerala Agricultural University.

3.3.4 GC-MS/MS and LC-MS/MS analysis of active fractions

All the four extracts (SP1, SP2, SP3 and SP4) were sent to Sophisticated Analytical Instrumentation Facility (SAIF), IIT, Mumbai for GC-MS/MS and LC-MS/MS analysis. GC-MS/MS analysis was done for hexane extracts, and LC-MS/MS was done for methanol extracts. Major compounds present in the extracts were recorded.

Results

4. RESULTS

The results of the study on "Antifeedant and growth regulatory activity of *Sphagneticola trilobata* (L) Pruski on *Spodoptera litura* (Fab)" conducted at the Department of Agricultural Entomology, College of Agriculture, Vellanikkara are presented in this chapter.

4.1. Yield of different extracts of *Sphagneticola trilobata*

All the extracts obtained from different parts of *S. trilobata* were dark green viscous and semisolids. The yield of methanol and hexane extract of aerial plant parts was higher as compared to extracts of plant roots. (Table 2).

Table 2. Yield of different solvent extracts of *Sphagneticola trilobata*

No	Solvent extracts	Yield (w/w)
1	Hexane extract of aerial parts (SP1)	1.96(%)
2	Methanol extract of aerial parts (SP2)	5.67(%)
3	Hexane extract of roots (SP3)	1.23(%)
4	Methanol extract of roots (SP4)	2.32(%)

4.2. Antifeedant activity of extracts from *Sphagneticola trilobata* against *Spodoptera litura* by no-choice method

4.2.1. Antifeedant activity of SP1 against *Spodoptera litura* by no-choice method

Antifeedant activity of 9.46 per cent was obtained at the lowest concentration of 0.005 per cent of SP1 after 24h of exposure (Table 3). Antifeedancy increased to 19.13 and 39.67 per cent at 0.05 and 0.1 per cent concentrations, respectively. Antifeedancy at 0.1 and 0.05 per cent were significantly different from each other, but for all other

concentrations, significant difference was not recorded. A similar trend of increasing antifeedancy with increasing extract concentration was also observed after 48h of exposure. When the observation period was doubled, the antifeedant activity of 4.97 per cent was recorded at the lowest dose of treatment, which was less than 24h exposure for the same concentration. Activity increased to 35.83 per cent at the highest dose of 0.1 per cent. Antifeedant activity at 0.05 and 0.1 per cent were significantly different from other lower concentrations. The activity after 24 and 48h of exposure was not significantly different.

4.2.2. Antifeedant activity of SP2 against *Spodoptera litura* by no-choice method

Antifeedant activity of SP2, when evaluated against third instar larvae *of S. litura*, highest antifeedant activity of 52.96 per cent was obtained at a concentration of 0.1 per cent after 24h of exposure (Table 4). At the lowest treatment dose, antifeedant activity of 5.19 per cent was obtained. Antifeedancy at 0.01 and 0.03 per cent were not statistically different, but for all other concentrations, antifeedant activity was significantly different from each other. Antifeedant activity at 0.005, 0.01, 0.03, 0.05 and 0.1 per cent were 5.16, 16.06, 21.64, 31.23 and 52.96 per cent, respectively. Activity increased in a dose-dependent manner for 24h experiment. Similar results were obtained after 48h of exposure. As the duration was doubled, a slight decrease in antifeedant activity was recorded for 0.005 per cent concentration, but for all other concentrations, activity was highest in 24h experiment. The activity was significantly different from each concentration except for 0.03 per cent. Antifeedant activity at 0.005, 0.01, 0.03, 0.05 and 0.1 per cent concentration was 6.76, 15.12, 21.05, 27.28 and 51.9 per cent, respectively, after 48h of feeding.

4.2.3. Antifeedant activity of SP3 against *Spodoptera litura* by no-choice method

The antifeedant activity of hexane extracts of roots (SP3) was less compared to aerial extract in the same solvent. Low antifeedancy of 3.21 and 3.16 per cent was obtained at 0.005 and 0.01 per cent concentrations after 24h of treatment, respectively (Table 5). At the highest dose of 0.1 per cent, a maximum antifeedant activity of 33.49 per cent was recorded after 24h of treatment. Antifeedant activity at 0.03, 0.05 and 0.1 per cent

concentrations was significantly different from each other. At lowest doses of 0.005 and 0.01 per cent, no significant difference in antifeedancy was recorded. Whereas after 48h of feeding, a slight increase in antifeedancy was recorded for 0.01 and 0.03 per cent concentrations. At the lowest dose of 0.005 per cent, antifeedant activity of 4.53 per cent was recorded. A gradual increase in antifeedant activity was recorded for 48h of the experiment. The highest activity of 30.67 per cent was obtained for 0.1 per cent of the extract, which was significantly different from all other concentrations. Antifeedancy at 0.05 and 0.03 per cent of the extract were not significantly different for 48h of feeding.

4.2.4. Antifeedant activity of SP4 against *Spodoptera litura* by no-choice method

Leaf disc treated with SP4 showed reduced antifeedant activity as compared to aerial extract of the same solvent. But at the lowest dose of treatment, antifeedant activity of 6.69 per cent was obtained for SP4, which was slightly higher than the activity obtained for aerial parts in same solvent (Table 6). At the highest dose of 0.1 per cent, a maximum antifeedant activity of 36.10 per cent was recorded, which was significantly different from all other concentrations. Antifeedancy at 0.03 and 0.05 per cent of SP4 extract was not significantly different from each other. The antifeedancy obtained at 0.03 and 0.05 per cent were 19.88 and 24.96 per cent, respectively. A concentration dependent increase in antifeedancy was observed after 48h of treatment, at the lowest concentration, an antifeedant activity of 7.96 per cent was observed after 48h of treatment. Antifeedancy attained to 26.18 and 33.76 per cent at 0.05 and 0.1 per cent concentrations, respectively. Antifeedant activity at 0.03, 0.05 and 0.1 per cent were significantly different, but at lowest doses of 0.005 and 0.01 per cent, no significant difference was recorded.

Table 3. Antifeedant activity of SP1 against *Spodoptera litura* by no-choice method

Concentration of extract (%)	Per cent feeding (24h)	Antifeedancy (%)	Per cent feeding (48h)	Antifeedancy (%)
0.005	82.42^{ab} (65.21)	9.462^{b} (17.91)	86.75^{ab} (68.65)	4.974^{c} (12.88)
0.01	82.98^{ab} (65.63)	10.00^{b} (18.43)	80.29^{bc} (63.64)	10.80^{bc} (19.18)
0.03	73.37^{bc} (58.93)	17.25^{b} (24.54)	79.15^{c} (62.83)	11.37^{b} (19.70)
0.05	71.41^{c} (57.67)	19.13^{b} (25.93)	66.55^{d} (54.66)	24.17^{a} (29.44)
0.1	51.55^{d} (45.88)	39.67^{a} (39.03)	57.62^{e} (49.38)	35.83^{a} (36.76)
Control	86.56^{a} (68.49)		90.74^{a} (72.28)	

Figures in the parenthesis are angular transformed. Figure in any column followed by same letter are not significantly different at $p < 0.05$ by Tukey's test

Table 4. Antifeedant activity of SP2 against *Spodoptera litura* by no-choice method

Concentration of extract (%)	Per cent Feeding (24h)	Antifeedancy (%)	Per cent Feeding (48h)	Antifeedancy (%)
0.005	79.63[b] (63.17)	5.169[d] (13.14)	82.18[ab] (65.03)	6.76[d] (15.07)
0.01	68.95[c] (56.13)	16.07[c] (23.63)	74.40[bc] (59.60)	15.12[c] (22.88)
0.03	63.95[d] (53.10)	21.64[c] (27.72)	69.78[cd] (56.65)	21.05[bc] (27.30)
0.05	55.78[e] (48.31)	31.23[b] (33.97)	64.20[d] (53.24)	27.28[b] (31.48)
0.1	36.81[f] (37.35)	52.96[a] (46.69)	43.16[e] (41.06)	51.9[a] (46.08)
Control	84.45[a] (66.77)		88.60[a] (70.26)	

Figures in the parenthesis are angular transformed, Figure in any column followed by same letter are not significantly different at $p < 0.05$ by Tukey's test

Table 5. Antifeedant activity of SP3 against *Spodoptera litura* by no-choice method

Concentration of extract (%)	Per cent Feeding (24h)	Antifeedancy (%)	Per cent Feeding (48h)	Antifeedancy (%)
0.005	85.61[a] (67.70)	3.21[d] (10.32)	91.23[b] (72.77)	4.53[d] (12.28)
0.01	84.32[a] (66.67)	3.16[d] (10.23)	87.36[b] (69.17)	9.44[c] (17.89)
0.03	79.98[b] (63.42)	12.38[c] (20.60)	79.39[c] (63.00)	16.92[b] (24.28)
0.05	71.65[b] (57.82)	21.56[b] (27.66)	75.32[c] (60.21)	22.31[b] (28.18)
0.1	61.32[c] (51.54)	33.49[a] (35.35)	66.32[d] (54.52)	30.67[a] (33.62)
Control	89.99[a] (71.55)		95.32[a] (77.50)	

Figures in the parenthesis are angular transformed. Figure in any column followed by same letter are not significantly different at $p < 0.05$ by Tukey's test

Table 6. Antifeedant activity of SP4 against *Spodoptera litura* by no-choice method

Concentration of extract (%)	Per cent Feeding (24h)	Antifeedancy (%)	Per cent Feeding (24h)	Antifeedancy (%)
0.005	84.98[a] (67.19)	6.693[c] (14.99)	84.32[b] (66.67)	7.96[d] (16.38)
0.01	82.61[a] (65.35)	9.287[c] (17.74)	82.56[b] (65.31)	9.700[d] (18.14)
0.03	71.30[b] (57.60)	19.88[b] (26.47)	76.95[c] (61.30)	17.47[c] (24.70)
0.05	65.58[b] (54.07)	24.96[b] (29.97)	71.10[d] (57.48)	26.18[b] (30.77)
0.1	57.19[c] (49.13)	36.10[a] (36.92)	63.83[e] (53.02)	33.76[a] (35.52)
Control	87.45[a] (69.25)		91.78[a] (73.33)	

Figures in the parenthesis are angular transformed. Figure in any column followed by same letter are not significantly different at $p < 0.05$ by Tukey's test

4.3.1. Antifeedant activity of SP1 against *Spodoptera litura* by choice method

Feeding deterrence activity was very less at 0.005, 0.01 and 0.03 per cent concentration of SP1 extract after 24h of experiment (Table 7). No significant difference was recorded for the three lower concentrations. A lowest deterrent activity of 0.712 per cent was obtained for 0.03 per cent of extract than 0.005 per cent. After 0.03 per cent, a sudden increase in activity to 11.95 per cent was recorded at 0.05 per cent. At highest dose of 0.1 per cent antifeedant activity reached up to 37.08 per cent. Antifeedant activity at 0.5 and 0.1 per cent were significantly different from all other treated concentrations. After 48 h of treatment, a slight increase in activity was observed for 0.005 to 0.03 per cent concentrations compared to 24h experiment. Highest antifeedant activity of 33.88 per cent was obtained for 0.1 per cent concentration of SP1 extract after 48h of feeding. Antifeedant activity recorded at 0.1 per cent was significantly different from all other concentration. Antifeedancy at 0.005, 0.01, 0.03, 0.05 and 0.1 per cent concentration were 1.32, 6.64, 12.76, 24.51 and 33.88 per cent after 48h of feeding. No significant difference in activity was found for any of the concentrations except 0.1 per cent.

4.3.2. Antifeedant activity of SP2 against *Spodoptera litura* by choice method

A lower deterrence effect of 6.32 per cent was observed at 0.005 per cent concentration of SP2 extract after24h of feeding (Table 8). At the highest dose of 0.1 per cent, a maximum antifeedant activity of 46.65 per cent was recorded, which was significantly different from all other treatments. Antifeedant activity at 0.05 per cent was 20.74 per cent and it was significantly different from other treatments. At lowest doses of 0.005, 0.01 and 0.03 per cent, the activities were 6.32, 12.04 and 14.31 per cent respectively after 24h of feeding. Compared to 24h experiment, a reduction in antifeedancy at 0.01 per cent concentration was recorded after 48h experiment. At 0.1 per cent of the extract, a maximum antifeedant activity of 38.81 per cent was recorded and it was significantly different from all other concentrations. Significant difference in activity was only observed

for 0.05 and 0.1 per cent of the extract. Antifeedant activity at 0.005, 0.01, 0.03, 0.05 and 0.1 per cent of SP2 were 3.32, 1.39, 8.82, 21.18 and 38.81, respectively after 48h of feeding

4.3.4. Antifeedant activity of SP3 against *Spodoptera litura* by choice method

A gradual increase in deterrent activity with increase in concentration was observed for SP3 extract after 24h of treatment. Feeding deterrence was not significantly different at lower concentrations of 0.005, 0.01 and 0.03 per cent after 24h of feeding but at 0.05 per cent and 0.1 per cent concentration a sudden increase in deterrent activity up to 30 per cent was observed (Table 9). Antifeedant activity at 0.05 and 0.1 per cent of SP3 extract were 12.83 and 30.38 per cent respectively. When the duration was doubled, all the concentrations up to 0.05 per shown a higher activity as compared to 24h experiment. At highest dose of 0.1 per cent, a maximum antifeedant activity of 28.65 per cent was recorded. The activities recorded at 0.05 and 0.1 per cent were significantly different from all other treatments in 48h experiment. Antifeedant activities at 0.005, 0.01, 0.03, 0.05 and 0.1 per cent were 0.75, 1.82, 7.56, 21.83 and 28.65 per cent respectively after 48h of feeding

4.3.3. Antifeedant activity of SP4 against *Spodoptera litura* by choice method

Very low deterrent activity was shown by 0.005, 0.01 and 0.03 per cent of SP4 extract after 24h of feeding. Antifeedant activities at 0.005, 0.01 and 0.03 per cent were 0.79, 1.96, 0.47 per cent respectively (Table 10). At highest concentration of 0.05 and 0.1 per cent, deterrent activities of 18.12 and 28.43 per cent were observed. Activity recorded at 0.05 and 0.1 per cent were significantly different from all other concentrations. No significant difference in activity was found at lower concentration of SP4 extract after 24h of feeding. Compared to methanol extract of aerial parts, a lower deterrent effect was noticed in root extract. When the duration of experiment was doubled, a slight decrease in antifeedant activity at 0.005 and 0.01 per cent. At highest dose of 0.1 per cent, a maximum antifeedant activity of 26.72 per cent was observed which was significantly different from

all other treatments in 48h experiment. Activity at 0.005 and 0.01 per cent were not significantly different for 48h experiment.

