BIOACTIVITY OF ESSENTIAL OILS AGAINST INSECT PESTS OF BRINJAL

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(2019-11-047)

DEPARTMENT OF AGRICULTURAL ENTOMOLOGY

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2022

DECLARATION

I, hereby declare that this thesis entitled "**BIOACTIVITY OF ESSENTIAL OILS AGAINST INSECT PESTS OF BRINJAL**" is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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Certified that this thesis entitled "**BIOACTIVITY OF ESSENTIAL OILS AGAINST INSECT PESTS OF BRINJAL**" is a record of research work done independently by Mr. Manoj K 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.

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Manoj K

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LIST OF ABBREVIATIONS

a.i	Active ingredient
ANOVA	Analysis of variance
&	And
et al.	And others
AI	Antifeedant Index
@	At the rate of
cm	Centimeter
CIBRC	Central Insecticides Board and Registration committee
CRD	Completely Randomized Design
CL	Confidence Limit
CD	Critical Difference
DAP	Days After Planting
DAS	Days After Spraying
DAT	Days After Treatment
^{o}C	Degree Celsius
^{o}C/min	Degree Celsius per minute
ECI	Efficiency of Conversion of Ingested Food
EPA	Environmental Protection Agency
EO’s	Essential oils
FDI	Feeding Deterrence Index
Fig.	Figure
FDA	Food and Drug Administration
GC-FID	Gas Chromatography Flame Ionization Detector
GC-MS	Gas Chromatography Mass Spectrometry
GRAS	Generally Recognized as Safe
g	Gram
h	Hour
HAT	Hour After Treatment

Kg	Kilogram
LC	Lethal Concentration
LD	Lethal Dose
LT	Lethal Time
L: D	Light : Dark
LRI	Linear Retention Indices
L ha^{-1}	Litre per hectare
μg	Microgram
μg cm^{-2}	Microgram per centimeter square
μL	Microlitre
mL cm^{-2}	Microlitre per centimeter square
μL L^{-1}	Microlitre per litre
mL min^{-1}	Microlitre per minute
mg cm^{-3}	Milligram per centimeter cube
mg cm^{-2}	Milligram per centimeter square
mg mL^{-1}	Milligram per milliliter
viz.	Namely
Op stat.	Operational Status
ppm	Parts per million
%	Per cent
PR	Per cent Repellency
RCR	Relative Consumption Rate
RGR	Relative Growth Rate
RH	Relative Humidity
SE	Standard Error
SC	Suspension Concentrate
i.e.	That is
$	US Dollar
v/v	Volume per volume
WG	Water-dispersible Granule
WASP	Web Agri Stat Package

Introduction

1. INTRODUCTION

Brinjal, *Solanum melongena* L. (Polemoniales: Solanaceae) is one of the major cultivating vegetable crops in India. Since its fruit resembles the shape of an egg, it's also referred as "Egg plant". Brinjal can be grown throughout the year in different agro-climatic zones. Brinjal is grown in an area of 7.41 lakh ha in India (Department of Agriculture and Farmers Welfare, 2020) with an annual production of 13.15 million metric tonnes (Statista Research Department, 2021). India is the second largest producer after China with productivity of 17.74 metric tons per hectare. Its high versatile use, high consumer preference and high productivity (Choudhary and Gaur, 2009) make brinjal fruit to be referred as king of vegetables and common man's vegetable.

Brinjal is infested by more than 36 insect pests from the nursery stage to harvest (Reghupathy *et al*., 1997). Among the insect pests, the important and the destructive ones are the spotted beetle, *Henosepilachna viginitioctopunctata* (Fabricius); brinjal ash weevil, *Myllocerus subfasciatus* (Guerin-Meneville); brinjal brown leafhopper, *Cestius phycitis* (Distant); shoot and fruit borer, *Leucinodes orbonalis* (Guen.); lacewing bug, *Urentius hystricellus* (Richter); stem borer, *Euzophera perticella* (Rag.); leaf roller, *Eublemma olivacea* (Wlk.); whitefly, *Bemisia tabaci* (Gennadius); mealy bug, *Coccidohystrix insolita* (Green); red spider mite, *Tetranychus ludeni* (Zacher).

Hadda beetle, *H. viginitioctopunctata* is a major chewing pest of brinjal. Grubs and adults scrape the green content of brinjal leaves and feed on the epidermis, making the leaves exceedingly papery. When the infestation is severe, the leaves become completely skeletonised and it also feeds on the flower calyx (Kunjwal and Srivastava, 2018). Fruit reduction in yield up to 60% has been reported in brinjal (Mall *et al*., 1992). Mealy bug, *C. insolita* is a major sucking pest of brinjal, where adults and nymphs congregate on the lower surface of the leaf and suck the green sap leading to yellowing, crinkling and drooping of leaves and flowers (Marimuthu *et al*., 2018). The per cent yield loss due to mealy bug in brinjal is upto 10-60% (Bose *et al.*, 2020).

Pest management is facing economic and ecological challenge worldwide due to human and environmental hazards caused by majority of chemical pesticides. The use of conventional insecticides even though helped in reducing pest incidence, lead to many problems including pollution to the environment, pesticide residue in the produce, development of resistance by major insect pests, pest outbreak and escalation of the cost of production. These limitations are pushing the discovery and development of less harmful products. One of the most promising solutions is the exploitation of plant-based pesticides. Currently, bio pesticides comprise a value of about $3 billion worldwide, accounting for five per cent of the total crop protection market (Marrone, 2014; Olson, 2015).

Essential oils (EO's) are important alternative of botanical pesticides. Their safety profile is guaranteed by the fact that most of EO's have been recognized as Generally Recognized as Safe (GRAS) substances by the Food and Drug Administration (FDA) and by the Environmental Protection Agency (EPA) of the United States (Brut, 2004). One of the most promising aspects in the exploitation of EO's is their lack of toxicity on mammals; they are generally harmless for the environment when compared with synthetic pesticides (Pavela and Benelli, 2016). For these reasons, a possible residue of EO-based pesticides on crop does not constitute a risk for human health. Biopesticidal potential of essential oils (EO's) from plant families Myrtaceae, Lamiaceae, Asteraceae, Apiaceae and Rutaceae have been reviewed by many authors (Isman, 2000; Rajendran and Sriranjini, 2007; Tripathi *et al*., 2009).

Basil oil is extracted from the leaves of *Ocimum basilicum* L. (Lamiaceae) mainly by steam distillation. There are several reports on the insecticidal activity of basil oil. Basil oil act as larvicide for control of mosquitoes (*Aedes aegypti* Linnaeus) (Chantraine *et al*., 1998). The essential oil also has a prominent insecticidal effect of storage pests, *Trogoderma granarium* Everts and *Tribolium castaneum* (Herbst) (Nenaah and Ibrahim, 2011). In addition to the proven contact toxicity, the essential oil has repellent (Shadia *et al.*, 2007) and antifeedant effect (Kostic *et al.*, 2008) on insect pests.

Eucalyptus oil is distilled from the leaves of *Eucalyptus globules* Labill, a genus of the Myrtaceae plant family native to Australia and widely grown worldwide. The oil has insecticidal (Maciel *et al.,* 2010), repellent effect (Yang and Ma, 2005), fumigation toxicity (Channoo *et al.*, 2002) against insect pests and also have antimicrobial (Damjanovic-Vratnica *et al.*, 2011), acaricidal (Pirali-Kheirabadi *et al.,* 2009) and nematicidal (Pandey *et al.,* 2000) activity.

Citronella oil is from the leaves and stems of various cymbopogon plants (Lemongrass) (Poaceae). Citronellal, citronellol and geraniol are some of the major chemical components found in the essential oil, which are responsible for insecticidal property (Arancibia *et al.*, 2014). Citronella oil is used in the management of post-harvest losses due to storage pests (Bean weevil, *Acanthoscelides obtectus* (Say)) (Rodriguez-Gonzalez *et al.,* 2019). The essential oil is used as a plant-based insect repellent in the US since 1948 (Maia and Moore, 2011).

Orange oil isolated from the fruit peel, leaf and flowers of sweet orange (*Citrus sinensis* (L.)) (Rutaceae) has more than 200 components that were identified and quantified. Limonene is the most abundant component in both the ripe and unripe fruit peel oils, accounting for 80.14 and 80.93 per cent respectively (Hamdan *et al.,* 2013). Orange oil has both contact and fumigant toxicity against various insect pests (*Sitophilus zeamais* Motschulsky and *T. castaneum*) (Kim and Lee, 2014).

In the present investigation entitled "Bioactivity of essential oils against insect pests of brinjal" where, four essential oils *viz.,* basil oil, eucalyptus oil, citronella oil and orange oil were screened for their effectiveness in managing pests of brinjal in laboratory and field conditions with the following objectives.

- Laboratory evaluation of the toxicity of essential oils against mealy bug and hadda bettle.
- Field evaluation of essential oils against insect pests of brinjal and its safety to natural enemies by pot culture experiment.
- Characterization of essential oils.

Review of literature

2. REVIEW OF LITERATURE

Brinjal, *S. melongena* is a year-round crop that grows in a variety of locations. Since the crop is cultivated throughout the year, pest infestation is a common problem in brinjal. Brinjal crop is infested by a plethora of insect pests in which mealy bug (*C. insolita*) and hadda beetle (*H. vigintioctopunctata*) are categorized as major pests. Despite the fact that many chemical pesticides have been designed to combat these pests, their lack of environmental safety and health risks to consumers lead to the search of more effective and safe botanical insecticides.

In the present investigation four essential oils *viz.*, basil oil, eucalyptus oil, citronella oil and orange oil were screened against the insect pests of brinjal through laboratory and field evaluation and the effective oils were further chemically characterised by GC-MS/GC-FID. The literatures on this topic are provided in this chapter under the headings below.

2.1 LABORATORY SCREENING OF ESSENTIAL OILS AGAINST INSECT PESTS

Since the literature on the laboratory screening of essential oils against the insect pests of brinjal is scanty, their effectiveness against pests of other crops is discussed here.

2.1.1 Basil Oil

Chang *et al.* (2009) reported that the lethal time for 90 per cent mortality or knockdown (LT_{90}) of the three fruit fly species *Ceratitis capitata* (Wiedemann), *Bactrocera dorsalis* (Hendel) and *Bactrocera cucurbitae* (Coquillett) to 10 per cent of the test chemicals i.e., basil oil, trans-Anethole, estragole and linalool were between 8 and 38 minutes. The toxic action of basil oil on *C. capitata* occurred significantly faster than on *B. cucurbitae* and *B. dorsalis* and showed a steep dose-response relationship.

An experiment on fumigant toxicity of *O. basilicum* oil on *Callosobruchus chinensis* (L.) reported that, the oil at a dose of 1 μL per 38.5 ml air caused 100 per cent mortality at adult stage. Regarding the oviposition deterrent activity, *O. basilicum* oil achieved 100 per cent oviposition deterrence at a dose of 0.5 μL per 38.5 mL air and the eggs failed to hatch on using oil at a dose of 0.6 μL per 38.5 mL of air (Abdel-Salam, 2010).

Karamaouna *et al*. (2013) reported that the LC_{50} values of the citrus, peppermint and thyme-leaved savory essential oils ranged from 2.7 to 8.1 mg mL^{-1}, and the LC_{50} values of lavender and basil oil ranged from 19.8 to 22.5 and 44.1 to 46.8 mg mL^{-1} respectively against grape vine mealy bug (*Planococcus ficus* Signoret). The citrus oil recorded high insecticidal activity and lack of any phytotoxic effect on grape vine and suggested that lemon and orange peels were the most attractive botanical sources for the production of alternative plant protection products against *P. ficus*.

Kim and Lee (2014) did an experiment where, basil and orange oils showed strong fumigant and contact toxicity against maize weevil (*S. zeamais*) and red flour beetle (*T. castaneum*). As a toxic fumigant, the basil oil was more effective (LC_{50} = 0.014 and 0.020 mg cm^{-3}) than the orange oil (LC_{50} = 0.106 and 0.130 mg cm^{-3}) against *S. zeamais* and *T. castaneum* adults respectively at 24 hours of treatment. The contact toxicity of basil oil was also more than orange oil.

Saroukolai *et al*. (2014) evaluated the toxicity of *O. basilium* essential oil against 4^{th} larvae and adults of *Leptinotarsa decemlineata* (Say). The LC_{50} values of the essential oil against 4^{th} instar larvae and adults of *L. decemlineata* were 103.58 and 196.35 ppm respectively.

de Souza *et al*. (2016) evaluated the LC_{50} and LC_{100} of four essential oils *viz., O. basilicum, Mentha spicata* L., *Croton pulegiodorus* Baill. and *Citrus aurantium* L. *on Rhizopertha dominica* (F.). The higher toxicity was in *O. basilicum* followed by *M. spicata*, *C. pulegiodorus* and *C. aurantium.* The essential oil of *O. basilicum* exhibited strong fumigant toxicity against *R. dominica* adults, with a LC_{50} value of 17.67 μL L^{-1} air and LC_{100} value of 27.15 μL L^{-1} air.

Mead (2018) investigated the toxicity of basil oil on first instar larvae of *Spodoptera littoralis* (Boisduval) through leaf dip bioassay method using castor bean leaf. Basil oil recorded LC_{50} and LC_{90} values as 1.176 and 9.08 % respectively after 72 hours of treatment. Rodriguez-Gonzalez *et al*. (2019) evaluated the insecticidal activity of basil and citronella oil on the bean weevil, *Acanthoscelides obtectus* Say. These essential oils affected the development of *A. obtectus* since the higher doses (more than 60 or 120 µL per sample) applied on beans decreased the emergence of the bean weevil.

Al-Harbi *et al.* (2021) studied the toxic effect of essential oils on *Sitophilus oryzae* L., where *O. basilicum* and *Lavandula angustifolia* (Lawrence) essential oils showed 100 per cent mortality at 6 mg cm^{-2} after 48 and 24 h respectively. The highest repellence activity was recorded for *O. basilicum* essential oil at 0.75 mg cm^{-2} with 82.3 per cent after an exposure time of 5 h.

2.1.2 Eucalyptus Oil

Isman (2000) reported the essential oils from eucalyptus (*E. camaldulensis* Dehnh) were effective as fumigants against two greenhouse pests, the cotton aphid (*Aphis gossypii* Glover) and the carmine spider mite (*Tetranychus cinnabarinus* Biosduval). Lee *et al.* (2001) evaluated the fumigant toxicity of eucalyptus essential oil against *S. oryzae.* The study reported the LD_{50} and LD_{95} of 28.9 and 43.8 µL L^{-1} air against *S. oryzae*.

Papachristosa and Stamopoulos (2004) investigated the fumigant toxicity of the essential oils from Lavandula hybrid, *Rosmarinus officinalis* L. and *E. globulus* against the larvae and pupae of *A. obtectus*. The essential oil vapors were toxic to all immature stages, with LC_{50} values ranging between 0.6 and 76 mL L^{-1} air, depending on the insect's stage and the essential oil. The same authors during 2004 reported insecticidal properties of eucalyptus oil against *A. obtectus*. Toxicity of eucalyptus oil vapours to *A. obtectus* 6 days old eggs reported the LC_{90} value of 10.2 µL L^{-1} at 24 h after exposure. At 24 h after exposure, cumulative toxicity (unhatched eggs + larval and pupal mortality) of *A. obtectus* to oil vapours have showed a LC_{50} and LC_{90} value of 3.1 and 9.5 µL L^{-1}.

Yang *et al*. (2004) did comparative study of eucalyptus leaf oil with δ-phenothrin and pyrethrum, which showed that eucalyptus leaf oil was 3.9 and 4.2-fold more toxic than either δ-phenothrin or pyrethrum, respectively on *Pediculus humanus capitis* Linnaeus. Toxicity of *E. globulus* leaf oil, δ-phenothrin and pyrethrum against female *P. h. capitis* using the filter paper contact lethal-time bioassay *reported* the LT_{50} value of 8.83, 34.57 and 36.73 minutes respectively for a dose of 0.125 mg cm^{-2}.

Nattudurai *et al*. (2012) reported that the eucalyptus oil had a LC_{50} and LC_{99} value of 4.95 and 171.63 µL L^{-1} respectively against *T. castaneum* adults at 24 h after exposure. Eucalyptus oil affected the fecundity of *T. castaneum* at the sub lethal concentration of LC_{10}, LC_{20}, LC_{30} and the number of eggs laid were 3.68, 1.86 and 1.22 per insect per day respectively. Egg hatchability was reduced from 80 to 66 per cent and larval survival percentage reduced from 48.6 to 32.3 per cent with increase in exposure concentrations of eucalyptus oil of 20 and 40 µL L^{-1} respectively. Adult emergence of *T. castaneum* decreased with increase in exposure concentrations of eucalyptus oil which was recorded as 53.3, 46.7 and 30 per cent at exposure concentrations of 20, 40 and 80 µL L^{-1} respectively.

Nowrouziasl *et al*. (2014) reported that the mortality of *S.oryzae* (1-7 days old adults) increased with increasing concentration and the exposure time of five essential oils from *Eucalyptus* spp. The LC_{50} values of essential oil of *Eucalyptus camaldulensis* Dehnh., *E. grandis* W., *E. viminalis* Labill., *E. microtheca* F. Muell. *and E. sargentii* Maiden. on adults of *S. oryzae* were found to be 17.49, 15.65, 14.73, 11.11 and 11.94 µL L^{-1} air respectively.

Slimane *et al.* (2014) reported that the essential oils of *Eucalyptus lehmannii* and *E. globulus* have a toxic action on nerves leading to a disruption of vital system of *Orgyia trigotephras* Boisduval. High toxic properties make these plant-derived compounds suitable for incorporation in integrated pest management programs.

Aref and Valizadegan (2015) reported the LC_{50} of *Eucalyptus kruseana* essential oil as 27.98 µL L^{-1} against *R. dominica*. The calculated LT_{50} of *E. kruseana* essential oil on *R. dominica* adults was 6.47 days. It was clear from the repellence index that *E. kruseana* essential oil has strong repellency at concentrations of 70, 140 and 280 µL L^{-1} air. Atanasova *et al.* (2018) evaluated eucalyptus oil against *Aphis* *gossypii* and reported the LC_{50} of eucalyptus oil as 3000-6000 ppm (0.3-0.6%) against *A. gossypii*.

Ebadollahi and Setzer (2020) investigated the fumigant toxicity of essential oils from *E. camaldulensis* against *Oryzaephilus surinamensis* (L.) and *S. oryzae*. The median lethal concentration (LC_{50}) of essential oil was estimated as 7.76 μL L^{-1} after 24 h on the adults of *O. surinamensis*, which was decreased to 3.36 μL L^{-1} at 72 h. The LC_{50} value against *S. oryzae* were 12.83 and 6.59 μL L^{-1} after 24 and 72 h respectively. The susceptibility of both pests to *E. camaldulensis* essential oil increased with the exposure time.