Table 7. Antifeedant activity of SP1 against *Spodoptera litura by* choice method

Concentration of extract (%)	Area consumed after 24h (cm^2)		Antifeedancy (%)	Area consumed after 48h (cm^2)		Antifeedancy (%)
	C	T		C	T	
0.005	7.68	7.61	0.880[c]	8.96	8.68	1.32[c]
0.01	8.21	7.92	2.160[c]	9.12	7.72	6.64[b]
0.03	8.12	8.10	0.712[c]	9.00	7.12	12.76[b]
0.05	6.96	5.32	11.95[b]	7.77	4.86	24.51[ab]
0.1	7.32	3.19	37.08[a]	8.12	4.12	33.88[a]

Figure in any column followed by same letters are not significantly different at $p < 0.05$ by Tukey's test

Table 8. Antifeedant activity of SP2 against *Spodoptera litura* by choice method

Concentration of extract (%)	Area consumed after 24h (cm^2)		Antifeedancy (%)	Area consumed after 48h (cm^2)		Antifeedancy (%)
	C	T		C	T	
0.005	7.34	6.21	6.32[d]	8.31	7.76	3.32[c]
0.01	7.98	6.31	12.04[cd]	7.21	7.12	1.39[c]
0.03	7.56	5.92	14.31[bc]	8.92	7.36	8.82[c]
0.05	8.21	5.26	20.74[b]	6.86	4.69	21.18[b]
0.1	8.66	3.23	46.65[a]	8.98	4.12	38.81[a]

Figure in any column followed by same letter are not significantly different at $p < 0.05$ by Tukey's test

Table. 9. Antifeedant activity of SP3 against *Spodoptera litura* by choice method

Concentration of extract (%)	Area consumed after 24h (cm^2)		Antifeedancy (%)	Area consumed after 48h (cm^2)		Antifeedancy (%)
	C	T		C	T	
0.005	9.21	9.10	0.027[c]	9.12	8.86	0.757[d]
0.01	7.88	7.69	0.271[c]	8.32	8.34	1.828[cd]
0.03	7.12	7.14	2.531[c]	8.61	7.57	7.567[c]
0.05	8.82	6.96	12.83[b]	9.23	6.21	21.83[b]
0.1	8.12	4.38	30.38[a]	8.78	5.01	28.65[a]

Figure in any column followed by same letter are not significantly different at $p < 0.05$ by Tukey's test

Table 10. Antifeedant activity of SP4 against *Spodoptera litura* by choice method

Concentration of extract (%)	Area consumed after 24h (cm^2)		Antifeedancy (%)	Area consumed after 48h (cm^2)		Antifeedancy (%)
	C	T		C	T	
0.005	8.31	8.26	0.790[c]	9.13	9.12	0.230[c]
0.01	9.22	8.98	1.960[c]	8.15	8.13	0.428[c]
0.03	7.98	7.99	0.477[c]	9.22	6.99	13.71[b]
0.05	8.22	5.62	18.12[b]	8.78	5.11	22.05[ab]
0.1	8.68	4.98	28.43[a]	7.98	3.92	26.72[a]

Figure in any column followed by same letter are not significantly different at $p < 0.05$ by Tukey's test

4.4. Insect growth regulatory activity of *Sphagneticola trilobata* against *Spodoptera litura*

Insect growth regulatory activity was evaluated by diet incorporation method. Different concentrations of extract were mixed with required amount of semi-synthetic diet and provided to 5-day old larvae of *Spodoptera litura*. Observations were made on

1) Larval weight after 5 days
2) Larval duration
3) Pupal weight
4) Pupal duration
5) Abnormal pupae
6) Dead larvae
7) Larval pupal intermediates (LPI)
8) Pupal mortality
9) Pupal adult intermediates (PAI)
10) Abnormal adults
11) Normal adults

Table 11. Insect growth regulatory activity of SP1 at various concentrations against *Spodoptera litura*

Conc (%)	Larval weight reduction (%)	Larval Duration (days)	Pupal weight reduction (%)	Pupal duration (days)	Abnormal Pupae (%)	Mortality					Normal adults (%)
						Larval (%)	LPI (%)	Pupal (%)	PAI (%)	Ab adults (%)	
0.005	3.92^{c}	16.76^{a}	6.28^{c}	11.80^{cd}	4.16^{a} (6.87)	0.00	0.00^{a} (1.15)	4.16^{b} (6.87	0.00	0.00^{a} (1.15)	95.83^{a} (78.22)
0.01	6.450^{c}	16.91^{a}	7.071^{bc}	12.12^{bcd}	0.00^{a} (1.15)	0.00	0.00^{a} (1.15)	4.16^{b} (6.87)	0.00	0.00^{a} (1.15)	95.83^{a} (78.22)
0.03	13.57^{b}	17.31^{a}	8.251^{b}	12.40^{abc}	4.16^{a} (6.87)	0.00	0.00^{a} (1.15)	16.67^{ab} (24.10)	0.00	4.16^{b} (6.87)	83.33^{ab} (65.87)
0.05	18.85^{ab}	17.28^{a}	13.92^{a}	12.95^{ab}	0.00^{a} (1.15)	0.00	4.16^{a} (6.87)	20.83^{ab} (27.15)	0.00	4.16^{b} (6.87)	70.83^{ab} (57.33)
0.1	19.62^{a}	17.62^{a}	15.96^{a}	13.35^{a}	4.16^{a} (6.87)	0.00	4.16^{a} (6.87)	41.61^{a} (40.16)	0.00	4.16^{b} (6.87)	50.00^{b} (45.00)
Ctl.		16.66^{a}		11.32^{d}	0.00^{a} (1.15)	0.00	0.00^{a} (1.15)	4.16^{b} (6.87)	0.00	0.00^{a} (1.15)	95.83^{a} (78.22)

Figures in the parenthesis are angular transformed

Figure in any column followed by same letter are not significantly different at $p < 0.05$ by Tukey's test

Table 12. Insect growth regulatory activity of SP2 at various concentrations against *Spodoptera litura*

Conc (%)	Larval weight reduction (%)	Larval Duration (days)	Pupal weight reduction (%)	Pupal duration (days)	Abnormal Pupae (%)	Mortality					Normal adults (%)
						Larval (%)	LPI (%)	Pupal (%)	PAI (%)	Ab adults (%)	
0.005	11.47^{c}	17.46^{b}	1.89^{d}	11.48^{cd}	0.00^{a} (1.15)	0.00	0.00^{a} (1.15)	4.16^{b} (6.87	0.00	0.00^{a} (1.15)	95.83^{a} (78.22)
0.01	17.27^{bc}	18.51^{b}	2.21^{d}	11.90^{bc}	4.16^{a} (6.87)	0.00	0.00^{a} (1.15)	4.16^{b} (6.87	0.00	0.00^{a} (1.15)	95.83^{a} (78.22)
0.03	22.15^{ab}	18.73^{b}	8.50^{c}	11.81^{bc}	0.00^{a} (1.15)	0.00	0.00^{a} (1.15)	4.16^{b} (6.87	0.00	0.00^{a} (1.15)	95.83^{a} (78.22)
0.05	28.75^{a}	19.36^{a}	19.87^{b}	12.15^{b}	4.16^{a} (6.87)	0.00	4.16^{a} (6.87)	16.67^{ab} (24.10)	0.00	4.16^{a} (6.87)	75.00^{ab} (60.00)
0.1	28.97^{a}	19.87^{a}	29.57^{a}	12.85^{a}	8.33^{a} (16.78)	0.00	8.33^{a} (16.78)	29.01^{a} (32.58)	0.00	8.33^{a} (16.78)	54.17^{b} (47.39)
Ctl.		16.66^{c}		11.32^{d}	0.00^{a} (1.15)	0.00	0.00^{a} (1.15)	4.16^{b} (6.87	0.00	0.00^{a} (1.15)	95.83^{a} (78.22)

Figures in the parenthesis are angular transformed

Figure in any column followed by same letter are not significantly different at $p < 0.05$ by Tukey's test

Table 13. Insect growth regulatory activity of SP3 at various concentrations against *Spodoptera litura*

Conc (%)	Larval weight reduction (%)	Larval Duration (days)	Pupal weight reduction (%)	Pupal duration (days)	Abnormal Pupae (%)	Mortality					Normal adults (%)
						Larval (%)	LPI (%)	Pupal (%)	PAI (%)	Ab adults (%)	
0.005	4.171^{d}	16.96^{c}	1.833^{d}	11.50^{c}	4.16^{a} (6.87)	0.00	0.00^{a} (1.15)	4.16^{a} (6.87)	0.00	0.00^{a} (1.15)	95.83^{a} (78.22)
0.01	7.452^{d}	17.43^{bc}	2.348^{cd}	11.69^{c}	0.00^{a} (1.15)	0.00	0.00^{a} (1.15)	4.16^{a} (6.87)	0.00	0.00^{a} (1.15)	95.83^{a} (78.22)
0.03	13.32^{c}	17.31^{bc}	3.215^{c}	11.86^{bc}	0.00^{a} (1.15)	0.00	0.00^{a} (1.15)	4.16^{a} (6.87)	0.00	4.16^{a} (6.87)	91.66^{a} (73.21)
0.05	19.35^{b}	18.25^{ab}	7.400^{b}	12.33^{ab}	0.00^{a} (1.15)	0.00	4.16^{a} (6.87)	8.33^{a} (16.78)	0.00	4.16^{a} (6.87)	83.33^{ab} (65.87)
0.1	26.87^{a}	19.34^{a}	11.168^{a}	12.60^{a}	0.00^{a} (1.15)	0.00	8.33^{a} (16.78)	16.67^{b} (24.10	0.00	8.33^{a} (16.78)	66.66^{ab} (54.73)
Ctl.		16.66^{c}		11.32^{c}	0.00^{a} (1.15)	0.00	0.00^{a} (1.15)	4.16^{a} (6.87)	0.00	0.00^{a} (1.15)	95.83^{b} (78.22)

Figures in the parenthesis are angular transformed

Figure in any column followed by same letter are not significantly different at $p < 0.05$ by Tukey's test

Table 14. Insect growth regulatory activity of SP4 at various concentrations against *Spodoptera litura*

Conc (%)	Larval weight reduction (%)	Larval Duration (days)	Pupal weight reduction (%)	Pupal duration (days)	Abnormal Pupae (%)	Mortality					Normal adults (%)
						Larval (%)	LPI (%)	Pupal (%)	PAI (%)	Ab adults (%)	
0.005	10.97^{c}	17.10^{c}	2.750^{d}	11.80^{cd}	0.00^{a} (1.15)	0.00	0.00^{b} (1.15)	4.16^{b} (6.87	0.00	4.16^{a} (6.87)	95.83^{ab} (78.22)
0.01	12.60^{c}	18.21^{b}	7.201^{c}	12.19^{bcd}	4.16^{a} (6.87)	0.00	0.00^{b} (1.15)	4.16^{b} (6.87)	0.00	4.16^{a} (6.87)	91.66^{ab} (73.21)
0.03	20.57^{b}	18.32^{b}	7.861^{c}	12.56^{bc}	4.16^{a} (6.87)	0.00	0.00^{b} (1.15)	4.16^{b} (6.87)	0.00	0.00^{a} (1.15)	95.83^{ab} (78.22)
0.05	28.15^{b}	18.63^{b}	13.77^{b}	12.97^{ab}	4.16^{a} (6.87)	0.00	4.16^{b} (6.87)	16.67^{a} (24.10)	0.00	4.16^{a} (6.87)	75.00^{bc} (60.00)
0.1	31.4^{a}	19.11^{a}	20.05^{a}	13.54^{a}	4.16^{a} (6.87)	0.00	16.67^{a} (24.10)	16.67^{a} (24.10)	0.00	4.16^{a} (6.87)	62.50^{c} (52.23)
Ctl.		16.66^{c}		11.32^{d}	0.00^{a} (1.15)	0.00	0.00^{b} (1.15)	0.00^{b} (1.15)	0.00	0.00^{a} (1.15)	100^{a} (90.00)

Figures in the parenthesis are angular transformed

Figure in any column followed by same letter are not significantly different at $p < 0.05$ by Tukey's test

4.4.1. Insect growth regulatory activity of SP1 against *Spodoptera litura*

When *S. litura* larvae were provided with diet treated with different concentrations of hexane extract of aerial parts (SP1), a maximum larval weight reduction of 19.62 per cent was observed for 0.1 per cent concentration (Table 11). Significant difference in larval weight reduction was observed for 0.01, 0.03 and 0.1 per cent concentration of SP1. A minimum larval weight reduction of 3.92 per cent was recorded for 0.005 per cent of the extract. The larval duration was extended to a maximum period of 17.62 days compared to control (16.66 days). No significant difference in larval duration was found for any of the treated concentrations of SP1. Pupal weight reduction ranged from 6.28 to 15.96 per cent was observed for concentrations ranged from 0.005 to 0.1 per cent. A significant difference in pupal weight reduction was recorded only with 0.05 and 0.1 per cent concentrations of the extract. An extended pupal duration of 13.35 days was observed in larvae provided with 0.1 per cent of SP1 treated diet, whereas in control, it was 11.32 days. Pupal duration recorded at 0.1 per cent concentration was significantly different from other treatments. Observations on abnormal pupae were not dose-dependent for SP1, and no significant difference was observed for any of the treatments.

No larval mortality was observed for any of the treatments. Larval pupal intermediates were observed only for 0.05 and 0.1 per cent concentration, but it was not significantly different from other treatments. Pupal mortality reached 41.61 per cent at 0.1 per cent of the extract, at 0.03 and 0.05 per cent, pupal mortality recorded were 16.67 and 20.83 per cent, respectively. A significant difference in pupal mortality was observed only for the highest dose of 0.1 per cent. As similar to larval mortality, no pupal adult intermediates were found for any of the treatments. No significant difference in abnormal adults was noticed for any of the concentrations. Abnormal adults were recorded only for 0.03, 0.05and 0.1 per cent concentrations. Normal adult emergence was 50 per cent at 0.1 per cent of SP1 treated diet, and it was significantly different from all other treatments.

4.4.2. Insect growth regulatory activity of SP2 against *Spodoptera litura*

A concentration-dependent increase in larval weight reduction ranging from 11.47 - 28.97 per cent was observed for all the concentrations of methanol extract of aerial plant parts (SP2) (Table 12). At the highest concentration of 0.1 per cent, pupal weight reduction reached 28.97 per cent, but it was not significantly different from the 0.05 per cent concentration. Compared to control, the total larval duration was extended in all the treatments. At the highest concentration of 0.1 per cent, larval duration increased to 19.87 days where as in control, it was 16.66 days. No significant difference in larval duration was recorded for any of the treatments. Pupal weight reduction was also dose-dependent. At lower concentrations of 0.005 and 0.01 per cent, pupal weight reduction was 1.89 and 2.21 per cent, respectively, and was not significantly different from each other. At the highest concentration, pupal weight reduction reached up to 29.57 per cent. A significant difference in pupal weight reduction was observed for 0.03, 0.05 and 0.1 per cent concentrations. An extended pupal duration of 12.85 days was observed in the larvae treated with 0.1 per cent SP2 extract, and it was significantly different from all other treatments. At the lowest dose of 0.005 per cent, pupal duration was 11.48 days, which was nearly similar to control. Abnormal pupae observed was not significantly different for any of the concentrations.

No larval mortality was observed for SP2 in any of the treated concentrations. Larval pupal intermediate was maximum (8.33%) at the higher concentration of 0.1 per cent, but it was not significantly different from other treatments. A dose-dependent increase in pupal mortality was observed in all the concentrations. Pupal mortality recorded up to 29.01 per cent at 0.1 per cent concentration of SP2 extract, and it was significantly different from all other treatments. No pupal adult intermediates were observed for any of the treatments. Normal adult emergence was not significantly different for 0.005, 0.01, and 0.03 per cent of extract, but it reached 75.00 per cent and 54.17 per cent at 0.05 per cent and 0.1 per cent concentrations, respectively.

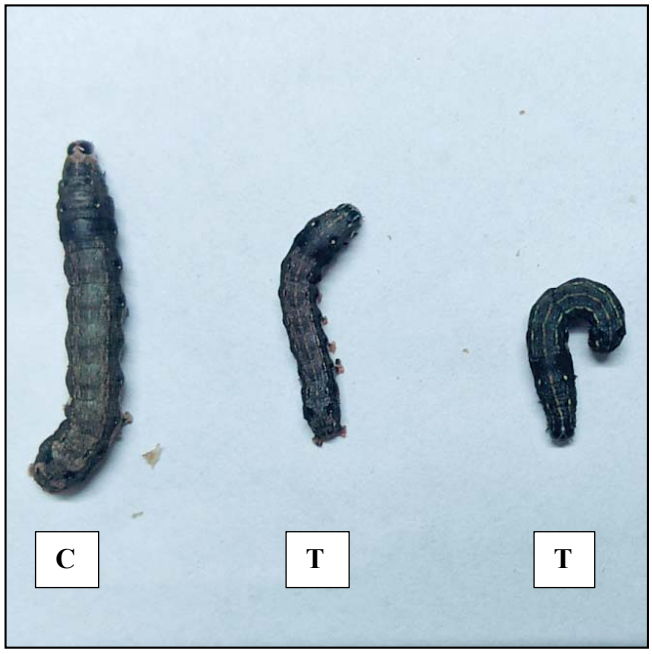


Plate 5a. Larval size and weight reduction

Plate 5b. Pupal size and weight reduction

Plate 5. Larval and pupal weight reduction of *Spodoptera litura*

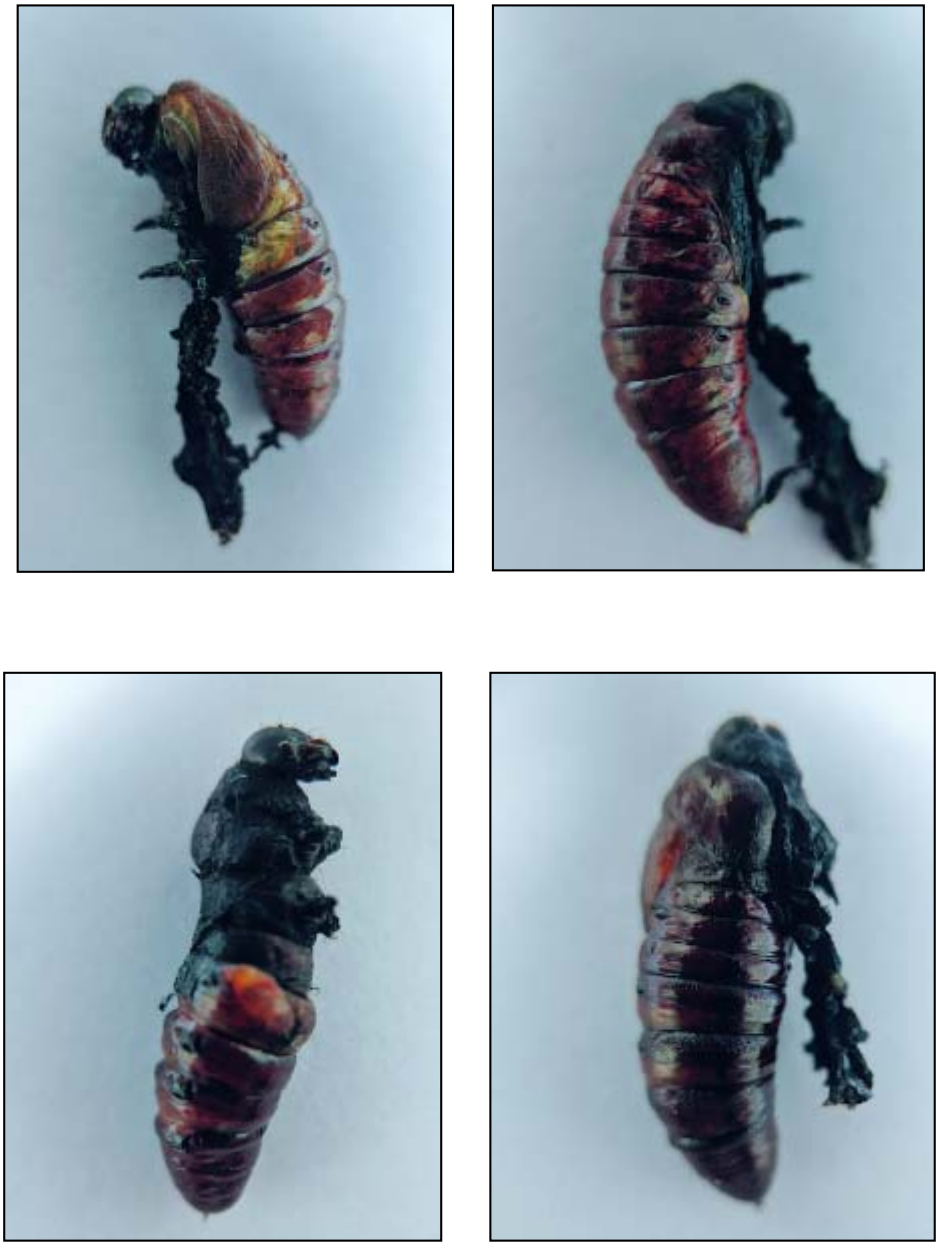


Plate 6. **Larval pupal intermediates (LPI) of *Spodoptera litura***

Abnormal pupa

Abnormal pupa

Normal pupa

Plate 7. Normal and abnormal pupae of *Spodoptera litura*

Abnormal adult

Abnormal adult

Normal adult

Plate 8. Abnormal and normal adults of *Spodoptera litura*

4.4.3. Insect growth regulatory activity of SP3 against *Spodoptera litura*

Different concentrations of hexane extract of roots of *S. trilobata* were mixed with artificial diet and provided to five-day old larvae of *S. litura*. After five days of treatment, larval weight reduction reached 19.35 and 26.87 per cent at 0.05 and 0.1 per cent concentrations of the extract. Larval weight reduction was significantly different at 0.03, 0.05 and 0.1 per cent concentrations. The larval duration was extended to 19.34 days at the highest dose of treatment, whereas in control, it was 16.66 days. No significant difference was recorded for the larval duration in any of the concentrations. Pupal weight reduction ranged between 1.83 per cent to 11.68 per cent for various concentrations of SP3 extract. Pupal weight reduction at 0.03, 0.05 and 0.1 per cent were 3.21, 7.40 and 11.16, respectively, and were significantly different from each other. An extended pupal duration of 12.60 days was observed for 0.1 per cent of the extract, and it was significantly different from all other treatments. Pupal duration at 0.005 per cent was not significantly different from control. Abnormal pupae recorded at 0.005 per cent concentration (4.16%) was not statistically significant from other treatments.

Similar to all other extracts, no larval mortality was observed in any of the concentrations of SP3. Pupal mortality was not significantly different in any of the concentrations except 0.1 per cent, where it was 16.67 per cent. Observations on larval pupal intermediates were not statistically different in any of the concentrations. No pupal adult intermediates were found for any of the treatments. Observations on abnormal adults were not significantly different in any of the treated concentrations. A minimum normal adult emergence of 66.66 per cent was recorded at the highest dose of treatment, and it was not significantly different from 0.05 per cent of the extract. At lower concentrations, no significant difference was recorded for normal adult emergence.

4.4.3. Insect growth regulatory activity of SP4 against *Spodoptera litura*

The highest larval weight reduction of 31.40 per cent was observed for *S. litura* larvae fed with 0.1 per cent of methanol extract of roots of *S. trilobata* (SP4). At 0.005 per cent, larval weight reduction was 10.97 per cent, and it increased to 12.60 per cent, 20.57 per cent and 28.15 per cent at 0.01, 0.03, 0.05 per cent concentrations, respectively. Larval weight reduction recorded at the highest concentration was significantly different from all other treatments. Larval duration was extended to 19.11 days at 0.1 per cent, and it was significantly different from other treatments. No significant difference in larval duration was recorded for 0.01, 0.03 and 0.05 per cent concentrations. Pupal weight reduction was not significantly different for 0.005 per cent and 0.01 per cent concentrations, but it reached 20.05 per cent at the highest concentration. Pupal weight reduction at 0.05 and 0.1 per cent extract were significantly different from other treatments. The pupal duration was extended to 13.54 and 12.97 days at 0.05 per cent and 0.1 per cent concentration of the extract, but significant difference was observed only at 0.1 per cent concentration.

At all the concentrations except 0.005 per cent and control, 4.16 per cent abnormal pupae were observed. Similar to other extracts no larval mortality was observed in any of the treated concentrations. Larval pupal intermediates obtained for a diet supplemented 0.1 per cent of the extract was 16.67 per cent, and it was significantly different from other treatments. The highest pupal mortality of 16.67 per cent was recorded for 0.1 per cent concentration compared to other lower concentrations. No pupal adult intermediates were recorded in any of the treated concentrations. Normal adult emergence was not significantly different at lower concentrations. Normal adult emergence of 62.50 per cent was recorded for 0.1 per cent concentration of extract, and it was significantly different from other concentrations.

4.5. Chemical characterization of various fractions of *Sphagneticola trilobata*

4.5.1. Chemical characterization of hexane extract of *Sphagneticola trilobata*

GC-MS/MS analysis of hexane extract of *S. trilobata* showed different peaks, which indicates the presence of seven phytochemical compounds from aerial parts and two from roots. Comparing the mass spectra of individual constituents with NIST (National Institute of Standards and Technology) library, various compounds were identified.

Table 15. Compounds identified through GC-MS/MS analysis of hexane extract of roots of *Sphagneticola trilobata*

S. No	Compound	Formula	Molecular weight
1	Cholestan-3-ol,5-chloro-6-nitro	$C_{27}H_{46}ClNO_3$	467
2	7,8-Epoxy-lanostan-11-ol,3-acetoxy	$C_{32}H_{54}O_4$	502

Comparing the results of GC-MS/MS analysis of hexane extract of *S. trilobata,* more number of phytochemicals were recorded from aerial extract than root. Most of the compounds recorded were derivatives steroids and sesquiterpene lactones. Only two compounds were reported from root extract.

The two compounds identified from hexane extract of roots which were cholestan-3-ol-5-chloro-6-nitro and 7, 8-epoxy-lanostan-11-ol-3-acetoxy. GC-MS/MS analysis of hexane extract of aerial parts revealed the presence of seven compounds which werecholest-2-enol(2,3-b)quinoxalin,6-nitro,cycloartanol, cholestan-3-ol,5-chloro-6-nitro, androstane-3,17-diol-16-(hydroxyl-methyl-acetic acid) lactone, 7,8-epoxy-lanostan-11-ol,3-acetoxy,benzoicacid,9-(adamantan-2-yliden-

methoxymethyl)6-oxoxanthen-3-yl ester and 1,3,2-dioxoborinane,2-(cholestan-3-yl-oxyl)4,6 dimethyl.

Table 16. Compounds identified through GC-MS/MS analysis of hexane extract of aerial parts of *Sphagneticola trilobata*

S. No	Compound	Formula	Molecular weight
1	Cholest-2-enol(2,3-b)quinoxalin,6-nitro	$C_{33}H_{47}N_3O_2$	517
2	Cycloartanol	$C_{30}H_{52}O$	428
3	Cholestan-3-ol,5-chloro-6-nitro	$C_{27}H_{46}ClNO_3$	467
4	Androstane-3,17-diol-16-(hydroxyl-methyl-acetic acid) lactone	$C_{22}H_{34}O_4$	362
5	7,8-Epoxy-lanostan-11-ol,3-acetoxy	$C_{32}H_{54}O_4$	502
6	Benzoicacid,9-(adamantan-2-yliden-methoxymethyl)6-oxoxanthen-3-yl ester	$C_{38}H_{32}O_5$	568
7	1,3,2-dioxoborinane,2-(cholestan-3-yl-oxyl)4,6 dimethyl	$C_{32}H_{57}BO_3$	500

4.5.2. Chemical characterization of methanol extract of *Sphagneticola trilobata*

LC-MS/MS analysis of methanol extract of *S. trilobata* showed the presence of 12 phytochemical compounds; xylitol, de-hydro epi androsterone, andrographolide, genistein, taxifolin, arachidonic acid, robinetin, emodin, galangin, methyl caffeate, (-)-caryophyllene oxide and artemisinin. Six compounds identified from methanol extract roots by LC-MS/MS analysis were, soyasaponin 1, (-)-caryophylleneoxide, arachidonic acid, 18-β-glycyrrhetinicacid, dehydroepiandrosterone (DHEA) and methyl caffeate.

Table 17. Compounds identified through LC-MS/MS analysis of methanol extract of aerial parts of *Sphagneticola trilobata*

S. No	Compound	Formula	Molecular weight
1	Xylitol	$C_5H_{12}O_5$	152.2
2	Genistein	$C_{15}H_{10}O_5$	270.05
3	Dehydroepiandrosterone (DHEA)	$C_{19}H_{28}O_2$	288.2
4	(-)-Caryophylleneoxide	$C_{15}H_{24}O$	220.18
5	Artemisinin	$C_{15}H_{22}O_5$	282.05
6	Taxifolin	$C_{15}H_{12}O_7$	304.05
7	Methyl caffeate	$C_{10}H_{10}O_4$	194.05
8	Robinetin	$C_{15}H_{10}O_7$	302.04
9	Emodin	$C_{15}H_{10}O_5$	270.5
10	Arachidonic acid	$C_{20}H_{32}O_2$	304.24
11	Andrographolide	$C_{20}H_{30}O_5$	350.20
12	Galangin	$C_{15}H_{10}O_5$	270.05

Table 18. Compounds identified through LC-MS/MS analysis of methanol extract of roots of *Sphagneticola trilobata*

S. No	Compound	Formula	Molecular weight
1	Soyasaponin I	$C_{48}H_{78}O_{18}$	942.5
2	(-)-Caryophylleneoxide	$C_{15}H_{24}O$	220.18
3	Dehydroepiandrosterone (DHEA)	$C_{19}H_{28}O_2$	288.2
4	18-β-Glycyrrhetinicacid	$C_{30}H_{46}O_4$	470.3
5	Arachidonic acid	$C_{20}H_{32}O_2$	304.2
6	Methyl caffeate	$C_{10}H_{10}O_4$	194.1

Discussion

5. DISCUSSION

The present study was carried out to investigate the biological activities of extracts of different parts of *S. trilobata* against the larvae of *S. litura*. Hexane and methanol were used for the extraction of active components from *S. trilobata*. Antifeedant activities of different extracts were tested against seven-day old larvae of *S. litura* by choice and no choice method. Insect growth regulatory activity (IGR) was tested by diet incorporation method against 5-day old larvae of *S. litura*. The results obtained from the present study is discussed here under the following headings.

1. Yield of different extracts from *S. trilobata*
2. Antifeedant activity of various extracts of *S. trilobata* against *S. litura*
3. Insect growth regulatory activity of different extracts of *S. trilobata* against *S. litura*
4. GC-MS/MS and LC-MS/MS analysis of active fractions

5.1. Yield of extracts from *Sphagneticola trilobata*

Comparing the yield of different extracts obtained from roots and aerial parts of *S. trilobata*, methanol extract of aerial parts was the highest (5.67 per cent w/w), followed by methanol extract of roots (2.32 per cent w/w), hexane extracts of aerial parts (1.96 per cent w/w) and hexane extract of root (1.23 per cent w/w). Polar solvents generally yield more extract as compared to non-polar solvents. Phankaen *et al*. (2017) extracted different parts of *Coffea arabica* in methanol and hexane, and they recorded yield of methanol extract as highest. Similarly, methanol extraction of *Andrographis paniculata* yielded more extract than hexane (Kumoro *et al.,* 2009).

5.2. Antifeedant activity of different extracts of *Sphagneticola trilobata* against *Spodoptera litura*

5.2.1. Antifeedant activity of different extracts of *Sphagneticola trilobata* against *Spodoptera litura* by no-choice method

The antifeedant activity of various extracts of *S. trilobata* was tested against *S. litura* by no-choice method. Concentrations ranging from 0.005 to 0.1 per cent were evaluated for 24 and 48h of exposure. Among the different extracts, methanol extract

of aerial parts exhibited maximum antifeedancy of 52.96 per cent at 0.1 per cent of the extract after 24h of feeding. For all other extracts, an antifeedant activity of less than 40 per cent was recorded. At lower concentrations, antifeedant activity of all the extract was not much prominent and was not statistically significant. The antifeedant activity was high in methanol extracts compared to hexane extracts. The lowest antifeedant activity was exhibited by hexane extract of roots. On comparing the activity of roots and aerial parts, aerial parts had superior antifeedancy. At the lowest concentration of 0.005 per cent, hexane extract of aerial parts exhibited higher antifeedant activity than the other three extracts. The decreasing order of antifeedancy of four extracts was SP2>SP1>SP4>SP3. (Fig 2)

Similar results were obtained after 48h of feeding. The highest antifeedant activity of 51.9 per cent was recorded for SP2 at the highest dose of 0.1 per cent. At lower concentrations, no significant difference in activity was recorded for any of the extracts. Similar to 24h treatments, root extracts exhibited lower antifeedant activity compared to aerial parts. At lowest concentrations of 0.005, 0.01 and 0.03 per cent, antifeedant activity was higher than 24h of treatment.

Junhirun *et al*. (2018) evaluated antifeedant activity of hexane and methanol extract of *S. trilobata* against *S. litura* and *Plutella xylostella.* Our results closely match their findings. They recorded that methanol extract was superior with a median antifeedant index of 0.33 mg/ml and 9.47 mg/ml against *P. xylostella* and *S. litura*, respectively. LC-MS/MS analysis of methanol extract of aerial parts of *S. trilobata* revealed the presence of artemisinin, andrographolide, taxifolin and genistein as important bio active molecules in the extract. The results recorded for methanol extract of *S. trilobata* are in close to Pathrose *et al*. (2006), who evaluated the antifeedant activity of andrographolide by no-choice method against *S. litura* and recorded a maximum antifeedance of 64.20 per cent at 0.1 per cent concentration after 24h of feeding. Similarly, antifeedant activity of artemisinin was evaluated by Maggi *et al.* (2005) by no choice method against *Spodoptera eridania* and recorded a maximum antifeedance of 87 per cent at 1.5 mg per ml of the test compound.

Methanol extract of *Wedelia chinensis* was tested for its antifeedant activity against third instar larvae of *S. litura* by no-choice method of bioassay (Wang *et al*., 2009). Similar to our observations, they also recorded a gradual increase in antifeedant activity from lower concentrations to higher concentrations. Highest antifeedant activity of 90.00 per cent was recorded at 5 per cent concentration of extract of *Wedelia chinensis*. Similarly, antifeedant activity of 80.00 per cent was obtained for 10 per cent methanol extract of *Wedelia chinensis* against larvae of *Cnaphalocrosis medinalis* (Qinglong *et al.,* 2012) which is similar to our findings. Reduced antifeedant activity of root extract of *S. trilobata* was agreeable with the findings of Caiyun *et al.* (2006), were they reported lower antifeedant activity for roots (AFC_{50} = 6618.8µg/ml) of *W. chinensis* against *Ostrinia furnacalis* compared to aerial parts including flowers (AFC_{50} = 3408.31µg/ml). Reduced antifeedant activity *S. trilobata* root extracts might be due to the presence of less bioactive molecules in roots compared to aerial parts. GC-MS/MS analysis of hexane extracts of roots showed the presence of only two compounds, whereas, from aerial parts, eight compounds were identified.