2.1.3 Citronella Oil

Manzoor *et al.* (2012) evaluated the contact toxicity of three plant oils (eucalyptus, mint and lemongrass) against American cockroach, *Periplanata americana* at different concentrations of 4, 5, 6 and 7 % at 2, 4, 6 and 24 h after treatment. Among the three oils lemon grass oil (citronella) showed the least LC_{50} values of 8.01, 7.05, 4.89 and 3.39 per cent at 2, 4, 6 and 24 h after treatment.

Arancibia *et al*. (2013) evaluated the insecticidal activity of biodegradable films containing clove or citronella oils by concentration response bioassay against Mediterranean fruit fly, *C. capitata*. The vapours of clove oil released from the film containing 3 per cent of essential oil were efficient to trigger the tumbling of flies i.e., 40 per cent after 4 h exposure and after 20 h of exposure 90 per cent of the flies were died.

Pinheiro *et al.* (2013) reported that the essential oil of citronella grass at 1% caused significant mortality to *Myzus persicae* (Sulzer) (96.9 ± 1.57%) and was less toxic to *Franklinella schultzei* (Trybom) (34.3 ± 3.77%). The LC_{50} and LC_{90} value against *M. persicae* were 0.36 and 0.66 % respectively. Silva *et al*. (2016) investigated the toxic effect of citronella oil on *Spodoptera frugiperda* (J. E. Smith). Citronella oil at a concentration of 50 mg mL^{-1} alter the structure of midgut epithelium of the third instar larvae of *S. frugiperda*. The essential oil also caused consequent reduction of fat body, glycogen content, reproduction and insect survival.

Chaisri *et al.* (2019) conducted an experiment, where micro emulsions of citronella oil were compared with conventional citronella oil with regard to their acaricidal toxicity against cattle ticks (*Rhipicephalus microplus* Canestrini). The larval mortality rate of 100 per cent was achieved at 0.78% of micro emulsion whereas, for conventional citronella oil 100 per cent mortality occurred at a concentration of 3.12% at 24 h after exposure. The study concluded that the micro emulsion of citronella oil was found more toxic than the conventional citronella oil.

Moustafa *et al.* (2021) reported the LC_{15} and LC_{50} values of *Cymbopogon citratus* L. essential oil as 427.67 and 2623.06 mg L^{-1} respectively against second instar larvae of *Agrotis ipsilon* (Hufnagel) after 96 h of treatment. The lemongrass oil showed significant effects on the mortality, developmental duration and expression level of chimeric acetyl transferase and lipid peroxidase after 96 h of treatment. The treated larvae also showed inhibition of detoxification enzymes compared with the control larvae.

2.1.3 Orange Oil

Raina *et al*. (2007) studied the effect of orange oil extract on Formosan subterranean termite. In laboratory experiments, 96 and 68 per cent of termites were killed in 5 days when orange oil extract at 5ppm was dispensed from the top and bottom respectively. Termites did not tunnel through glass tubes filled with sand treated with 0.2 and 0.4% of orange oil extract.

Karamaouna *et al*. (2013) tested for the lethal concentration of the orange essential oils against vine mealy bug *P. ficus* and reported the LC_{50} value ranging from 2.7 to 8.1 mg mL^{-1}. Martins *et al.* (2017) evaluated bioassay of orange oil on coffee pest *Dysmicoccus brevipes* (Cockerell). The LC_{50} and LC_{95} of the essential oil against the pest was 2.21 and 3.55% respectively.

Al-Antary *et al*. (2018) evaluated the efficacy of orange oil with four different solvents namely, acetone, ethanol, n-hexane and chloroform against green peach aphid, *M. persicae*. Results showed orange oil with acetone was the most effective in reducing population of first and second nymphal instars.

Ali *et al.* (2019) evaluated the bioassay of orange oil, lemon oil, basil oil and eucalyptus oil against red palm weevil larvae. Sugarcane pieces were dipped in concentrations of 1, 3, 5, 7 and 9 % for 20 s and allowed to feed for red palm weevil larvae. Results showed that orange oil was highly toxic to the larvae with a LC_{50} of 3.53%, followed by eucalyptus oil (5.61%), basil (5.36%) and lemon oil (4.37%).

2.2. REPELLENT AND ANTIFEEDANT EFFECT OF ESSENTIAL OILS

2.2.1. Repellent Effect of Essential Oils

Labinas and Crocomo (2002) reported the repellent effect of Java grass (*Cymbopogon winterianus* Jowitt) essential oil at 1% and above on larvae of corn fall army worm, *S. frugiperda*.

Silva *et al*. (2016) evaluated citronella oil as an effective repellent and insecticide against *S. fugiperda* larvae. Kumar *et al*. (2011) evaluated insecticidal properties of essential oils (*M. piperita*, *E. globulus* and *C. citratus*) on house fly, *Musca domestica* Linnaeus. *M. piperita* (RC_{84}, 61.0 µg cm^{-2}) followed by *E. globulus* (RC_{84}, 214.5 µg cm^{-2}) and *C. citratus* (RC_{84}, 289.2 µg cm^{-2}) were found to be effective in repelling the fly. Formulated *M. piperita* and *E. Globules* recorded LC_{50} of 5.12 µg cm^{-2} and 6.09 µg cm^{-2} respectively at 72 hours after treatment. The crude oils of *M. piperita* and *E. globulus* suppressed the emergence of adult flies by 100 per cent.

Wei *et al*. (2018) evaluated the contact toxicity and repellent effect of essential oil from the roots of *Bupleurum bicaule* Helm against *Lasioderma serricorne* and *Liposcelis bostrychophila* Badonnel adults. Essential oil exhibited contact toxicity against *L. serricorne* (LD_{50} = 11.91 µg/adult) and also showed strong repellent activity of 38 per cent at concentration of 0.13 mL cm^{-2} and 100 per cent at 78.63 mL cm^{-2} against *L. serricorne*. Thus, percent repellency values declined with the decrease in the oil concentrations.

Abdelkader *et al*. (2020) conducted an experiment to test the repellent effect of *E. Globules* on apterous adults of the black bean aphids, *Aphis fabae* Scopoli. Five concentrations of *E. globulus* (1.25%, 2.50%, 5%, 10% and 15%) were checked for the repellent effect using area preference method in laboratory condition. The tested concentrations showed good repellency against *A. fabae* after 2 h and maximum repellence of 87.5 per cent was shown at 15% concentration of oil.

Chandel *et al.* (2021) investigated the fumigation toxicity and repellent activity of eucalyptus oil against lesser grain borer *R. dominica.* The concentration and exposure time increased the mortality and repellence. At the lowest concentration of eucalyptus oil of 0.08 µL cm^{-2}, the repellent activity was found to be 42.5 per cent in 36 hours. When dose was increased to 0.16, 0.24, and 0.32 µL cm^{-2}, the repellency was found to be 43.5, 47.5 and 62.5 per cent respectively after 36 h of exposure.

2.2.2. Antifeedant Effect of Essential Oils

Kostic *et al.* (2008) tested the antifeedant effect of basil oil on larvae of *Lymantria dispar* by choice test. Basil oil at 0.5% showed antifeedant effect of 60 and 85.71 per cent after 24 and 48 h of treatment respectively.

Saroukolai *et al*. (2014) investigated the effects of *O. basilium* essential oils on nutritional indices like Relative Growth Rate (RGR), Relative Consumption Rate (RCR) and Efficiency of Conversion of Ingested Food (ECI) and Feeding Deterrence Index (FDI) on the fourth instar larvae of *L. decemlineata* in potato leaves. The basil oil recorded 0.02 mg $mg^{-1}h^{-1}$, 0.03 mg $mg^{-1}h^{-1}$ and 7.81%; 0.01 mg $mg^{-1}h^{-1}$, 0.26 mg $mg^{-1}h^{-1}$ and 5.58%; 0.004 mg $mg^{-1}h^{-1}$, 0.13 mg $mg^{-1}h^{-1}$ and 3.22% of RGR, RCR, ECI and FCI values at concentration of 0, 12 and 16 ppm respectively. At 16 ppm of concentration, 60.28 per cent of FDI was observed.

E. kruseana essential oil showed anti-nutritional effects on adults of *R. dominica*. Relative growth rate, relative consumption rate and efficiency of conversion of ingested food reduced with increase in essential oil concentration. Feeding Deterrence Index (FDI) increased with increase in *E. kruseana* essential oil concentration, which showed 43.28 and 91.47 per cent of FDI values at 12.5 and 75 µL L^{-1} air concentration (Arefand Valizadegan, 2015).

2.3 PHYTOTOXICITY EVALUATION OF ESSENTIAL OILS

de Almeida *et al.* (2010) tested for the phytotoxicity effect of twelve essential oils *viz.*, *Hyssopus officinalis* L. , *Lavandula angustifolia* (Lawrence), *Majorana hortensis* (M.), *Melissa officinalis* L. , *O. basilicum*, *Origanum vulgare* L. , *Salvia officinalis* L. , *Thymus vulgaris* L. , *Verbena officinalis* L. , *Pimpinella anisum*L. , *Foeniculum vulgare* Mill. *and Carum carvi* L. on seeds of *Raphanus sativus* L. ,*Lactuca sativa* L.and *Lepidium sativum* L. . The germination and radicle growth of tested seeds at initial stages were affected significantly by the essential oils.

Karamaouna *et al.* (2013) conducted bioassay of basil and citrus oil on vine mealy bug, *P. ficus*, a pest in grape vine. The LC_{50} values of citrus and basil oil ranged from 2.7-8.1 and 44.1-46.8 mg mL^{-1} respectively. No phytotoxicity symptoms were observed when citrus essential oil was used whereas; highest phytotoxicity was observed when basil oil was sprayed on grape vine.