The antifeedant activity of methanol extract of roots reached 36 per cent at 0.1 per cent of the extract. It might be due to the presence of soyasaponin-1 in the extract. Choudary *et al.* (2014) evaluated antifeedant activity of saponins isolated from *Gymnema sylvestre* against *S. litura*. At 0.1 per cent of the test compound, 65.00 per cent antifeedancy was recorded in no-choice method. Lower intake of saponin treated leaves was recorded for caterpillars by Taylor *et al*. (2004). Ishaaya (1986) reported that saponins slow down the movement of food through insect alimentary canal.

Deterrent effect of different extracts of *S. trilobata* was tested against *S. litura* by choice leaf disc bioassay. The highest deterrent activity of 46.65 per cent was obtained for methanol extract of aerial parts (SP2). For all the extracts, a deterrent activity of less than 50 per cent was recorded. Compared to the no-choice method, antifeedant activity was less in the choice method**.** Deterrent activity ranging from 6.32 to 46.65 per cent was shown by methanol extract of aerial parts. Similar to the no-choice method, antifeedant activity was less for root extracts than aerial parts. At the lowest concentrations of 0.005, 0.01 and 0.03, no significant difference in antifeedant activity was recorded. Hexane extract of aerial parts (SP1) exhibited an antifeedant activity of

37.00 per cent after 24h of feeding. The decreasing order of antifeedant activity of the various extracts in the choice method after 24h of feeding was SP2>SP1>SP4>SP3 (Fig 3)

Similar results were obtained after 48h of feeding. At lowest concentrations of 0.005, 0.01 and 0.03 per cent the choice preference was negligible, a low antifeedant activity was recorded for these concentrations of all the extracts. For all the extracts, antifeedant activity reduced after 48h of exposure at highest concentration of 0.1 per cent. Similar to no choice method, root extracts *S. trilobata* exhibited low level of antifeedant activity against *S. litura.*

Our results are in close proximity with Yanping *et al*. (2003), who reported that methanol extract from *W. chinensis* exhibited repellent activity of 65 per cent at 1 per cent concentration against *Aphis medicaginis.* In the case of SP2 and SP3 extracts, lower activity was obtained for 0.03 per cent concentration than 0.005 per cent, it may be due to the low dose of treatment. At lower concentrations, choice preference was negligible. The results obtained for methanol extract of aerial parts (SP1) are in close proximity with Pathrose (2006), who recorded a maximum antifeedant activity of 55.2 per cent at 0.1 per cent of andrographolide against *S. litura* larvae in the choice method. Similarly, Choudary *et a*l. (2014) reported a maximum antifeedant activity of 36 per cent at 0.1 per cent concentrations of saponin isolated from *Gymenema sylvestre* against *S. litura* in choice leaf disc bioassay.

Zhang *et al*. (2014) conducted choice bioassay to test the repellent effect of volatiles of twenty non-host plants against females of brown plant hopper *Nilapartvata lugens*. Volatiles from *Eucalyptus exsetrta*, *Lantana camara*, *Ageratum conezoides*, *Wedelia chinensis*, *Kaya senagalensis, etc*. were tested using a Y-tube olfactometer. Volatiles of *W. chinensis*, showed significant repellent activity of 87.50 per cent against females of *Nilaparvata lugens.*

Fig 2. Comparative antifeedant activity of different extracts *Sphagneticola trilobata* by no-choice method (24h)

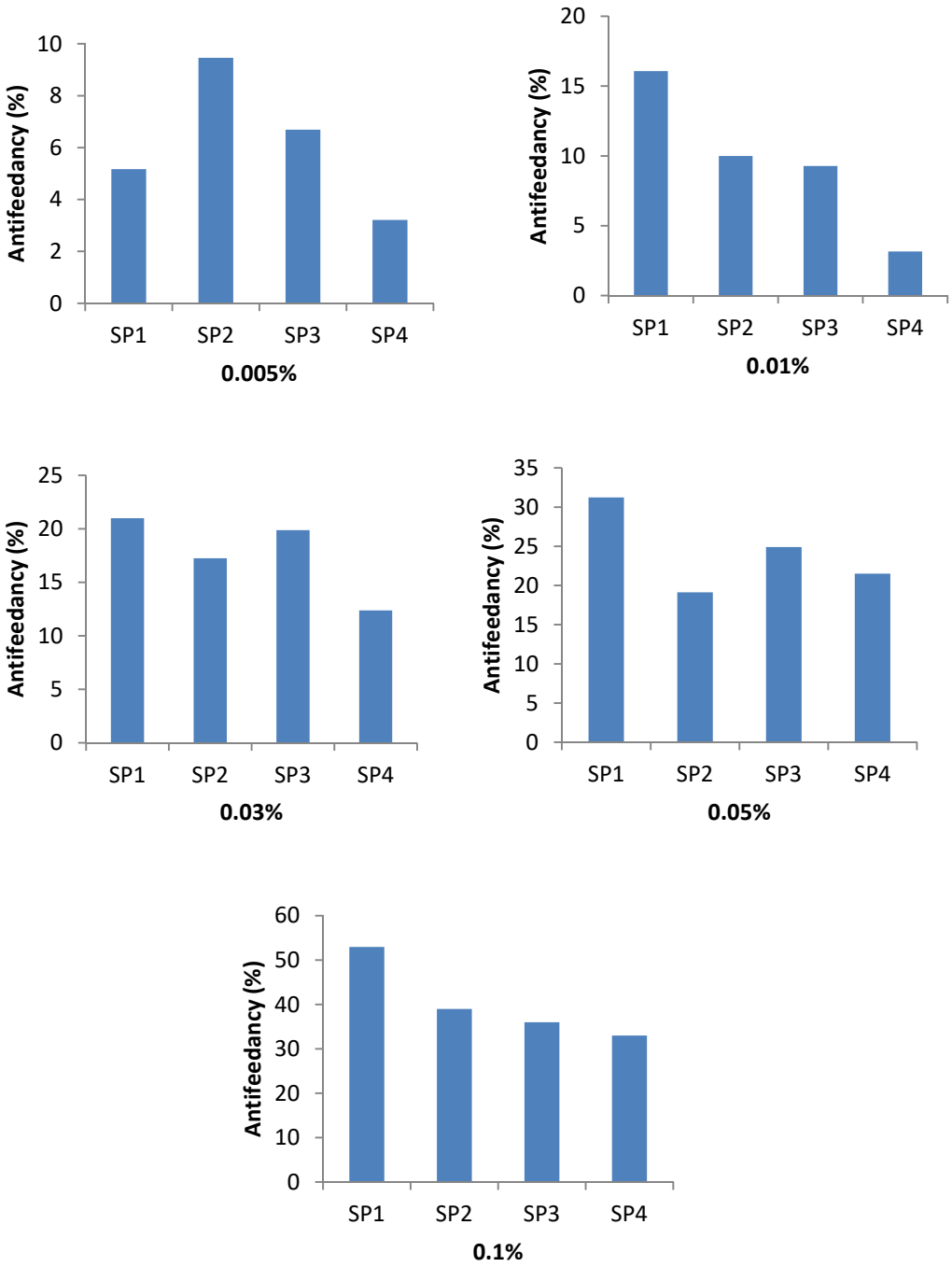


Fig 3. Comparative antifeedant activity of different extracts of *Sphagneticola trilobata* by no-choice method (48h)

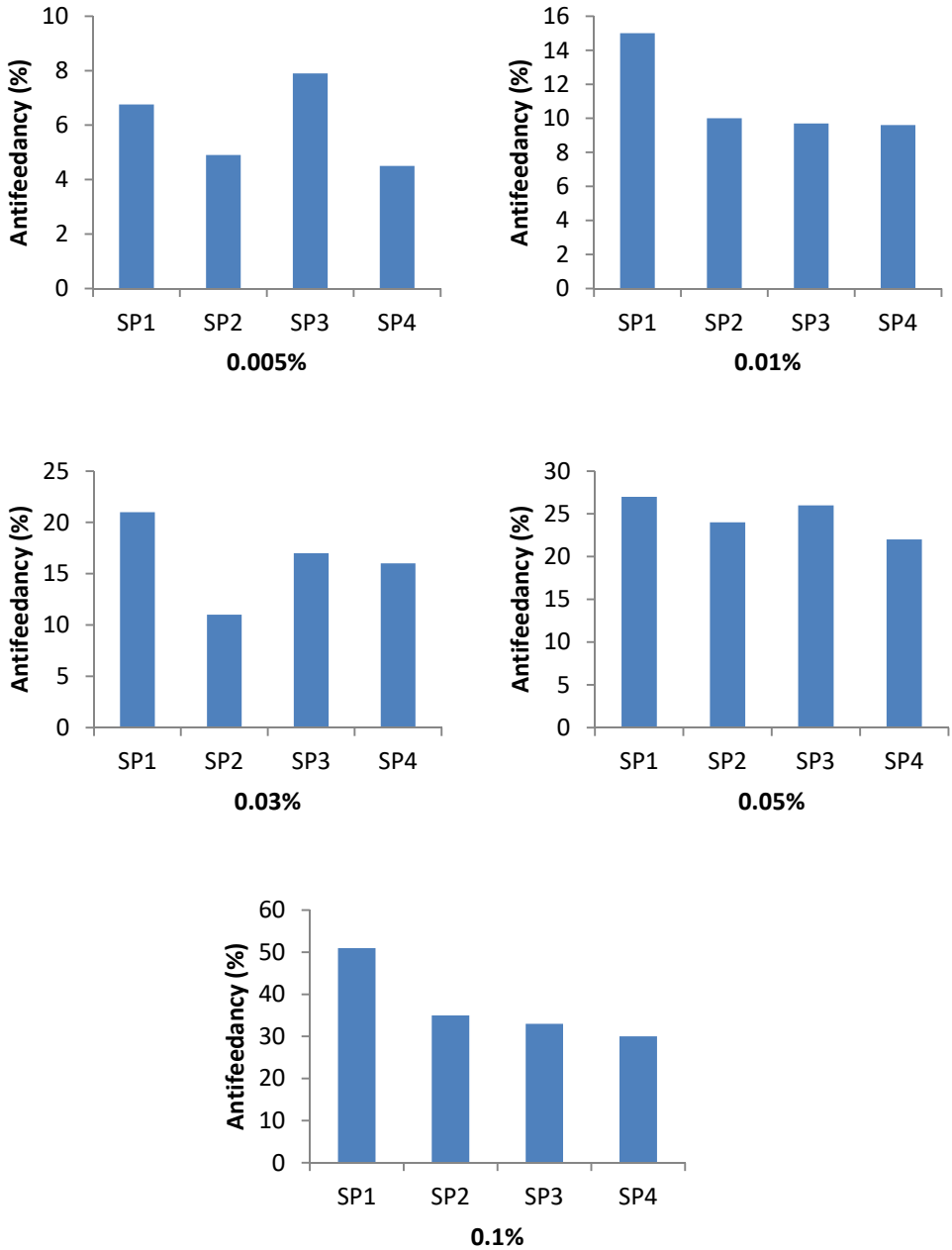


Fig 4. Comparative antifeedant activity of different extracts of *Sphagneticola trilobata* by choice method (24h)

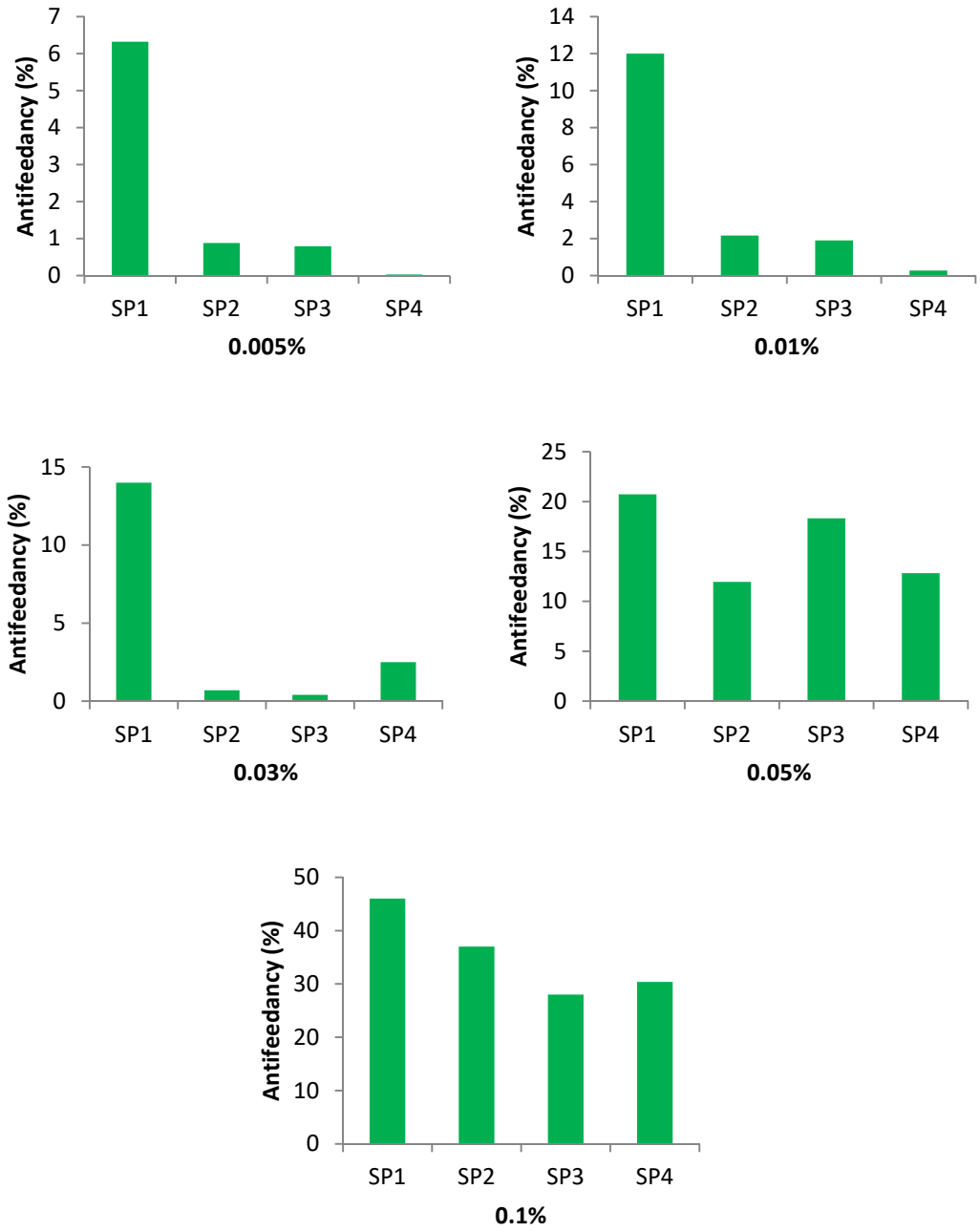
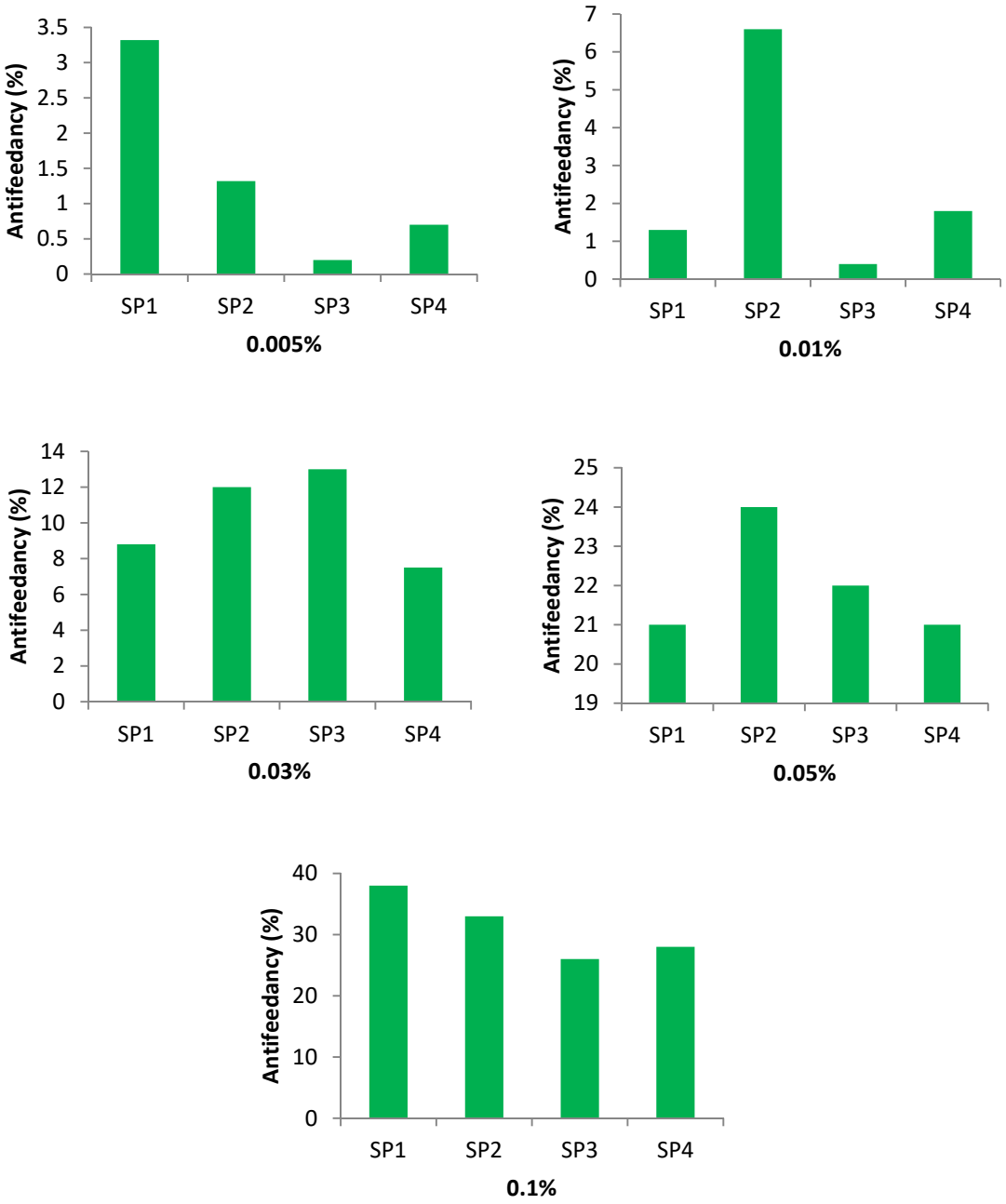


Fig 5. Comparative antifeedant activity of different extracts of *Sphagneticola trilobata* by choice method (48h)



5.3. Insect growth regulatory activity of different extracts of *Sphagneticola trilobata* against *Spodoptera litura.*

Insect growth regulatory activity (IGR) was studied by diet incorporations of various concentrations of methanol and hexane extract of *S. trilobata* against 5-day old larvae of *S. litura*. Different parameters like larval weight reduction, larval duration, pupal weight reduction, pupal duration, abnormal pupae, larval pupal intermediates, pupal mortality, pupal adult intermediates, abnormal adults and normal adults were recorded.

All the extracts showed a dose-dependent larval weight reduction after five days of treatment. The maximum larval weight reduction of 31.4 per cent was recorded for 0.1 per cent concentration of hexane extract of roots (SP3). For all the extracts, at concentrations of 0.005 and 0.01 per cent, larval weight reduction was not significantly different. Methanol extract of aerial parts (SP1) exhibited a maximum weight reduction of 28.97 per cent at the highest dose of treatment (Fig 4). The weight reduction observed might be due to the reduced intake of food by the larvae. Kaur *et al.* (2019) evaluated the growth regulatory effect of hexane extract of *Inula racemosa* (Asteraceae) against *S. litura*. A dose dependent larval weight reduction was recorded for all the concentrations ranging from 1000 to 2500ppm. Schulter (1985) reported that the reduction of weight gain of *Epilachna varivestris* treated with azadiractin may be due to inhibition of fat body storage proteins. Similar results were obtained for Pathrose (2006), who conducted diet incorporation bioassay of andrographolide against *S. litura* and recorded 30.00 per cent weight reduction of larvae at 0.01 per cent of the test compound. Andrographolide at lower doses caused more than 25 per cent larval weight reduction of *S. litura.* A significant reduction in larval weight of *S. eridania* was observed by Maggi *et al*. (2005) with a diet supplemented with 0.15 per cent of artemisinin isolated from methanolic extract of *Artemisia annua.*

The larval duration was significantly extended with SP1, SP2 and SP3, whereas with SP2, no significant difference was recorded. The longest duration of 19.87 days was recorded for the highest concentration of methanol extract of aerial parts. The extended duration of the larval period may be due to an imbalance of growth stimulating

and inhibiting hormone. GC-MS/MS analysis of hexane extract of aerial parts revealed the presence of steroid hormone precursors like cholest-2-enol (2, 3-b) quinoxalin-6-nitro and cholestan-3-ol, 5-chloro-6-nitro in the extract, which may be the reason for the maximum extended larval duration recorded for SP1 extract at 0.1 per cent concentration (Fig 5). The result of the present investigation is in agreement with the results of Baskar *et al*. (2011), who reported extended larval duration of *S. litura* larvae treated with chloroform and hexane extract of *Hygrophila auriculata* and *Blames mollis*. Similar results were recorded by Choudary *et al*. (2014), who evaluated the bio-activity of saponin isolated from *G. sylvestre* against *S. litura*. A maximum extended duration of 20.34 days was recorded at 0.1 per cent of the test compound against *S. litura.*

Similar to larval weight reduction, a dose-dependent pupal weight reduction was also observed for all the extracts. The highest pupal weight reduction of 29.57 per cent was recorded for methanol extract of aerial parts (SP1) (Fig 5). For all the extracts, pupal weight reduction ranged between 1 to 30 per cent. At lower doses, pupal weight reduction was not significantly different for all the extracts. A similar reduction in pupal weight of *S. litura* was reported by Govindachari *et al.* (1996), who tested bioactivity of salannin by diet incorporation method. The decrease in pupal weight may be due to a reduction in total lipids. The findings were similar to those of Abu El-Ghar *et al.* (1996). They found that treating sixth instar larvae of *Agrotis ipsilon* with ethanol extract of *Melia azedarach* resulted in a significant reduction in total lipids and pupal weight.

A maximum extended pupal duration of 13.54 days as compared to control (11.32 days) was recorded for methanol extract of roots (SP3). For all the extracts, at lower concentrations, pupal duration was not significantly different (Fig 6). Similar observation was reported by Torres *et al*. (2003) with extract of *Myrtillocactus geometrizans* on *S. frugiperda* and *Yucca periculosa* (Cespedes *et a*l., 2005). A prolonged pupal period of 13.08 days was observed for the leaf extract of *A. tagala* against *S. litura* by Baskar *et al.* (2011).

No larval mortality could be observed with any of the extracts; it may be due to the low dose of treatment. The results suggest that extracts from *S. trilobata* have only less contact toxicity. In contrast to our results, larval mortality was recorded by Junhirun *et al*. (2018) with ethyl acetate extract of leaves of *S. trilobata a*gainst *S. litura*, *S. frugiperda* and *P. xylostella* by topical application. It may be due to the difference in concentration and type of bioassay performed. Compounds like andrographolide, artemisinin and saponin have previous record of larval mortality on *S. litura*. The lower concentration of these compounds may be the reason for no larval mortality of *S. litura*. The increase in mortality over time suggest that the extracts have more physiological action than contact toxicity.

Abnormal pupae observed for all the extracts were not statistically different. A maximum of 4.16 per cent of abnormal pupae was recorded, but it was not dose dependent for any of the extract. Abnormal pupae were characterized by partial sclerotization, wrinkled appearance, *etc.*

Diet mixed with 0.1 per cent methanol extracts of roots (SP3) produced a maximum of 16.67 per cent larval pupal intermediates (Fig 7). Larval pupal intermediates are characterized by the presence of larval skin attached to partially emerged pupae. For all other extracts, no significant difference could be found for any of the treated concentrations. Similar results were obtained by Lit and Rejesuis (1990), who tested crude extract of *Aristalochia elegens* against *S. litura.*

Significant pupal mortality was recorded for all the extracts at the highest dose of treatment. Hexane extract of aerial parts (SP2) exhibited 41.61 per cent pupal mortality (Fig 8). It might be due to the presence of steroidal precursors in the extract. Our GC-MS/MS analysis shows that hexane extract of aerial parts contains more number of steroidal derivatives. Cholestane type steroids isolated from yams and *Colacasia esculenta* have biological activities against insects. Secondary metabolites like stigma sterol and sitosterol have larvicidal and pupicidal activity against *S. litura* (Abaza and Gaber, 2017). At lower concentrations of 0.005, 0.01 and 0.03 per cent pupal mortality was not significantly different, and it may be due to low doses of treatment. Malarvannan *et al.* (1999) reported that methanol extract of *Argemone*

mexicana increased pupal mortality in *S. litura* larvae fed with 0.1 per cent concentration of leaf extract. The growth regulatory activity increased over time suggests that the extracts may have activity on the endocrine system.

From all the extracts tested, more than 50 per cent of normal adults emerged. A minimum normal adult emergence was recorded for hexane extract of aerial parts (SP2). For methanol and hexane extract of roots of *S. trilobata,* maximum normal adult emergence was recorded than aerial extracts. GC-MS/MS and LC-MS/MS analysis of different extracts of *S. trilobata* revealed that a few phytochemical compounds are known to occur in roots. The lack active chemicals might be the reason for the reduced bioactivity of root extract as compared to aerial parts. There is no study on the activity of roots of *S. trilobata* were found for comparison.

5.4. GC-MS/MS and LC-MS/MS analysis *Sphagneticola trilobata* extracts

GC-MS/MS analysis of hexane extract of *S. trilobata* revealed the presence of derivatives of phytosteroids, derivatives of sesequiterpene lactones and derivatives of flavonoids. Silva *et al*., (2012) conducted GC-MS/MS analysis of essential oil obtained from *S. trilobata* and their results shows that *S. trilobata* is a good source of diterpenes, monoterpene and other lactone derivatives. They also found seasonal variation in active compounds in the essential oil. Our results of GC-MS/MS analysis revealed fewer compounds from hexane extract of roots of *S. trilobata*. The study of active constituents from roots of *S. trilobata* is lacking for a comparison. The less bioactivity of roots compared to aerial parts is attributed to the less bioactive compounds present in the roots.

LC-MS/MS analysis of methanol extract of *S. trilobata* revealed the presence of more number of phytochemicals compared to hexane extract. Bio active compounds like andrographolide, artemisinin, genistein, taxifolin were identified from methanol extract. Most of the compound were derivatives of terpenoids. Azizan *et al*. (2016) recorded similar results, who conducted LC-MS/MS analysis of water extract of *S. trilobata*. Their results shows the presence of diterpenoids, sesterpenoids, and compounds involved caffeine metabolism (methyl caffeate), alkaloids and

Fig 6. Effect of various extracts of *Sphagneticola trilobata* at various concentration on larval weight reduction of *Spodoptera litura*

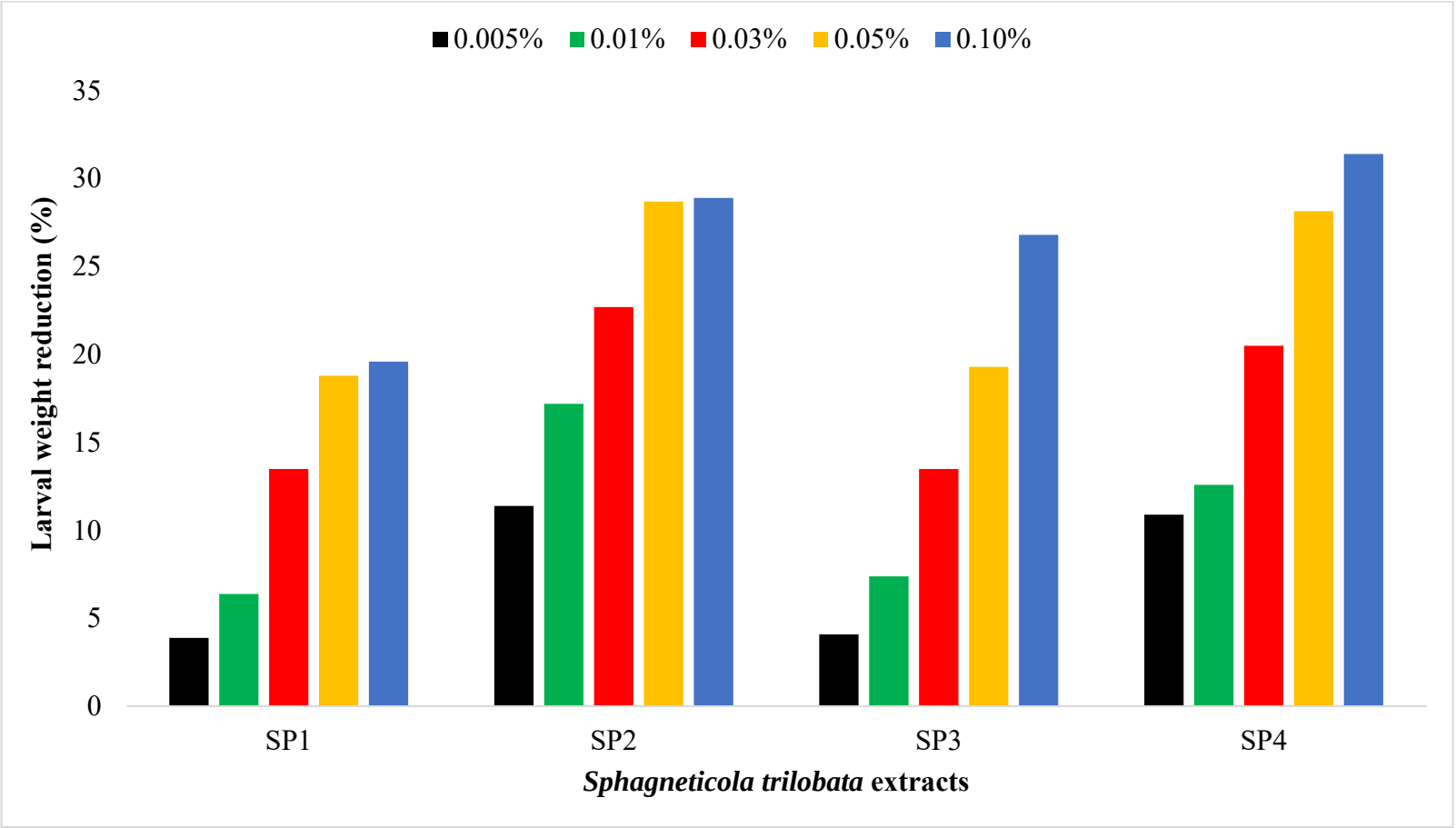


Fig 7. Effect of various extracts of *Sphagneticola trilobata* at various concentration on larval duration of *Spodoptera litura*