Poonpaiboonpipat *et al*. (2013) investigated phytotoxic effect of citronella oil on *Echinochloa crusgalli* (L.). Citronella oil at 1.25, 2.5, 5, and 10 % was applied on leaf of 28 days old seedlings of *E. crusgalli* raised in glass house at a spray volume of 1000 L ha^{-1}. Leaf wilt was observed after 6 h of treatment. Chlorophyll a, b and carotenoid content decreased with increase in concentration of essential oil. The essential oil caused membrane disruption and integrity loss due electrolyte leakage. This phytotoxic effect of essential oil suggested the utilisation of the essential oil as herbicides.

2.4. MANAGEMENT OF PESTS IN FIELD USING ESSENTIAL OIL

Shadia *et al.* (2007) evaluated the insecticidal properties of the American basil oil against the black cutworm, *A. ipsilon* in the laboratory and in a semi field trail. They found positive correlation between the concentration of basil oil and the percentage of larval mortality and malformed pupae and adult. At basil oil 3% only 35 per cent of the larvae reached the pupal stage and 13 per cent of the pupae were deformed. In the semi field experiment, basil oil was more effective on adults and it had repellent effect on *A. ipsilon* moths with 66.42 per cent repellency at 3%.

Sammour *et al*. (2018) evaluated the efficiency of formulations of basil oil (*O. basilicum*), turmeric oil (*Curcurma longa* L.), rosemary oil (*R. officinalis*) and thyme oil (*T. vulgaris*) and with Nimbecidine (commercial botanical) against *Tuta absoluta* (Meyrick) under laboratory and field conditions. The laboratory evaluation showed that rosemary oil was the most potent with LC_{50} value of 0.04%, followed by thyme oil (0.07%), basil oil (0.12%) and turmeric (0.19%) compared to nimbecidine (0.06%). The most promising formulations of rosemary and thyme oil showed 84.79 and 80.80 per cent reduction of *T. absoluta* populations respectively, while nimbecidine showed a reduction of 87.37 per cent after 7 days of treatment in field study.

Mardiningsih and Mamun (2021) conducted a field experiment by spraying citronella-clove and lemongrass-clove mixtures, each at the ratio of 1:1 on the rice plants. These formulations at the concentration of 10 mL L^{-1} deterred oviposition of *Nilaparvathalugens* (Stal) by 64.6 and 57.4 per cent respectively. Both essential oils caused 60 to 64 and 43.3 per cent mortality in nymphs and adults of *N. lugens*. The formulations applied twice in a week were effective to control *N. lugens* and safe to natural enemies in the field.

Setiawati *et al*. (2011) conducted an experiment on the insecticidal properties of citronella oil against *Helicoverpa amrigera* (Hubner) on chili pepper under both lab and field condition. In laboratory 4,000 ppm of citronella oil reduced egg laying by 53 to 66 per cent. The field experiment showed, that the citronella oil at 2.0 mL L^{-1} significantly reduced the fruit damage by *H. armigera* and was on par with spinosad at the recommended dose of 60 g ai. ha^{-1}. Application of citronella oil significantly reduced fruit damage by 72 per cent.

Telaumbanua *et al*. (2021) reported that citronella based pesticides can significantly reduce the insect pest population in rice *viz.,* brown plant hopper, green grass hopper, stink bug and armyworm. Citronella oil was applied after 21 days of planting and was repeated for four times in a week. At first spray (21 DAP), only grasshoppers were found in plot A, while no pests were found in plot B and all four types of pests were found in control plot. After fourth spray (49 DAP) number of predatory insects in plot A and B was 3 and 5 respectively and in control plot 10 were found. The predatory insect reduction was due to both toxicity and lack of food in plot A and B.

2.5. CHARACTERISATION OF ESSENTIAL OILS

Singh and Kumar (2017) evaluated the composition of the Java Citronella essential oil using Gas Chromatography Mass Spectrometry (GC-MS). The results of this study showed the yield of citronella essential oil as 0.79 per cent. The recovered essential oil was richer in citronellal (29.15%) followed by geraniol (22.52%), citronellol (7.43%), geranyl acetate (2.63%), neral (6.52%), geranial (5.20%) elemol (1.92%) and limonene (1.27%).

Setiawati *et al*. (2011) characterized the citronella oil chemically by using GC-MS and the results showed that major chemical compounds of the citronella oil used were citronella (35.97%), nerol (17.28%), citronellol (10.03%), geranyle acetate (4.44%), elemol (4.38%), limonene (3.98%) and citronellyl acetate (3.51%).

Wogiatzi *et al.* (2011) extracted basil oil via hydro distillation and analysed using gas chromatography. The chemical components varied among basil cultivars. Regarding the basil oil active substances, linalool and eugenol were dependent on the cultivar and year of cultivation. The broad leaf basil cultivar had an average of 3.8 mg g^{-1} linalool and 0.5 mg g^{-1} eugenol and the narrow leaf basil had 2.8 mg g^{-1} linalool and 0.7 mg g^{-1} eugenol.

Vivekanandhan *et al*. (2020) evaluated the chemical composition of essential oil (*E. globulus*) using GC-MS and FT-IR analysis shows the presence of 1, 8-cineol, (71.7%), α-pinene, (9.14%) as major compounds.

Mead (2018) conducted GC-MS profiling of basil oil and reported that linalool (48.26%), eucalyptol (9.21%) and estragole (5.16%) were found as the three principal active components with 0.37% of linalool oxide content.

Sajjadi (2006) reported chemnical components of *O. basilicum* cv. purple and *O. basilicum* cv. green from Iran. Methyl chavicol (52.4%), linalool (20.1%), epi-α-cadinol (5.9%) and trans-α-bergamotene (5.2%) were major constitruents of *O. basilicum* cv. Purple whereas, in *O. basilicum* cv. green methyl chavicol (40.5%), geranial (27.6%), neral (18.5%) and caryophyllene oxide (5.4%) were the major constituents. In both the essential oil methyl chavicol was the major component.

Wany *et al.* (2014) classified citronella oil chemotype as Ceylon citronella oil (*C. nardus* (inferior type)) and Java citronella oil (*Cymbopogon winterianus* Jowitt (superior type)). Geraniol (18-20%), limonene (9-11%), methyl isoeugenol (7-11%), citronellol (6-8 %), and citronellal (5-15 %) were found to be the major component of Ceylon citronella oil whereas, java citronella oil was containing citronellal (32-45%), geraniol (11-13%), geranyl acetate (3-8%), and limonene (1-4%) as major component.

Materials and methods

3. MATERIALS AND METHODS

The present investigation entitled "Bioactivity of essential oils against insect pests of brinjal" was carried out at Department of Agricultural Entomology, College of Agriculture, Vellayani during 2019-2021. The toxicity bioassays of the essential oils viz., basil oil, eucalyptus oil, citronella oil and orange oil were done against mealy bug (*C. insolita*) and hadda beetle (*H. vigintioctopunctata*) under laboratory conditions. The two effective oils selected based on their LC_{50}/LC_{90} values were further evaluated for their antifeedant and repellent properties against hadda beetle.

Before field application, the two selected essential oils based on the laboratory assays were further tested for the emulsion formation (bloom test) and phytotoxicity evaluation on brinjal leaves. Two effective doses of the selected essential oils based on the laboratory evaluation along with chemical control (chlorantraniliprole 18.5% SC and thiamethoxam 25.5% WG) and an untreated control were further evaluated on potted brinjal plants.

The chemical fingerprinting/characterization of the effective essential oils based on lab and field trial experiment were done using GC-FID/ GC-MS (Gas Chromatography-Flame Ionization Detector / Gas Chromatography-Mass Spectrometry.

3.1 LABORATORY LEAFDIP BIOASSAY FOR EVALUATING TOXICITY OF ESSENTIAL OILS AGAINST THE PESTS OF BRINJAL

The toxicity bioassays of the four oils *viz.*, basil oil, eucalyptus oil, citronella oil and orange oil were done against one sucking (mealy bug) and one chewing insect (hadda beetle) under the laboratory condition using leaf dip bioassay method (Elbert and Nauen, 1996).

3.1.1 Procurement of Essential Oils

Essential oils *viz.* basil oil (*Ocimum bascilicum* L.), eucalyptus oil (*E. globulus* Labill.), citronella oil (*Cymbopogon nardus* Rendle) and orange oil (*Citrus sinensis* L.) were procured from Synthite Industries Private Limited, Kadayiruppu, Ernakulam.

3.1.2 Maintenance of Test Insect

All the available stages of the test insects (egg mass, immature stages and adults of mealy bug and hadda beetle) were collected from the campus of College of Agriculture, Vellayani and were reared under laboratory conditions to get the second generation (F2). The F2 generation were used for the laboratory evaluation.

3.1.2.1 Coccidohystrix insolita (Green)

The mealy bug (both nymphs and adults) was collected from the unsprayed brinjal fields within college campus. Later the population were inoculated to the sprouted potatoes which were kept on the sterile soil filled trays as medium to grow the potatoes. Frequent watering was done to prevent drying and support the growth of potato (Plate 1a). Once the population has multiplied on the potatoes, the insects were collected and used for bioassay experiments.

3.1.2.2. Henosepilachna vigintioctopunctata (Fabricius)

Egg mass, grubs and beetles were collected from the campus premises and were kept in different plastic boxes of size one litre. The boxes were covered with muslin cloth for proper aeration. Fresh leaves were provided on alternative days to grubs and adults. Once the adult started mating and laying eggs on the leaves provided, the leaves with eggs were separated and observed for egg hatching. The emerged first instars were given fresh leaves and further reared to later instars and adults (Plate 1b and 1c). The late instar of the F2 generation were used for bioassay studies.