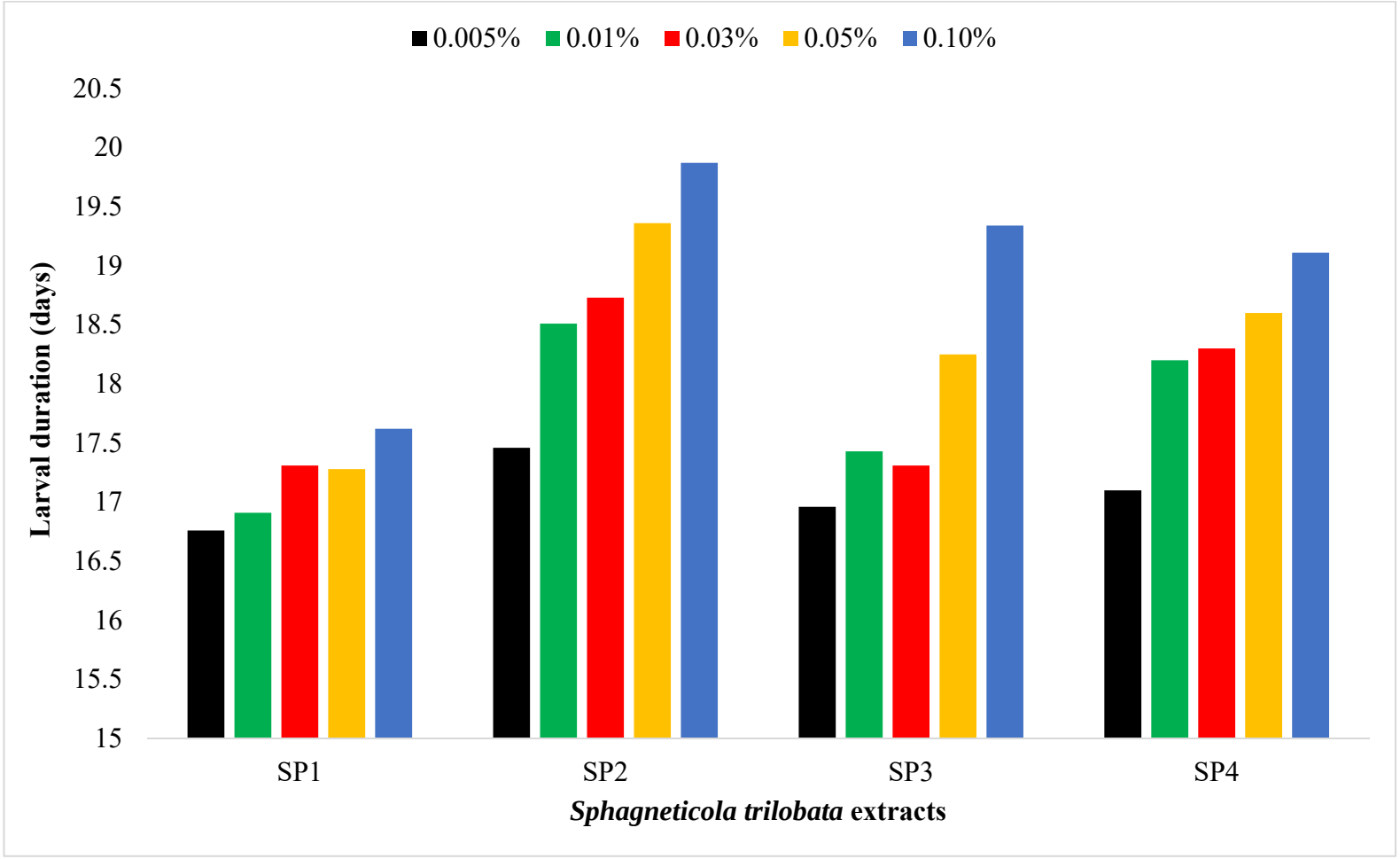


Fig 8. Effect of various extracts of *Sphagneticola trilobata* at various concentration on pupal weight reduction of *Spodoptera litura*

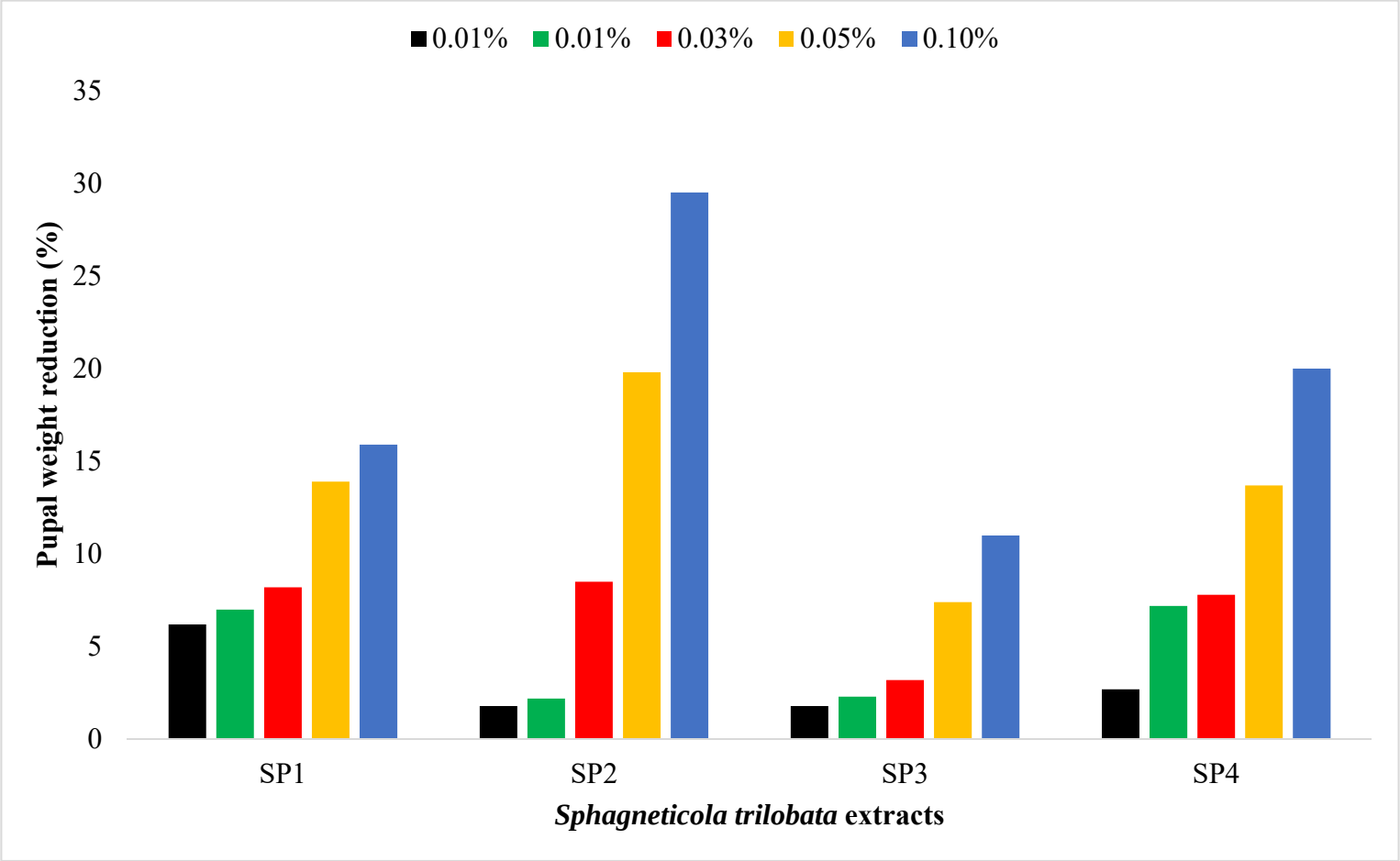


Fig 9. Effect of various extracts of *Sphagneticola trilobata* at various concentration on pupal duration of *Spodoptera litura*

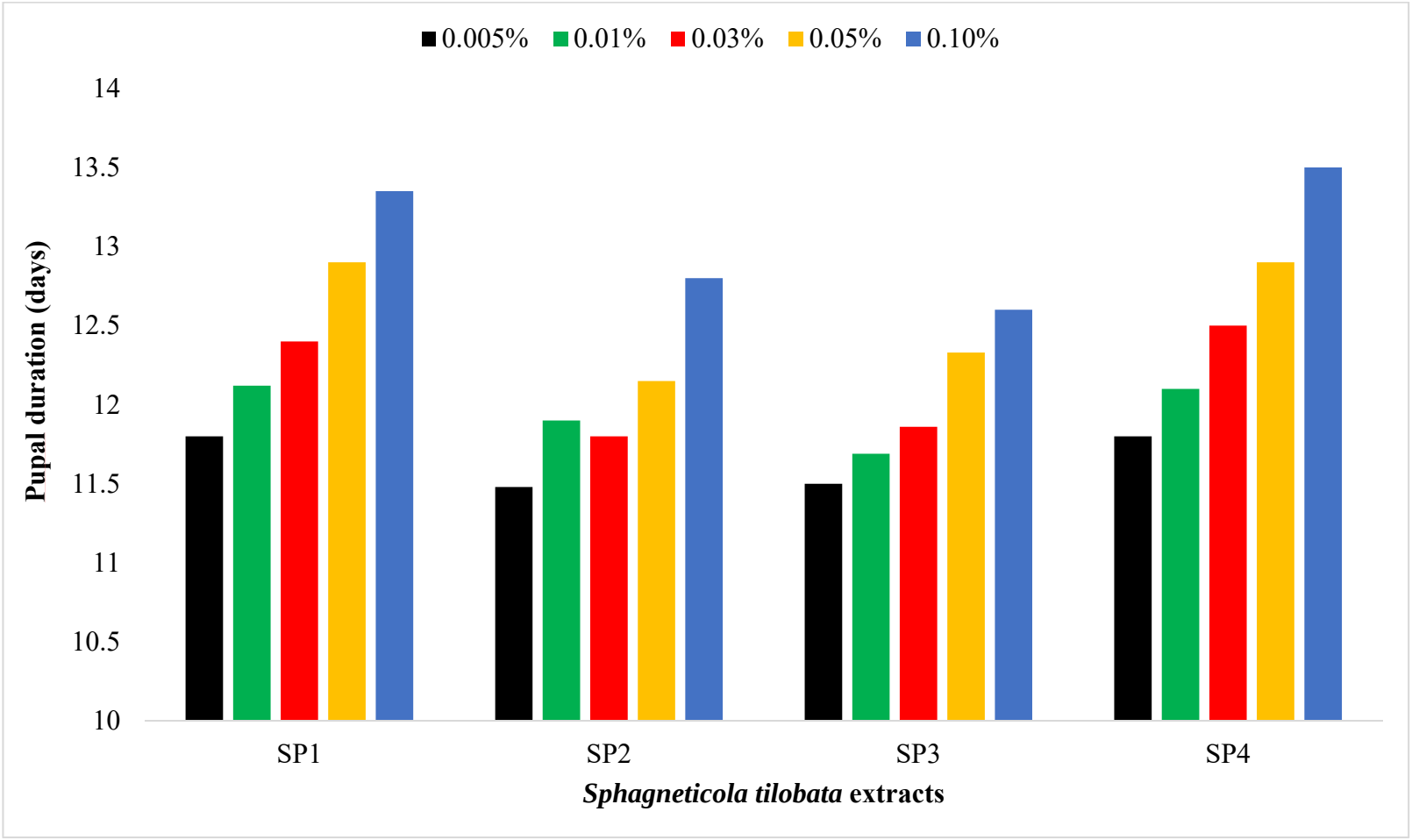


Fig 10. Effect of various extracts of *Sphagneticola trilobata* at various concentration on abnormal pupae (%) of *Spodoptera litura*

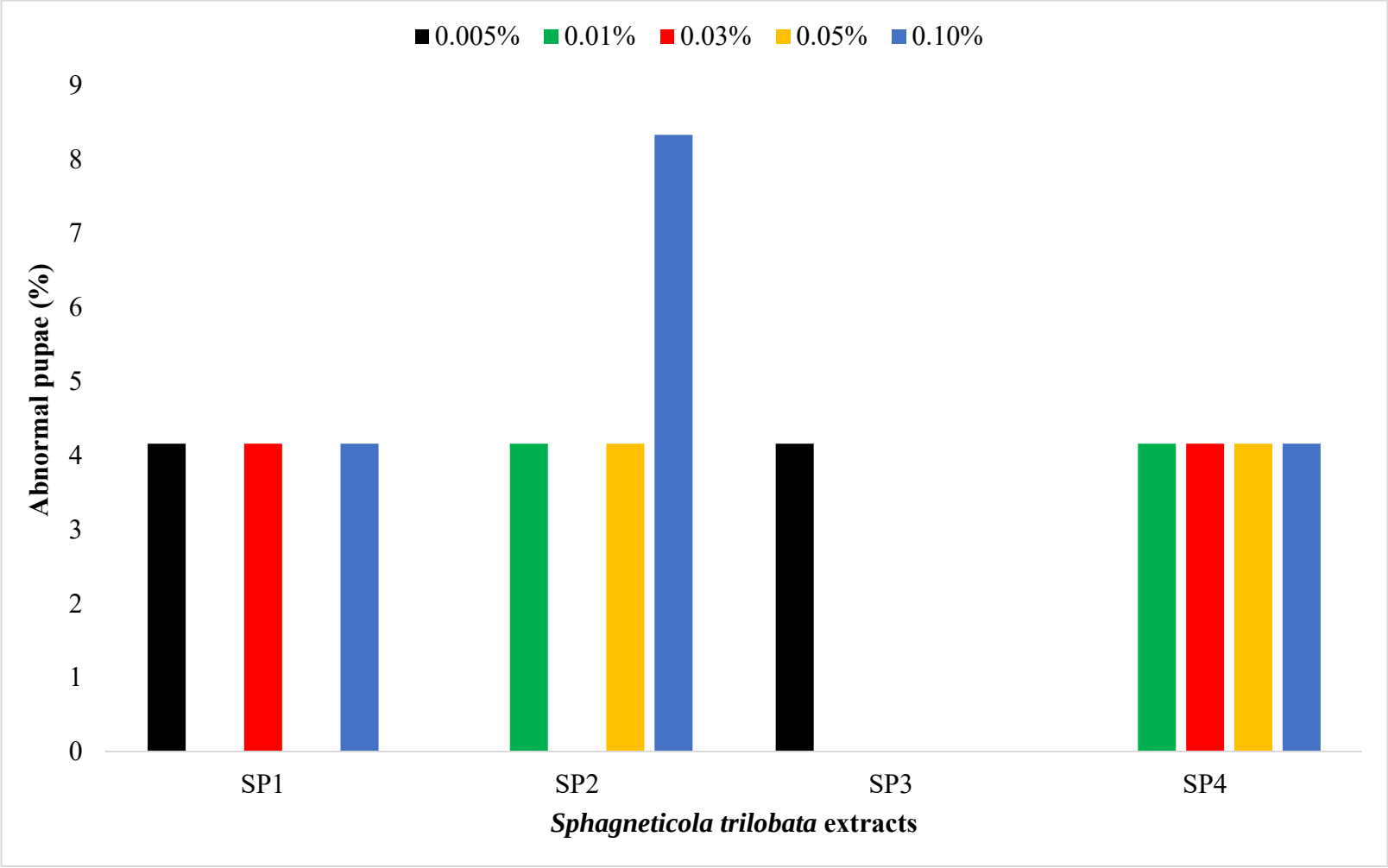


Fig 11. Effect of various extracts of *Sphagneticola trilobata* at various concentration on larval pupal intermediate (%) of *Spodoptera litura*

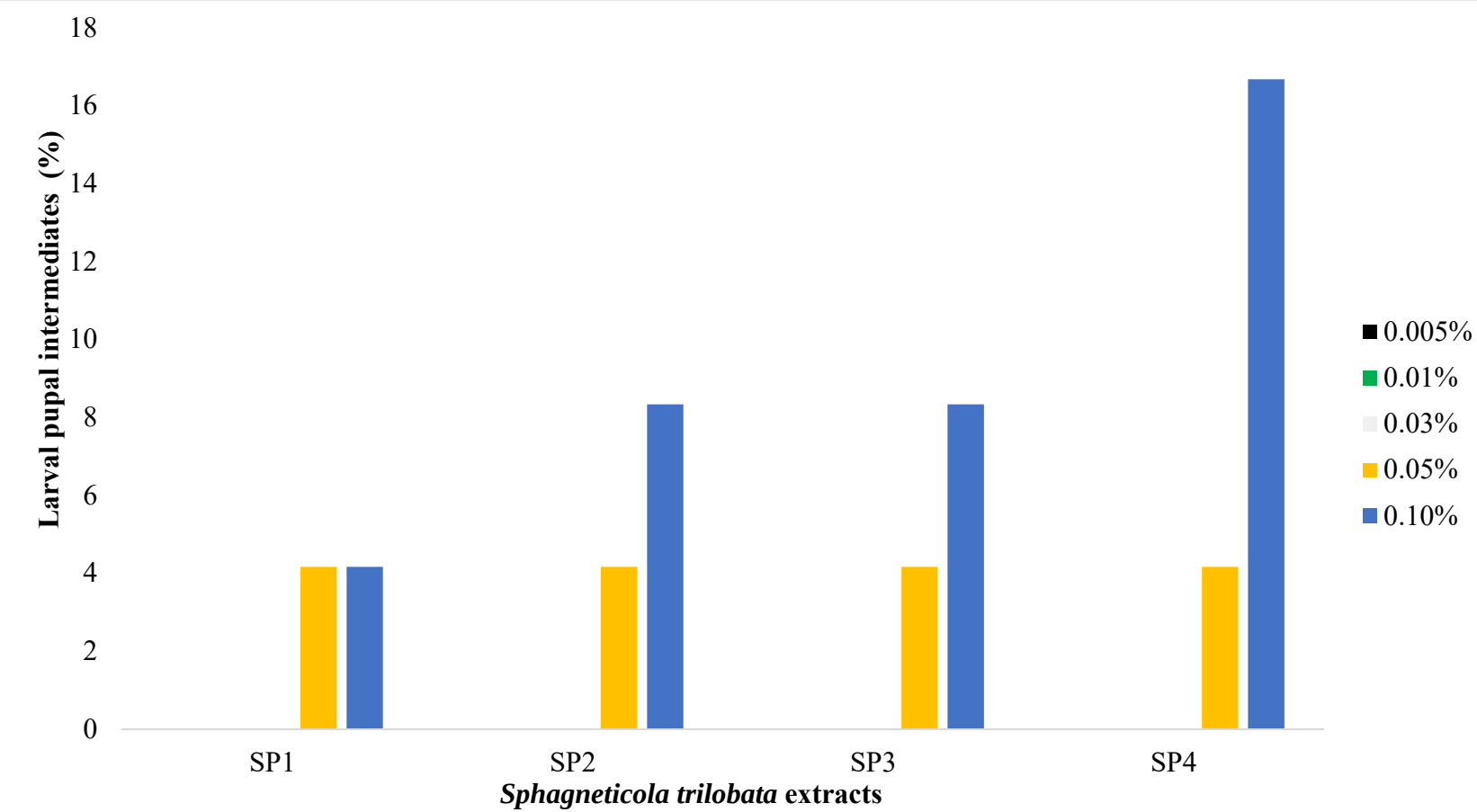


Fig 12. Effect of various extracts of *Sphagneticola trilobata* at various concentration on pupal mortality of *Spodoptera litura*