(a) Mealy bug rearing on potato sprouts.

(b) Egg mass of hadda beetle collected from brinjal field.

(c) Late instar grubs of hadda beetle reared for lab evaluation.

Plate 1: Test insect culture maintained for laboratory experiments.

3.1.3 Bioassay of Essential Oils against Test Insects

Acute toxicity of essential oil was determined by modifying the leaf dip bioassay method of Elbert and Nauen (1996). Preliminary range tests were done to fix the test dose range causing 20 to 80 per cent mortality approximately. Based on this, 5 doses along with a control were taken. The stable emulsions of essential oils were prepared by mixing oil with tween 80 (1:1v/v) as surfactant and then with double distilled water using a magnetic stirrer. Fresh brinjal leaf piece dipped in essential oil emulsions were kept on filter paper wetted with double distilled water and kept in Petri plates of diameter 9 cm (Plate 2). Control experiment was maintained with only water and surfactant. Ten adults of the mealy bugs and five grubs of hadda beetle from each culture were released over the leaf disc inside each glass Petri dish as separate experiments. These glass Petri dishes were then covered with lid to prevent insect escape. The inner side of the lid was coated with Vaseline to prevent insect staying on the lid. The experiment was done in three replicated trials to reduce the error. The insects were kept at 27± 5^{o}C, 60 ± 5% RH, photoperiod of 12:12 (L:D) and after 24 h and 48 h of exposures the number of dead insects were recorded and recognized as such by prodding with a fine hair brush. The insects were considered as dead if unable to move their appendages.

3.2 EVALUATION OF THE REPPELLENT AND ANTIFFEDANT EFFECT OF SELECTED ESSENTIAL OILS AGAINST HADDA BEETLE

The effective essential oils from the previous experiment were again evaluated for their repellent and antifeedant effect.

3.2.1 Evaluation of Repellent Action of Essential Oils against Hadda Beetle Grubs

The design followed for this experiment was completely randomized design (CRD) and values were subjected to arcsine transformation and further analysis of variance (ANOVA) was done.

Repellent toxicity of the essential oils was evaluated by slightly modifying the method of Wei *et al*. (2018). Two equal halves of filter paper were placed on opposite sides of Petri plates (diameter-15 cm.) with 5cm gap between two filter paper halves and different concentrations of essential oils emulsions (1mL) were poured on one half of the filter paper, while the other half was poured with control (surfactant + water) (Plate 3a). Later, hadda beetle (late instar grubs) was released into the Petri plate and observations were made at 30 and 60 MAT (Minutes After Treatment).

Per cent Repellency (PR (%)) was calculated using following formula,

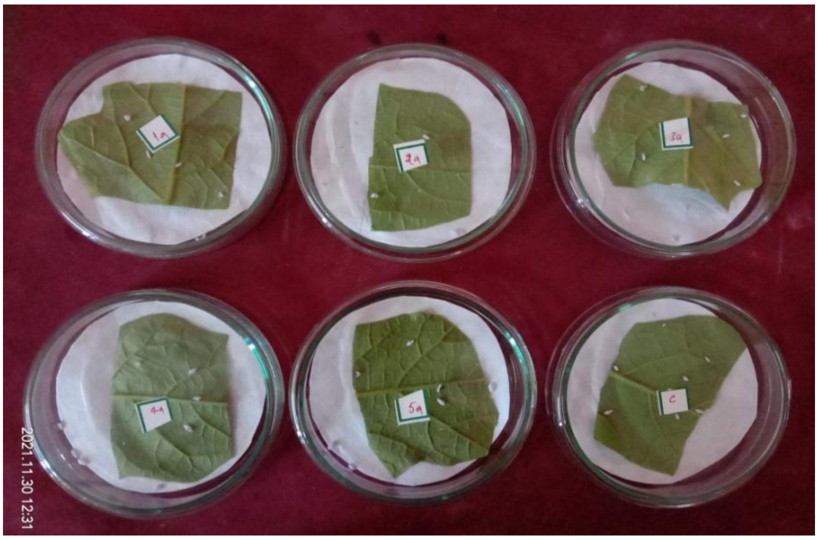
$$PR(\%) = \frac{Nc - Nt}{Nc + Nt} * 100$$

Where, PR= Per cent Repellency (%), Nc= Number of grubs in control half, Nt= Number of grubs in treated half.

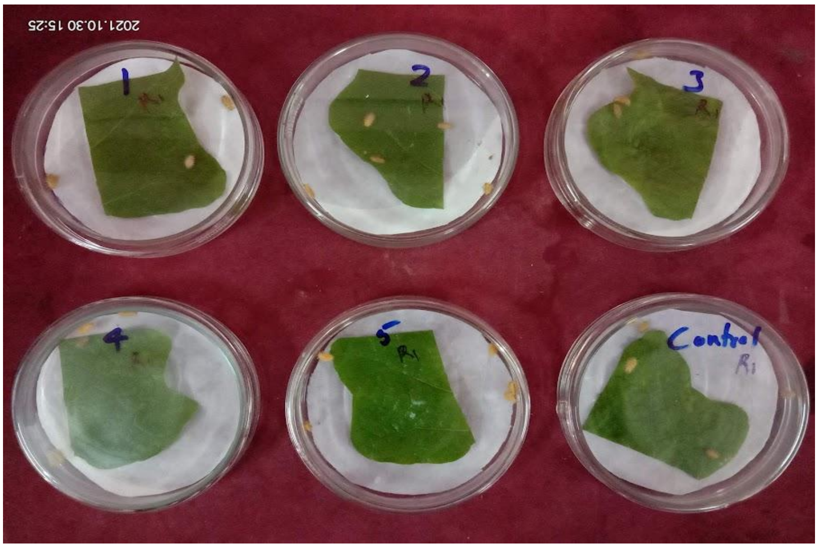
3.2.2 Evaluation of antifeedant Effect of Essential Oils against Hadda Beetle Grubs

The design followed for this experiment was CRD and values were subjected to arcsine transformation and further ANOVA was done.

The antifeedant properties of essential oil on hadda beetle grub was studied by no choice method (Duraipandiyan *et al*., 2011). Brinjal leaves were cut into square piece of $16cm^2$ and then dipped in various concentrations of essential oil. The leaf



(a) Leaf dip bioassay method for screening essential oils against mealy bug.



(b) Leaf dip bioassay method for screening essential oils against hadda beetle.

Plate 2. Leaf dip bioassay method followed for screening essential oil against test insects.

slices were placed on Petri plates, laid with filter paper. Each trial was replicated thrice. A control was maintained with surfactant and water without any oil (Plate 3b). The leaf area eaten was computed using the graph.

Antifeedant Index (AI) was calculated using the formula,

$$AI = \frac{C-T}{C+T} * 100$$

Where, C and T represent the leaf area eaten by late instar grubs of hadda beetle at control and essential oil treated leaf bit.

3.3. BLOOM TEST OF SELECTED ESSENTIAL OILS

The bloom test was conducted to evaluate the emulsion formation of essential oils. 1mL of essential oil formulation (essential oil + surfactant) was added to 99mL of distilled water in a measuring cylinder. Micropipettes were used to add the formulation by dipping the tip in 2 cm depth of distilled water taken in the measuring cylinder and slowly releasing the oil. Later, the bloom formation was examined and scored as described below (Table 1).

Table 1. Bloom test code, bloom rating and observations (Bessette, 2007).

Code	Bloom Rating	Observations
5	Excellent	White billowing emulsion, no droplets observed.
4	Good	White billowing emulsion, very few droplets break away.
3	Fair	White emulsion, some droplets break away.
2	Poor	Poor emulsion, many oil droplets observed.
1-0	Unacceptable	No emulsion, all oil droplets observed.

3.4. PHYTOTOXICITY EVALUATION OF SELECTED ESSENTIAL OILS ON BRINJAL

The phytotoxicity effects of selected essential oils on brinjal plant were evaluated at various doses.

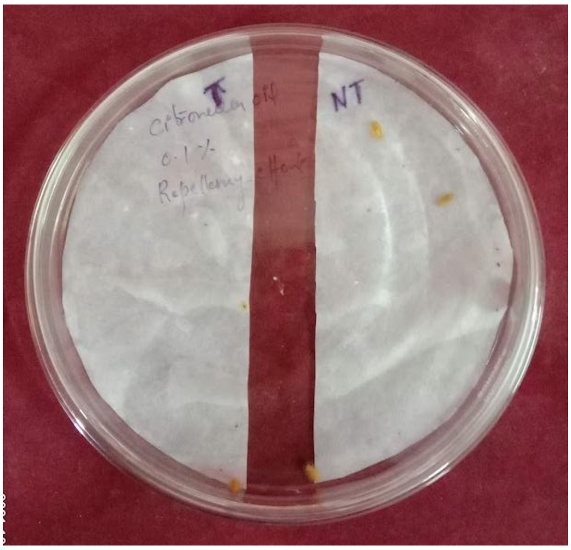
The phytotoxicity evaluation was done by spraying essential oil formulations of above-mentioned concentration on brinjal plants raised in grow bags. Each concentration was tested on three plants and control plants were sprayed with water. The treated plants were checked for phytotoxic effect one day (24 hours) after spraying and rated according to the protocol of Central Insecticides Board and Registration committee (CIBRC) (Table 2) as detailed below,

Table 2. Rating of essential oil based on phytotoxicity symptoms (%).