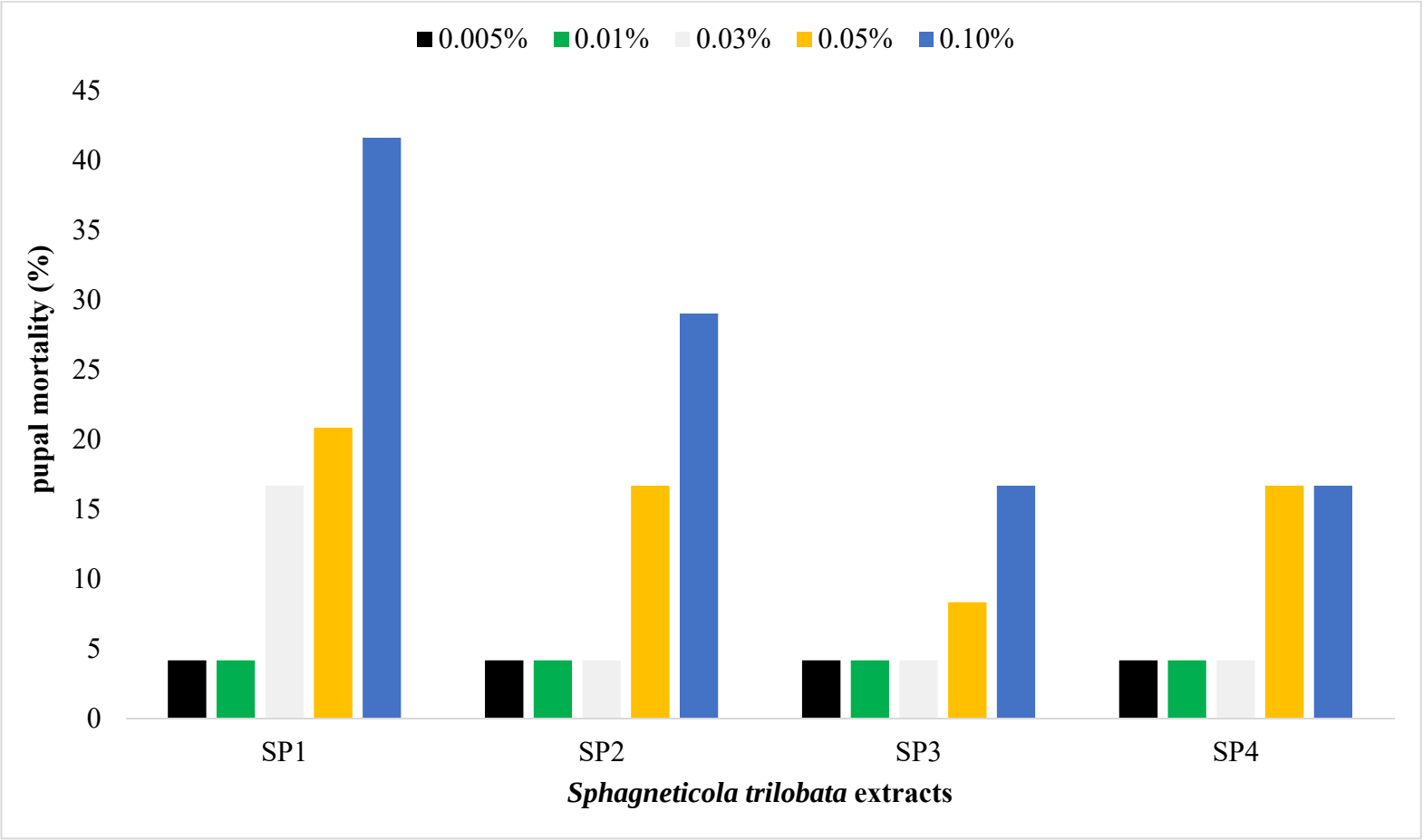
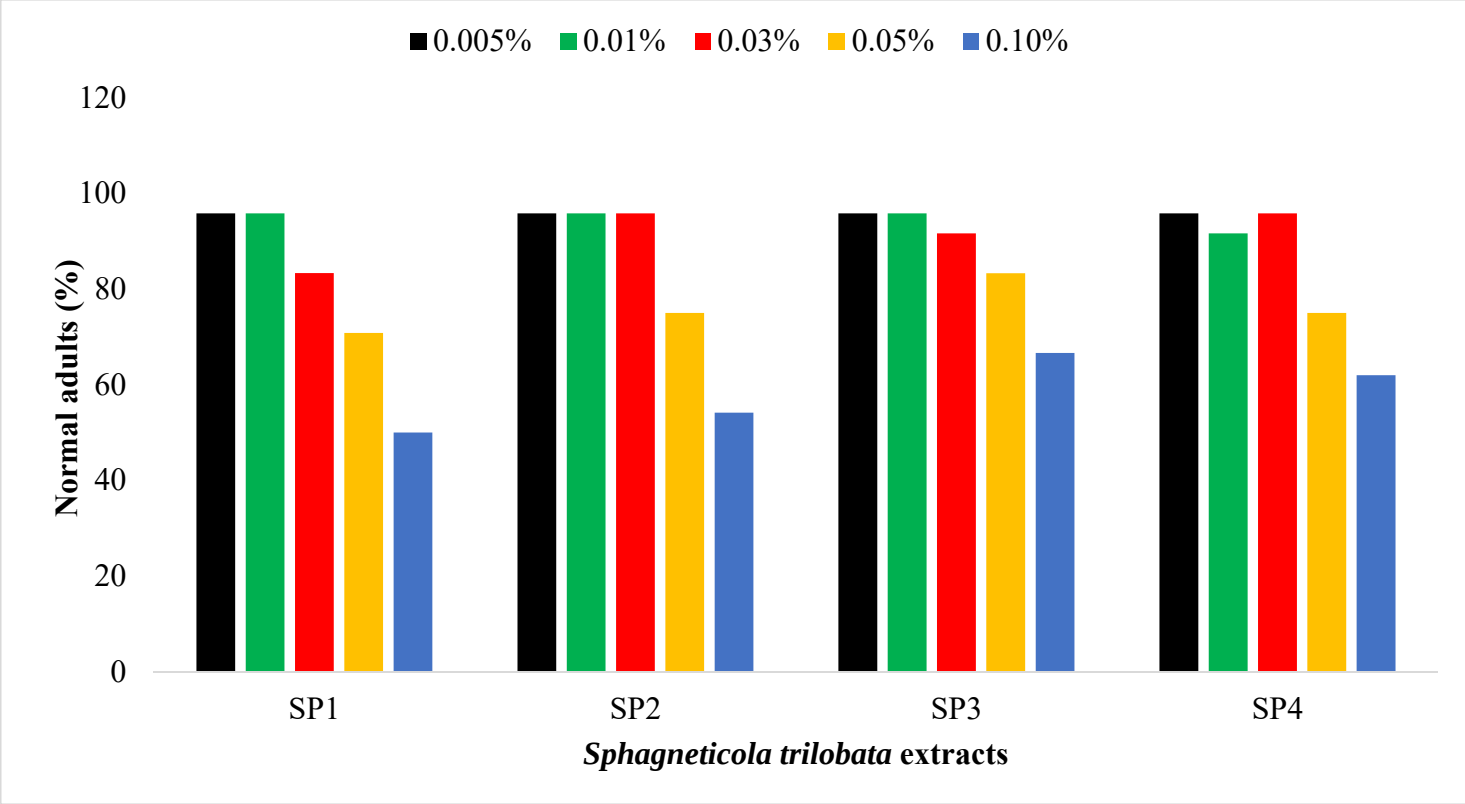


Fig 13. Effect of various extracts of *Sphagneticola trilobata* at various concentration on normal adult emergence of *Spodoptera litura*



monosaccharides in *S. trilobata* extract. A study on LC-MS/MS analysis of roots of *S. trilobata* is lacking for comparison.

Summary

6. SUMMARY

The study on the "Antifeedant and growth regulatory activity of *Sphagneticola trilobata* (L) Pruski on Tobacco caterpillar, *Spodoptera litura* (Fab) (Lepidoptera: Noctuidae)" was carried out at the Department of Agricultural Entomology, College of Agriculture, Vellanikkara, Thrissur, Kerala during 2020 to 2021. The objective was to evaluate biological activities of aerial and root extracts of *Sphagneticola trilobata* against *Spodoptera litura* and characterization of its constituents. Antifeedant activity of various extracts of *S. trilobata* were investigated by no-choice and choice leaf disc bio assay against seven days old *S. litura* larvae. Insect growth regulatory activity was tested by diet incorporation against five days old larvae of *S. litura.*

The salient findings of the present study are summarized below

- Methanol extract (SP2) was superior in both choice and no-choice method of antifeedant assay (maximum antifeedancy of 52.96 per cent in no-choice method).
- Compared to aerial parts, root extracts exhibited low level of antifeedant activity against *S. litura*.
- Lower concentrations of all the extracts of *S. trilobata* were not effective against *S. litura.*
- Larval weight reduction (LWR) increased in a dose dependent manner for all the extracts. Maximum larval weight reduction was recorded for SP4 (31.4 per cent) at 0.1 per cent of the extract.
- All the extracts extended the larval duration compared to control. Maximum extended larval duration of 19.87 days was observed with 0.1 per cent of SP2.
- Similar to LWR, pupal weight reduction (PWR) was also increased in a dose dependent manner with a maximum at 0.1 per cent of SP1 (29.57 per cent)
- No significant difference in pupal duration was observed for most of the treatments, a maximum extended duration was recorded for SP4 (13.54 days) at 0.1 per cent concentration.
- Observations on abnormal pupae were insignificant for any of the extracts
- No larval mortality could be obtained with any of the extracts

- Observations on larval pupal intermediates (LPI) were not dose dependent. Significant increase in formation of LPI was observed only for 0.05 per cent and 0.1 per cent of SP4.
- Pupal mortality increased in a dose dependent manner for all the extracts. Root extracts exhibited low level of mortality. Maximum pupal mortality of 41.61 per cent was recorded for 0.1 per cent of SP1.
- A minimum normal adult emergence of 50 per cent was observed for SP1 at 0.1 per cent of the extract. Methanol and hexane extracts of roots have less effect on adult emergence.
- Bio active compound from *S. trilobata* included terpenoids, phytosteroids and saponins. Among identified phytochemicals, taxifolin, artemisinin, andrographolide, soya saponin-1 have previous record of bio activity against *S. litura*.

The study points out that, among different extracts of *S. trilobata,* methanol extract of aerial parts (SP2) was strong antifeedant and hexane extract of aerial parts (SP1) was having more growth regulatory activity against *S. litura*. Roots of *S. trilobata* was less active against *S. litura*

REFERENCES

Abaza, A. M. and Gaber, M. M. 2017. Secondary metabolites of *Colocasia esculenta* extract as green insecticide against the cotton leaf worm, *Spodoptera littoralis* (Boisd.). *Egyptian Acad. J. Biol. Sci. F. Toxicol. Pest Control,* 9(1): 99-113.

Abu El-Ghar, G. A., Khalil, M. E., and Eid, T. M. 1996. Some biochemical effects of plant extracts in the black cutworm, *Agrotis ipsilon* (Hufnagel) (Lep: Noctuidae). *J. Appl. Entomol.* 120(1-5): 477-482.

Akthar, Y and Isman, M. B. 2004. Comparative growth regulatory and antifeedant effects of plant extracts and pure allelochemicals on four polyphagous insect species. *J. Appl. Entomol.* 128:32-38.

Armes, N. J., Wightman, J. A., Jadhav, D. R. and Ranga Rao, G. V., 1997. Status of insecticide resistance in *Spodoptera litura* in Andhra Pradesh, India. *Pesticide Sci*. 50(3): 240-248.

Arvigo, R and Balik, M., 1993. Bursera simaruba. *Rainforest Remedies. One*.

Asif, M., Parihar, K., Rehman, B., Ashraf Ganai, M., Usman, A., and Siddiqui, M. A., 2014. Bio-efficacy of some leaf extracts on the inhibition of egg hatching and mortality of *Meloidogyne incognita*. *Arch. Phytopathol. Plant Prot.* 47(8): 1015-1021.

Azizan, K. A., Ibrahim, S., Ghani, N. H. A. and Nawawi, M. F., 2016. LC-MS based metabolomics analysis to identify potential allelochemicals in *Wedelia trilobata*. *Rec. Natural Products*, 10(6):788.

Balekar, N., Nakpheng, T., and Srichana, T. 2014. *Wedelia trilobata* (L.): A phytochemical and pharmacological review. *Chiang Mai. J. Sci.* 41(3): 590-605.

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. Biiol. Sci.* 18(1): 23-27.

Benelli, G., Govindarajan, M., Altaha, M. S., Devanesan, S., and Maggi, F. 2018. High toxicity of camphene and γ-elemene from *Wedelia prostrata* essential oil against larvae of *Spodoptera litura* (Lepidoptera: Noctuidae). *Environ. Sci. Pollut. Res.* 25(11): 10383-10391.

Boussaada, O., Kamel, M. B. H., Ammar, S., Haouas, D., Mighri, Z., and Helal, A. N. 2008. Insecticidal activity of some Asteraceae plant extracts against *Tribolium confusum*. *Bull. Insectol.* 61(2): 283-289.

Caiyun, Z., Minglong, L., Jing, G., and Xinnian, Z. 2006. Study of the bioactivity of extracts from *Wedelia chinensis* Merr on Larvae of *Ostrinia furnacali*. *Chinese Agric. Sci. Bull.* 1:12-14.

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: 2481-2493.

Cespedes, C. L., Vázquez, M. M., Calderón, J. S., Salazar, J. R., and Aranda, E. 2001. Insect growth regulatory activity of some extracts and compounds from *Parthenium argentatum* on fall armyworm *Spodoptera frugiperda*. *Zeitschrift für Naturforschung C* 56(1&2): 95-105.

Choudhary, J. S., Srivastava, C., and Walia, S. 2014. Screening for antifeedant activity of *Gymnema sylvestre* leaf extracts against *Spodoptera litura* F. (Lepidoptera: Noctuidae). *The Bioscan*, 9(2): 633-638.

Fabrick, J. A., Yool, A. J., and Spurgeon, D.W. 2020. Insecticidal activity of marigold *Tagetes patula* plants and foliar extracts against the Hemipteran pests, *Lygus hesperus* and *Bemisia tabaci. PLoS One*, 15(5): 233-511. Available: https://journals.plos.org/ plosone/article?id=10.1371/journal.pone.0233511 [Accessed 23 march 2021].

Fucina, G., Rocha, L. W., Silva, G. F., Hoepers, S. M., Ferreira, F. P., Guaratini, T., Cechinel Filho, V., Lucinda-Silva, R. M., Quintão, N. L. M. and Bresolin, T. M.

B., 2016. Topical anti-inflammatory phytomedicine based on *Sphagneticola trilobata* dried extracts. *Pharmaceutical Biol* 54(11): 2465-2474

Gebbinck, E. A. K., Jansen, B. J. and de Groot, A., 2002. Insect antifeedant activity of clerodane diterpenes and related model compounds. *Phytochem*. *61*(7): 737-770.

Govindachari, *T.* R., Narasimhan N. S., Suresh, G., Patho, P. O., Gopalakrishnan, G., and Krishnakumari, G. N. 1996. Structure related antifeedant activity of some limonoids. *J. Chem. Ecol*. 21(10): 1585-1600.

Hernandez-Aro, M., Hernandez-Pérez, R., Guillen-Sanchez, D., and Torres-Garcia, S. 2020. Allelopathic Influence of Residues from *Sphagneticola trilobata* on Weeds and Crops1. *Planta daninha*, 34:81-90.