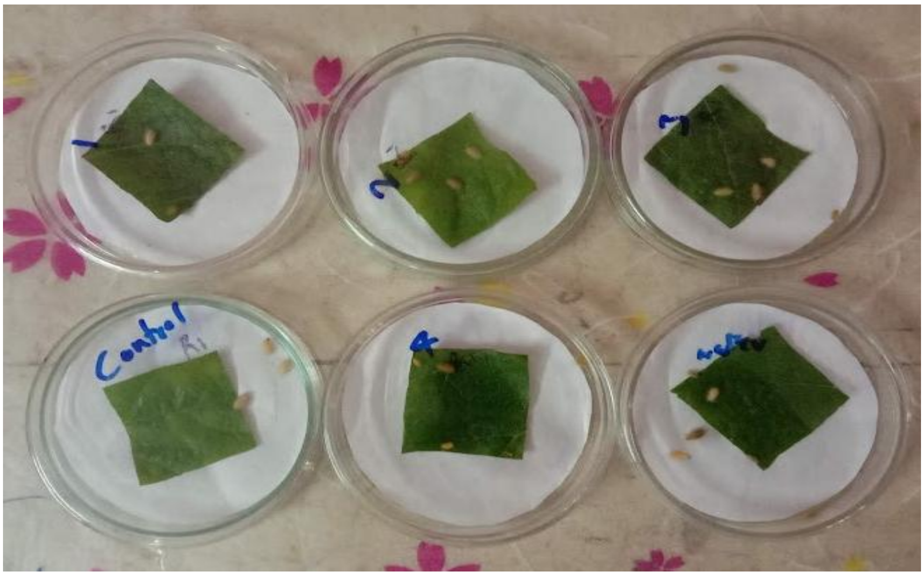
Rating	Phytotoxicity symptom (%)
0	No symptom
1	0-10
2	11-20
3	21-30
4	31-40
5	41-50
6	51-60
7	61-70
8	71-80
9	81-90
10	91-100

3.5. FIELD EVALUATION OF ESSENTIAL OILS AGAINST INSECT PESTS OF BRINJAL AND ITS SAFETY TO NATURAL ENEMIES

Based on the results of laboratory and phytotoxic evaluation, two doses of the two selected essential oils were further evaluated on brinjal plants raised in poly bags.



(a) Modified preference method to evaluate the repellent effect of essential oils.



(b) Leaf disc no choice method to evaluate the antifeedant effect of essential oils.

Plate 3: Repellent (a) and antifeedant (b) effect of essential oils against hadda beetle grubs.

This experiment was conducted with 7 treatments which include two doses each of two selected essential oil, two chemical checks and an untreated control. Thirty days old brinjal seedlings procured from the instructional farm, College of Agriculture Vellayani and were transplanted in grow bags filled with sand, soil and farm yard manure in proportion of 1:2:1. The experiment was laid out in completely randomized design with three replications.

T1: Essential oil 1 (Dose 1)

T2: Essential oil 1 (Dose 2)

T3: Essential oil 2 (Dose 1)

T4: Essential oil 2 (Dose 2)

T5: Chlorantraniliprole 18.5% SC @ 40 g a.i ha^{-1}

T6: Thiamethoxam 25% WG @ 50 g a.i ha^{-1}

T7: Untreated check

3.5.1 Observations

Population of pests and natural enemies were counted before treatment application and 1, 3, 5, 7 and 14 days after treatment application.

3.5.2 Biometric Observations of Brinjal Plant

Biometric observations *viz.,* total number of leaves per plant, total number of damaged leaves per plant and plant height (in cm.) were noted.

3.5.3 Fruit Yield

Fruit yield (in gram) per plant was recorded.

3.6. CHARACTERISATION OF SELECTED ESSENTIAL OILS

The characterisation of essential oil was done on GC-FID and GC-MS to know the major constituents of essential oil.

3.6.1. GC-FID

A Shimadzu GC-2010 Plus Gas Chromatograph (Shimadzu, Japan) with AOC-20i auto injector and flame ionisation detector, fitted with a Rxi-5 Sil MS capillary column (5% phenyl and 95% dimethyl polysiloxane, non-polar, 30 m x 0.25

mm i.d., 0.25 μm film thickness, Restek, USA) was used. 30 μL of basil and citronella oil were diluted in acetone to 1.5 mL, and 1 μL of this solution was injected into a Shimadzu GC-2010 Plus Gas Chromatograph. Injection method is split; split ratio is 50:1; injector temperature is 270°C; oven temperature programme is 60-250°C (3°C/min); hold time is 2 minutes at 250°C; carrier gas is N_2 at 3 mL min^{-1}; detector temperature is 270°C. Under the same experimental circumstances, essential oil infusions (1 μL each) were performed three times. The peak area-percent report of volatiles from GC-FID data was used to calculate relative percentages of specific components in each essential oil.

3.6.2. GC-MS

The essential oil was analysed using a Shimadzu GC-MS system (QP2020NX, Japan) equipped with a Rxi-5 Sil MS capillary column (5 percent phenyl 95 percent dimethylpolysiloxane, non-polar, 30 m x 0.32 mm, i.d., 0.25 μm film thickness). Injector temperature 250^0C, transfer 240^0C, oven temperature programme 60-250^0C (3°C/min), carrier gas He 1.4 mL min^{-1}, mass spectra-electron impact (EI+) mode 70 eV and ion source temperature 240 ^{0}C were the operating settings for the GC-MS. Using typical C5-C30 hydrocarbons, the linear retention indices (LRIs) of essential oil constituents in the Rxi-5 column were calculated (Aldrich Chemical Company, USA). Wiley 275.L database matching, comparison of LRIs, and comparison of constituent mass spectra with published data were used to identify individual components (Adams, 2007; Dool and Kratz, 1963).

3.7. STATISTICAL ANALYSIS

Data of each experiment were analysed with the help of suitable analytical methods. One-way analysis of variance (ANOVA) for all experiments was done after suitable data transformations in Web Assisted Statistical Package (WASP 2.0) software. Data on per cent mortality were analysed by ANOVA and further probit analysis (Finney, 1971) was performed using the OP stat to find out LC_{50}/LC_{90}. Data on per cent Repellence and Antifeedant Index were analysed by ANOVA after arc sine transformation. Mean population of aphid, leaf webber, natural enemies (after square root transformation) and mean damage of shoot and fruit borer were analysed by ANOVA.

Results

4. RESULTS

Brinjal, *S. melongena* is infested by many sucking and chewing pests in its ecosystem. In the present investigation, toxicity bioassays of four essential oils *viz.*, basil oil, eucalyptus oil, citronella oil and orange oil were conducted against one sucking pest (mealy bug, *C. insolita*) and one chewing pest (hadda beetle, *H. vigintioctopunctata*) on brinjal. The two effective essentials oil selected based on the toxicity, from the first experiment was further evaluated for its repellent and antifeedant activities against hadda beetle in the laboratory. Before conducting field evaluation of essential oils on potted brinjal plants, bloom test was conducted to check the emulsification of essential oil formulation with surfactant and further phytotoxicity evaluation was also done. Based on the laboratory and phytotoxicity evaluation, two doses of selected essential oils were used for the pot culture experiment along with two chemical checks and an untreated control. Further, the chemical components of the essential oils were identified through GC-FID/GC-MS.

4.1 LABORATORY LEAF DIP BIOASSAY FOR EVALUATING TOXICITY OF ESSENTIAL OILS AGAINST THE PESTS OF BRINJAL

4.1.1 Leaf dip Bioassay of Essential Oils on Mealy Bug

When the mealy bugs were exposed to varied concentrations of basil oil from 0.3 to 1.2%, the maximum per cent mortality was recorded in 1.2% and 1% (90) followed by 0.8% (63.33), 0.5% (46.66) and 0.3% (33.33) concentrations at 24 HAT. The per cent mortality corresponding to the same concentration (1.2%, 1%, 0.8%, 0.5% and 0.3%) at 48 HAT was 100, 96.66, 76.66, 76.66 and 53.33 respectively. The highest per cent mortality was at 1% and 1.2% and they were on par with each other (Table 3).

At 24 HAT, the per cent mortality of mealy bugs exposed to varied concentrations of eucalyptus oil *viz.*, 1%, 1.5%, 2%, 2.5% and 3% were 23.33, 36.66, 46.66, 73.33 and 80 respectively. The corresponding per cent mortality at 48 HAT was 73.33, 73.33, 93.33, 96.66 and 100 respectively. Maximum mortality was reported at 2.5% and 3% and they were on par to each other (Table 4).

When the mealy bugs were exposed to various concentrations of citronella oil ranging from 0.1 to 1%, the percentage mortality ranges from 6.66 to 90 and 20 to 100 at 24 and 48 HAT respectively. The highest per cent mortality (100) of citronella oil was recorded at 1 per cent concentration (Table 5).

Similarly, the per cent mortality of mealy bugs exposed to various concentrations of orange oil *viz*. 0.5%, 1%, 1.5%, 2%, 2.5% and 3% were 6.66, 33.33, 56.66, 70.00 and 96.66 respectively at 24 HAT. The corresponding mortality at 48 HAT was 13.33, 43.33, 90.00, 93.33 and 100 respectively. At 3% orange oil, higher per cent mortality was reported (Table 6).

Probit analysis was done to calculate the LC_{50} and LC_{90} (with 95% confidence limit) values of basil oil, eucalyptus oil, citronella oil and orange oil against mealy bug at 24 and 48 HAT and were presented in the table 7 and 8 respectively.

The LC_{50} and LC_{90} of basil oil at 24 HAT were 0.49% and 1.33% with fiducial limits in the range of 0.37-0.65 and 1.01-1.76 respectively. The LC_{50} and LC_{90} values of eucalyptus oil at 24 HAT were 1.92% and 4.25% with fiducial limits in the range of 1.55-2.39 and 3.43-5.27 respectively. Citronella oil at 24 HAT recorded LC_{50} and LC_{90} values of 0.67% and 2.11% with fiducial limits in the range of 0.48-0.95 and 0.83-1.81 respectively. Orange oil at 24 HAT recorded LC_{50} and LC_{90} values of 1.54% and 3.53% with fiducial limits in the range of 1.20-1.98 and 2.76-4.53 respectively (Table 7).

The LC_{50} and LC_{90} values of basil oil at 48 HAT were 0.30% and 0.91% with fiducial limits in the range of 0.22-0.43 and 0.65-1.27 respectively. Similarly, the LC_{50} and LC_{90} values of eucalyptus oil at 48 HAT were 0.76% and 1.98% with fiducial limits of 0.55-1.05 and 1.44-2.72 respectively. Citronella oil at 48 HAT recorded the LC_{50} and LC_{90} values of 0.32% and 1.23% with fiducial limits in the range of 0.21-0.47 and 0.83-1.81 respectively. Likewise, the LC_{50} and LC_{90} values of orange oil at 48 HAT were 1.11% and 2.17% with fiducial limits in the range of 0.81-1.40 and 1.71-2.74 respectively (Table 8).

Table 3. Per cent mortality of mealy bug to basil oil.

Basil oil concentration (%)	Per cent mortality	
	24 HAT	48 HAT
0.3	33.33 (35.21)^{c}	53.33 (46.92)^{c}
0.5	46.66 (43.07)^{bc}	76.66 (61.22)^{b}
0.8	63.33 (53.06)^{b}	76.66 (61.71)^{b}
1.0	90.00 (74.69)^{a}	96.66 (83.25)^{a}
1.2	90.00 (71.56)^{a}	100.00 (89.09)^{a}
Control	3.33 (6.74)^{d}	3.33 (12.59)^{d}
CD (0.05)	(14.27)	(11.86)

Values in parenthesis were subjected to arc sine transformation; HAT-Hours after treatment.

Table 4. Per cent mortality of mealy bug to eucalyptus oil.

Eucalyptus oil concentration (%)	Per cent mortality	
	24 HAT	48 HAT
1.0	23.33 (28.78)^{c}	73.33 (59.21)^{b}
1.5	36.66 (37.22)^{bc}	73.33 (59.70)^{b}
2.0	46.66 (43.07)^{b}	93.33 (77.40)^{a}
2.5	73.33 (59.00)^{a}	96.66 (83.25)^{a}
3.0	80.00 (63.93)^{a}	100.00 (89.09)^{a}
Control	3.33 (6.74)^{d}	3.33 (6.74)^{c}
CD (0.05)	(10.52)	(15.83)

Values in parenthesis were subjected to arc sine transformation; HAT-Hours after treatment.

Table 5. Per cent mortality of mealy bug to citronella oil.

Citronella oil concentration (%)	Per cent mortality	
	24 HAT	48 HAT
0.1	6.66 (12.59)[de]	20.00 (26.07)[d]
0.2	13.33 (21.14)[cd]	46.66 (43.07)[c]
0.5	30.00 (33.00)[bc]	63.33 (53.06)[bc]
0.8	40.00 (39.14)[b]	73.33 (59.00)[b]
1.0	90.00 (74.69)[a]	100.00 (89.09)[a]
Control	0.00 (0.90)[e]	3.33 (6.74)[e]
CD (0.05)	(13.99)	(11.97)

Values in parenthesis were subjected to arc sine transformation; HAT-Hours after treatment.

Table 6. Per cent mortality of mealy bug to orange oil.

Orange oil concentration (%)	Per cent mortality	
	24 HAT	48 HAT
0.5	6.66 (12.59)[d]	13.33 (21.14)[c]
1.0	33.33 (35.21)[c]	43.33 (41.15)[b]
2.0	56.66 (48.84)[bc]	90.00 (74.69)[a]
2.5	70.00 (56.99)[b]	93.33 (80.54)[a]
3.0	96.66 (83.25)[a]	100.00 (89.09)[a]
Control	3.33 (6.74)[d]	3.33 (6.74)[c]
CD (0.05)	(13.98)	(16.67)

Values in parenthesis were subjected to arc sine transformation; HAT-Hours after treatment.

Table 7. LC_{50} and LC_{90} of essential oils against mealy bug at 24 HAT.

Essential oil	χ2	d.f.	LC_{50} (%) (95% CL)	LC_{90} (%) (95% CL)	Slope ± SE
Basil oil	4.09	4	0.49 (0.37-0.65)	1.33 (1.01-1.76)	2.98 ± 0.90
Eucalyptus oil	1.91	4	1.86 (1.48-2.33)	4.28 (3.42-5.36)	3.54 ±(-0.95)
Citronella oil	13.78	4	0.64 (0.43-0.94)	2.39 (1.63-3.51)	2.24 ± 0.43
Orange oil	6.39	4	1.54 (1.20-1.98)	3.53 (2.76-4.53)	3.57 ±(-0.67)

Table value of χ^2 at 4 df= 9.48, χ^2 is non-significant at: $p < 0.05$.
LC_{50}-Lethal Concentration causing 50 per cent mortality; LC_{90}-Lethal Concentration causing 90 per cent mortality; HAT-Hours After Treatment; CL-Confidence Limit; SE-Standard Error.

Table 8. LC_{50} and LC_{90} of essential oils against mealy bug at 48 HAT.

Essential oil	χ2	d.f.	LC_{50} (%) (95% CL)	LC_{90} (%) (95% CL)	Slope ± SE
Basil oil	5.14	4	0.29 (0.21-0.41)	0.89 (0.63-1.26)	2.67 ± 1.41
Eucalyptus oil	3.28	4	0.72 (0.51-1.01)	1.92 (1.37-2.67)	3.02 ± 0.42
Citronella oil	6.12	4	0.28 (0.18-0.42)	1.22 (0.80-1.86)	2.00 ± 1.10
Orange oil	0.52	4	1.06 (0.88-1.35)	2.17 (1.71-2.74)	4.16 ±(-0.10)

Table value of χ^2 at 4 df= 9.48, χ^2 is non-significant at: $p < 0.05$.
LC_{50}-Lethal Concentration causing 50 per cent mortality; LC_{90}-Lethal Concentration causing 90 per cent mortality; HAT-Hours After Treatment; CL-Confidence Limit; SE-Standard Error.

4.1.2 Leaf dip Bioassay of Essential Oils on Hadda Beetle

When the hadda beetles were exposed to varied concentrations of basil oil from 0.5 to 2.2%, the highest per cent mortality was recorded in 2.2% (93.33) followed by 2% (73.33), 1.5% (53.33), 1% (26.66), and 0.5% (13.33) at 24 HAT. The per cent mortality corresponding to the same concentration (0.5%, 1%, 1.5 %, 2% and 2.2 %) at 48 HAT was 100, 100, 93.33, 33.33 and 26.66 respectively. At 2.2% and 2% basil oil, significantly higher per cent mortality was reported (Table 9).

At 24 HAT the per cent mortality of hadda beetle when exposed to varied concentrations (2%, 2.5%, 3%, 3.5% and 4 %) of eucalyptus oil were 6.66, 13.33, 46.66, 46.66 and 73.33 respectively. The corresponding per cent mortality at 48 HAT were 46.66, 60.00, 73.33 93.33 and 100 respectively. Highest per cent mortality was recorded at 3.5% and 4% and they were on par with each other (Table 10).

Per cent mortality of hadda beetle when exposed to varied concentrations of citronella oil ranging from 0.6 to 1.5% were in the range of 20 to 100 and 53.33 to100 respectively at 24 and 48 HAT. Highest per cent mortality was reported at 1.5% (100) at 24 and 48 HAT (Table 11).

Likewise, the per cent mortality of hadda beetle when exposed to varied concentrations of orange oil (1%, 1.5%, 3%, 4% and 4.5%) were 6.66, 13.33, 33.33, 53.33 and 73.33 respectively. The corresponding per cent mortality at 48 HAT was 6.66, 40, 60, 93.33 and 100 respectively. Highest per cent mortality was reported at 4% and 4.5% and they were on par with each other (Table 12).

Probit analysis were done to calculate the LC_{50} and LC_{90} values of basil oil, eucalyptus oil, citronella oil and orange oil against hadda beetle at 24 and 48 HAT and were presented in the table 13 and 14 respectively.

The LC_{50} and LC_{90} values of basil oil at 24 HAT were 1.31% and 2.62% with fiducial limits in the range of 0.98-1.75 and 1.9 6-3.50 respectively. Eucalyptus oil at 24 HAT recorded the LC_{50} and LC_{90} values of 3.35% and 5.09% with fiducial limits in the range of 2.82-3.96 and 4.30-6.02 respectively. Similarly, the LC_{50} and LC_{90} values of citronella oil at 24 HAT were 0.93% and 1.52% with fiducial limits in the range of 0.77-1.14 and 1.24-1.85 respectively. Orange oil at 24 HAT recorded LC_{50} and LC_{90} values of 3.48% and 9.11% with fiducial limits in the range of 2.35-5.14 and 6.16-13.47 respectively (Table 13).