Huang, X., Shiyi, O. U., Shuze, T. A. N. G., Liang, F. U., and Jianzhong, W. U. 2006. Simultaneous determination of Trilobolide-6-O-Isobutyrates A and B in Wedelia trilobata by gas chromatography. *Chinese J. Chromatography,* 24(5): 499-502.

Ishaaya, I. 1986. Nutritional and allelochemical insect-plant interactions relating to digestion and food intake: Some examples. In: I. R. Miller and T. A. Miller (ed.) Insect-plant interactions. Springer, New York, pp. 191-22.

Isman, M. B., Wheeler, D. A., Vindas, P. E., and Arnason, J. T. 1990. Screening of *Costa rican* Trichilia species for biological activity against the larvae of *Spodoptera litura* (Lepidoptera: Noctuidae). *Biochem. Syst. Ecol.* 29: 347-358.

Junhirun, P., Pluempanupat, W., Yooboon, T., Ruttanaphan, T., Koul, O., and Bullangpoti, V. 2018. The study of isolated alkane compounds and crude extracts from *Sphagneticola trilobata* (Asterales: Asteraceae) as a candidate botanical insecticide for Lepidopteran larvae. *J. Econ. Entomol.* 111(6): 2699-2705.

Kaur, M., Saraf, I., Kumar, R., Singh, I. P., and Kaur, S. 2019. Bioefficacy of hexane extract of *Inula racemosa* (Asteraceae) against *Spodoptera litura* (Lepidoptera: Noctuidae). *Gesunde Pflanzen*, 71(3): 165-174.

Khater, K. S. and El-Shafiey, S. N., 2015. Insecticidal effect of essential oils from two aromatic plants against *Tribolium castaneum* (Herbst), (Coleoptera: Tenebrionidae). *Egyptian J. Biol. Pest Control*, 25(1): 129.

Kumoro, A. C., Hasan, M., and Singh, H. 2009. Effects of solvent properties on the Soxhlet extraction of diterpenoid lactones from *Andrographis paniculata* leaves. *Sci. Asia* 35: 306–330

Leite, A. G. B., Farias, E. T. N., Oliveira, A. P. D., Abreu, R. E. F. D., Costa, M. M. D., Almeida, J. R. G. D. S., Estevao, L. R. D. M., and Evencio-Neto, J. 2019. Phytochemical screening and antimicrobial activity testing of crude hydroalcoholic extract from leaves of *Sphagneticola trilobata* (Asteraceae). *Ciencia Rural,* 49(4): e20180639. Available: https://doi.org/10.1590/0103-8478cr20180639 [Accessed 14 February 2020]

Lit, M. C. and Rejesuis, B. M. 1990. Effects of *Aristalochia elegens* on common cut worm *Spodoptera litura. Philipp. Entomologist,* 8(2): 761-769.

Maggi, M.E., Mangeaud, A., Carpinella, M.C., Ferrayoli, C.G., Valladares, G.R. and Palacios, S.M., 2005. Laboratory evaluation of *Artemisia annua* L. extract and artemisinin activity against *Epilachna paenulata* and *Spodoptera eridania*. *J. Chemical. Ecol*, *31*(7):1527-1536

Mani, C. and Rao, P.J. 1998. Comparative biology of *Spodoptera litura* (Fab.) on semi-synthetic diet and natural food. *Shashpa*. 5:141-144.

Malarvannan, S., Senthil Kumar, S., Giridharan, R. and Nair, S., 1999. Bio-efficacy of *Argemone mexicana* against American bollworm, *Helicoverpa armigera* (Hubner), (Noctuidae: Lepidoptera). *Hexapoda. 2008a*, *15*:49-55.

Maldini, M., Sosa, S., Montoro, P., Giangaspero, A., Balick, M.J., Pizza, C. and Della Loggia, R., 2009. Screening of the topical anti-inflammatory activity of the bark

of *Acacia cornigera* Willdenow, *Byrsonima crassifolia* Kunth, *Sweetia panamensis* Yakovlev and the leaves of *Sphagneticola trilobata* Hitchcock. *J. Ethnopharmacol*, *122*(3): 430-43

Mardina, V., Ilyas, S., Harmawan, T., Halimatussakdiah, H., and Tanjung, M. 2009. Antioxidant and cytotoxic activities of the ethyl acetate extract of *Sphagneticola trilobata* (L.) J. F Pruski on MCF-7 breast cancer cell. *J. Adv. Pharma. Technol. Res.* 11(3): 123.

Matloub, A., Maamoun, A., Aziz, N. A., Samour, E., and El-Rafie, H. 2021. Eco-friendly secondary metabolites from *Conyza dioscoridis* against *Spodoptera littoralis. Egyptian J. Chem.* 64(1): 341-357.

Meena, A. K., Rao, M. M., Meena, R. P. and Panda, P., 2011. Pharmacological and phytochemical evidences for the plants of Wedelia Genus-A Review. *Asian J. Pharmaceutical Res*. 1(1): 7-12.

Meilan, W., Dezhi, Z., Qingjin, X., Rongrong, X., and Qianqiong, L. 2009. Chemical constituents from flower of *Wedelia trilobata*. *Asia-Pac. Tradit. Med.* 8: 321-323.

Miles, D. H., Chittawong, V., Payne, A. M., Hedin, P. A., and Kokpol, U. 1990. Cotton boll weevil antifeedant activity and antifungal activity (*Rhizoctonia solani* and *Pythium ultimum*) of extracts of the stems of *Wedelia biflora*. *J. Agric. Food Chem.* 38(7): 1591-1594.

Nie, C. R., Zeng, R. S., Luo, S. M., Li, H. S., Hong, M. Q., and Cheng, L. Q. 2004. Allelopathic potentials of *Wedelia trilobata* L. on rice. *Acta Agronomica Sinica,* 30(9): 942-946.

Nithin, R., Padmaja, V., Shijikumar, P. S., Shiji S. C. D., and Anny, P. 2011. Comparing the pharmacological activities of *Sphagneticola calendulaceae* and *Sphagneticola trilobata* an over view. *World J. Pharmacy Pharma. Sci.* 6: 457-467.

Pathrose, B. 2006. Antifeedant and growth regulatory effects of kalmegh, *Andrographis paniculata* against *Spodoptera litura* (Fab). MSc (Ag) thesis, Indian Agricultural Research Institute, New Delhi, 92p.

Phankaen, Y. A., Manaprasertsak, W., Pluempanupat, O., Koul, Y., Kainoh, and Bullangpoti, V. 2017. Toxicity and repellent action of *Coffea arabica* against *Tribolium castaneum* (Herbst.) adults under laboratory conditions. *J. Stored Product Res.* 71: 112–118.

Prasanna, K. S., Reddy, G. J., Kiran, M., and Raju, K. T. 2019. Biological activities and phytochemical constituents of trailing *Daisy trilobata*: A review. *J. Drug Delivery Ther.* 9(4): 888-892.

Qi, S. S., Dai, Z. C., Zhai, D. L., Chen, S. C., Si, C. C., Huang, P., Wang, R. P., Zhong, Q. X., and Du, D. L. 2014. Curvilinear effects of invasive plants on plant diversity: Plant community invaded by *Sphagneticola trilobata*. *PLoS One*9 (11):113964.Available:https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0113964 [Accessed 12 march 2020]

Qinlong, G. U ., KE, M., and Wang, F. 2012. Antifeedant and toxic effects of methanol extracts from *Wedelia chinensis* on *Cnaphalocrocis medinalis* Larvae. *Acta Scientiarum Naturalium Universitatis Sunyatseni*, 51:103-106.

Rathi, J. and Gopalakrishnan, S. 2005. Insecticidal activity of aerial parts of *Synedrella nodiflora* Gaertn (Compositae) on *Spodoptera litura* (Fab.). *J. Cent. Eur. Agric.* 6(3): 223-228.

Ray, D. P., Dutta, D., Srivastava, S., Kumar, B., and Saha, S. 2013. Insect growth regulatory activity of *Thevetia nerifolia* Juss. against *Spodoptera litura* (Fab.). *J. Appl. Bot. Food Qual.* 85(2): 212.

Roopa, M. S., Rhetso, T., Shubharani, R., and Sivaram, V. 2021. Insecticidal potentials of dry powder and solvent extracts of *Tithonia diversifolia* (Hemsl.) A. Gray flowers against rice meal moth, *Corcyra cephalonica* (Stainton). *Acta fytotechn zootechn*, 24(2): 94-100.

Schoonhoven, L. M. 1982. Biological aspects of antifeedants. *Entomologia Experimentalis et Applicata*, 31: 57-69.

Schoonhoven, L. M., Jermy, T and Van, J. J. A. 1998. Insect Plant Biology, from physiology to evolution. Chapman and Hall, London.

Schulter U. 1985. Occurrence of weight gain reduction and inhibition of metamorphosis and storage protein formation in last instars of Mexican bean beetle, *Epilachna varivestris*, after injection of azadiractin. *Entomologia Experimentalis et Applicata,* 39: 191-195.

Silva, C.J.D., Barbosa, L.C., Demuner, A.J., Montanari, R.M., Francino, D., Meira, R.M. and Souza, A.O.D., 2012. Chemical composition and histochemistry of *Sphagneticola trilobata* essential oil. *Revista Brasileira de Farmacognosia*, 22(1):482-48Simarmata, M., Susanti, L. and Setyowati, N., 2016. Utilization of manure and green organic composts as alternative fertilizers for cauliflower production. *J. Agric. Technol.* 12(2): 311-319.

Singh, R. P. and Pant, N. C. 1980. *Hymenocallis littoralis* Salisb as antifeedant to desert locust, *Schistocera gregaria* Forsk. *Indian J. Entomol.* 42(3): 460-464.

Taylor, W. G., Fields, P. G., and Sutherland, D. H. 2004. Insecticidal components from field pea extracts: Soyasaponins and lysolecithins. *J. Agric. Chem.* 52: 7484-7490.

Torres, P., Avila, G., De Vivar, A. R., Garcia, A. M., Marin, J. C., Aranda, E., and Cespedes, C. L. 2003. Antioxidant and insect growth regulatory activities of stilbenes and extract from *Yucca periculosa*. *Phytochem*. 64: 463-473.

Vats, T. K., Rawal, V., Mullick, S., Devi, M. R., Singh, P., and Singh, A. K. 2019. Bioactivity of *Ageratum conyzoides* (L.) (Asteraceae) on feeding and oviposition behaviour of diamondback moth *Plutella xylostella* (L.) (Lepidoptera: Plutellidae). *Int. J. Trop. Insect Sci.* 39(4): 311-318.

Verma, R. S., Padalia, R. C., Chauhan, A., and Sundaresan, V. 2014. Essential oil composition of *Sphagneticola trilobata* (L.) Pruski from India. *J. Essential Oil Res.* 26(1): 29-33.

Wang, L.Y., Lu, Y. Q., and Luo, Y. P. 2009. Antifeeding activities of 45 south herbs extracts against *Spodptera litura* Fabricius. *Hubei Agric. Sci*. *48*: 628-630.

Wanna, R., Darikaunphan, W. K., Wongsawas, M., and Khaengkhan, P. 2021. The toxicity and bioactivity of climbing Wedelia powder and essential oil against maize weevil. *J. Sustain. Sci. Manag.* 16(2): 105-113.

Yanping, C. Z. F. Y. L. 2003. Repellent and control effect of extract from *Wedelia chinensis* Merr. On *Aphis medicaginis* Koch. *Chinese J. Trop. Crops,* 3:15-17.

Zhang, X., Huo, Z., You, C., and Hu, F. 2014. Effects of volatiles in twenty non-host plants on the repellent and attractive behaviours of brown plant hopper, *Nilaparvata lugens*. *J. South China Agric. Uni*. 35(3): 63-68.

ANTIFEEDANT AND GROWTH REGULATORY ACTIVITY OF *Sphagneticola trilobata* (L) Pruski ON TOBACCO CATERPILLAR *Spodoptera litura* (Fab) (Lepidoptera: Noctuidae)

by

RAHUL RAJ M

(2019-11-134)

ABSTRACT OF THE THESIS

Submitted in partial fulfillment of the requirement for the degree of

Master of Science in Agriculture

Faculty of Agriculture

Kerala Agricultural University

DEPARTMENT OF AGRICULTURAL ENTOMOLOGY

COLLEGE OF AGRICULTURE

VELLANIKKARA, THRISSUR – 680656

KERALA, INDIA

2021

ABSTRACT

One of the most important aspects of agriculture is pest management. Pesticides used in the past produced a variety of environmental problems, including ecological imbalances, soil fertility loss, and marine life degradation. Conventional pesticides have also been linked to a number of significant and detrimental effects on human and animal health, including severe malignancies, neurological diseases, hormone disruptions, and reproductive troubles. Botanical pesticides are found to be an effective alternative to conventional pesticides for pest management. *Sphagneticola trilobata* (L.) Pruski, is an herb included in the Asteraceae and is well known for its medicinal properties, information about its bioactivity against insects is very less. Hence, in this study we evaluated the antifeedant and growth regulatory activity of various extract of *Sphagneticola trilobata* against *Spodoptera litura*.

Dried and powdered *S. trilobata* plant materials were sequentially extracted with hexane and methanol followed by concentrated in *vacuo* in a rotary evaporator at a lower temperature to obtain crude extracts of *S. trilobata.* Hexane extracts of aerial parts and roots were named as SP1 and SP3 and methanol extract of aerial parts and roots were named as SP2 and SP4. The yield of methanol extract of aerial parts (5.67% w/w) was higher followed by methanol extract of roots (2.32% w/w), hexane extract of aerial parts (1.96% w/w) and hexane extract of roots (1.23% w/w)

The antifeedant activity of various extracts of *Sphagneticola trilobata* tested in no-choice leaf disc bioassay revealed that methanol extract of aerial parts of *S. trilobata* had the maximum antifeedant activity of 52.10 per cent at 0.1 per cent of the extract against *S. litura* after 24h of feeding. Compared to aerial extracts, root extracts were less active against *S. litura*. At lower concentrations, the antifeedant activity of all the extract were not much prominent and are not statistically significant. The antifeedant activity of all the extract increased in 48h experiment except for 0.1 per cent concentration. The decreasing order of antifeedancy of four extracts was SP2>SP1>SP4>SP3.

Similar results were recorded in the choice method of bioassay of extracts against *S. litura*. Maximum antifeedant activity of 46.65 per cent was obtained for methanol extract of aerial parts (SP2) after 24h of feeding. At lowest concentrations of 0.005, 0.01 and 0.03 per cent none of the extracts exhibited significant difference in activity. Increasing concentration beyond 0.03 per cent had a significant effect on antifeedancy in the choice method. As similar to the no-choice method, reduced activity was recorded after 48h of feeding. Maximum antifeedant activity of 38.81 per cent was recorded for SP2 after 48h of feeding. Similar to the no-choice method, root extracts exhibited a low level of activity against *S. litura.*

Insect growth regulatory activity of various extracts was evaluated by diet incorporation method against five-day old larvae of *S. litura.* A maximum larval weight reduction of 31.4 per cent was recorded for SP4 at 0.1 per cent of the extract. A significant increase in larval duration compared to control was recorded for SP2 extracts at higher concentrations. Similar to larval weight reduction, a dose-dependent pupal weight reduction was recorded for SP1 (29.57 per cent at 0.1 per cent of the extract). Compared to the other three extracts, pupal weight reduction was very less for SP3. Pupal duration increased to a maximum period of 13.54 days at 0.1 per cent of SP4. Observations on abnormal pupae were not significant for any of the extracts. No larval mortality could be obtained with any of the treatments. A maximum of 16.7 per cent larval pupal intermediates was recorded for SP4 at the highest dose, but it was not significantly different from other treatments. Similar to larval mortality, no pupal adult intermediates were recorded for any of the extracts. Maximum pupal mortality of 41.00 per cent was recorded at 0.1 per cent of SP1, and it was significantly different from other treatments. For all the extracts, pupal mortality was dose-dependent. Minimum adult emergence was noticed for SP1 at 0.1 per cent of the extract.

GC-MS/MS and LC-MS/MS analysis of active fractions revealed that more phytochemicals are present in aerial parts than roots. The higher activity of aerial parts might be due to the presence bioactive compounds like andrographolide, artemisin,

genistein and taxifolin. Most of the chemicals identified were derivatives of steroids, terpenoids and flavonoids.

The study results indicate that methanol extract of aerial parts of *S. trilobata* has strong antifeedant activity against *S. litura*. All other extracts were relatively less active against *S. litura*. Insect growth regulatory activity was maximum for hexane extract of aerial parts. All the extracts adversely affected the growth and devolvement of *S. litura*. The absence of larval mortality indicates that all the extracts have less contact toxicity.