At 48 HAT, the LC_{50} and LC_{90} values of basil oil were 0.98% and 1.75% with fiducial limits in the range of 0.75-1.30 and 1.33-2.31, whereas the LC_{50} and LC_{90} values of eucalyptus oil were 2.09% and 3.51% with fiducial limit of 1.67-2.60 and 2.82-4.38 respectively. Citronella oil at 48 HAT recorded LC_{50} and LC_{90} values of 0.63% and 1.14% with fiducial limits in the range of 0.48-0.83 and 0.87-1.50 respectively. At 48 HAT, the LC_{50} and LC_{90} values of orange oil were 2.02% and 4.05% with fiducial limits in the range of 1.48-2.57 and 2.96-5.57 respectively (Table 14).

Table 9. Per cent mortality of hadda beetle to basil oil.

Basil oil concentration (%)	Per cent mortality	
	24 HAT	48 HAT
0.5	13.33 (18.13)[de]	26.66 (30.78)[b]
1.0	26.66 (30.78)[cd]	33.33 (35.00)[b]
1.5	53.33 (46.92)[bc]	93.33 (80.29)[a]
2.0	73.33 (59.23)[b]	100.00 (88.71)[a]
2.2	93.33 (80.29)[a]	100.00 (88.71)[a]
Control	0.00 (1.28)[e]	0.00 (1.28)[c]
CD (0.05)	(17.45)	(12.99)

Values in parenthesis were subjected to arc sine transformation; HAT-Hours after treatment.

Table 10. Per cent mortality of hadda beetle to eucalyptus oil.

Eucalyptus oil concentration (%)	Per cent mortality	
	24 HAT	48 HAT
2.0	6.66 (9.70)[b]	46.66 (42.70)[b]
2.5	13.33 (18.13)[b]	60.00 (51.14)[b]
3.0	46.66 (43.07)[a]	73.33 (59.21)[b]
3.5	46.66 (43.07)[a]	93.33 (80.29)[a]
4.0	73.33 (59.21)[a]	100.00 (88.71)[a]
Control	0.00 (1.28)[b]	0.00 (1.28)[c]
CD (0.05)	(17.31)	(17.91)

Values in parenthesis were subjected to arc sine transformation; HAT-Hours after treatment.

Table 11. Per cent mortality of hadda beetle to citronella oil.

Citronella oil concentration (%)	Per cent mortality	
	24 HAT	48 HAT
0.6	20.00 (26.56)[d]	53.33 (46.92)[b]
0.9	46.66 (43.07)[c]	73.33 (59.21)[b]
1.0	60.00 (51.14)[be]	93.33 (80.29)[a]
1.3	73.33 (59.21)[b]	93.33 (80.29)[a]
1.5	100.00 (88.71)[a]	100.00 (88.71)[a]
Control	0.00 (1.28)[e]	0.00 (1.28)[c]
CD (0.05)	(11.35)	(16.62)

Values in parenthesis were subjected to arc sine transformation; HAT-Hours after treatment.

Table 12. Per cent mortality of hadda beetle to orange oil.

Orange oil concentration (%)	Per cent mortality	
	24 HAT	48 HAT
1.0	6.66 (9.70)[d]	6.66 (9.70)[c]
1.5	13.33 (18.13)[cd]	40.00 (39.23)[b]
3.0	33.33 (35.00)[bc]	60.00 (51.14)[b]
4.0	53.33 (46.92)[ab]	93.33 (80.29)[a]
4.5	73.33 (59.21)[a]	100.00 (88.71)[a]
Control	0.00 (1.28)[d]	0.00 (1.28)[c]
CD (0.05)	(17.45)	(17.38)

Values in parenthesis were subjected to arc sine transformation; HAT-Hours after treatment.

Table 13. LC_{50} and LC_{90} of essential oils against hadda beetle at 24 HAT.

Essential oil	χ2	d.f.	LC_{50} (%) (95% CL)	LC_{90} (%) (95% CL)	Slope ± SE
Basil oil	3.25	4	1.25 (0.90-1.74)	2.85 (2.04-3.97)	3.5 ±(-0.35)
Eucalyptus oil	1.43	4	3.35 (2.82-3.96)	5.09 (4.30-6.02)	7.05 ± 1.74
Citronella oil	1.96	4	0.93 (0.77-1.14)	1.52 (1.24-1.85)	6.11 ± 1.34
Orange oil	1.32	4	3.48 (2.35-5.14)	9.11 (6.16-13.47)	3.06 ± 0.76

Table value of χ^2 at 4 df= 9.48, χ^2 is non-significant at: $p< 0.05$.
LC_{50}-Lethal Concentration causing 50 per cent mortality; LC_{90}-Lethal Concentration causing 90 per cent mortality; HAT-Hours After Treatment; CL-Confidence Limit; SE-Standard Error.

Table 14. LC_{50} and LC_{90} of essential oils against hadda beetle at 48 HAT.

Essential oil	χ2	d.f.	LC_{50} (%) (95% CL)	LD_{90} (%) (95% CL)	Slope ± SE
Basil oil	7.44	4	0.85 (0.62-1.17)	1.70 (1.24-2.35)	4.27 ± 0.28
Eucalyptus oil	1.78	4	2.09 (1.67-2.60)	3.51 (2.82-4.38)	5.67 ± 1.67
Citronella oil	1.47	4	0.63 (0.48-0.83)	1.14 (0.87-1.50)	4.98 ± 1.38
Orange oil	0.76	4	2.02 (1.48-2.75)	4.05 (2.96-5.52)	4.24 ± 0.76

Table value of χ^2 at 4 df= 9.48, χ^2 is non-significant at: $p< 0.05$.
LC_{50}-Lethal Concentration causing 50 per cent mortality; LC_{90}-Lethal Concentration causing 90 per cent mortality; HAT-Hours After Treatment; CL-Confidence Limit; SE-Standard Error.

From the four tables (Table 7, 8, 13 & 14) it is evident that the LC_{50} and LC_{90} values of basil oil and citronella oil were lower than that of eucalyptus oil and orange oil against the two test insects *viz.,* mealy bug and hadda beetle at 24 and 48 HAT. It may be concluded that the effectiveness of basil and citronella oil in terms of toxicity to insects were higher than eucalyptus and orange oil. Hence, after the first laboratory experiment, basil oil and citronella oil were selected for further evaluations in the lab and field.

4.2 EVALUATION OF THE REPELLENT AND ANTIFFEDANT EFFECT OF SELECTED ESSENTIAL OILS AGAINST HADDA BEETLE

The effective essential oils selected from the first experiment were further evaluated for its repellent and antifeedant effect against hadda beetle.

4.2.1 Repellent Effect of Basil and Citronella Oil

The per cent repellence of basil oil against hadda beetle was presented in table 15. Basil oil at 0.7% was showing 100 per cent repellence at 30 and 60 MAT (Minutes After Treatment) while 0.5% of basil oil was showing 90 and 100 per cent repellence respectively at 30 and 60 MAT and were statistically on par with each other. The per cent repellence of 0.2%, 0.1% and 0.05% of basil oil were (50, 70), (20, 40) and (10, 20) per cent respectively at 30 and 60 MAT. The per cent repellence at 0.1 and 0.05% concentration were on par with each other.

The per cent repellence of citronella oil against hadda beetle was presented in table 16. Citronella oil at 0.7% was showing 100 per cent repellence at 30 and 60 MAT. 0.5% of citronella oil was showing 90 and 100 per cent of repellence at 30 and 60 MAT respectively. The per cent repellence at 0.7 and 0.5 per cent concentration were statistically on par with each other at 30 and 60 MAT. Citronella oil at 0.2%, 0.1% and 0.05% recorded (70, 70), (40, 50) and (15, 20) per cent repellence respectively at 30and 60 MAT.

From the experiment it can be concluded that basil oil and citronella oil at 0.7 and 0.5% showed statistically significant repellence at 30 and 60 MAT (Plate 4).

4.2.2 Antifeedant Effect of Basil and Citronella Oil

Antifeedant Index (%) of basil and citronella oil against hadda beetle were presented in the table 17. 0.7% and 0.5% of basil oil were showing 100 per cent of antifeedant index and were on par with each other. Basil oil at 0.2%, 0.1% and 0.05% were showing an antifeedant index of 43.37, 10.31 and 1.56 per cent respectively.

100 per cent of antifeedant Index was recorded when the leaves were treated with 0.7% and 0.5% of citronella oil and the results were on par with each other. The antifeedant index of 0.2%, 0.1% and 0.05% citronella oil were 80.12, 52.68 and 11.85 per cent respectively.

From the antifeedant index, it may be concluded that basil oil and citronella oil at 0.5% and 0.7% showed significantly higher antifeedant effect against hadda beetle (Plate 5).

Table 15. Repellent effect of basil oil against hadda beetle.

Basil oil concentration (%)	Per cent Repellence (%)	
	30 MAT	60 MAT
0.05	10 (13.92)c	20 (26.56)c
0.10	20 (26.56)c	40 (38.66)c
0.20	50 (44.71)b	70 (60.25)b
0.50	90 (79.23)a	100 (88.71)a
0.70	100 (88.71)a	100 (88.71)a
1.00	100 (88.71)a	100 (88.71)a
CD (0.05)	(16.27)	(14.29)

Values in parenthesis were subjected to arc sine transformation; MAT-Minutes After Treatment.

Table 16. Repellent effect of citronella oil against hadda beetle.

Citronella oil concentration (%)	Per cent Repellence (%)	
	30 MAT	60 MAT
0.05	15 (20.24)c	20 (26.56)d
0.10	40 (38.66)c	50 (44.71)c
0.20	70 (60.25)b	70 (60.25)b
0.50	90 (79.23)ab	100 (88.71)a
0.70	100 (88.71)a	100 (88.71)a
1.00	100 (88.71)a	100 (88.71)a
CD (0.05)	(19.88)	(13.65)

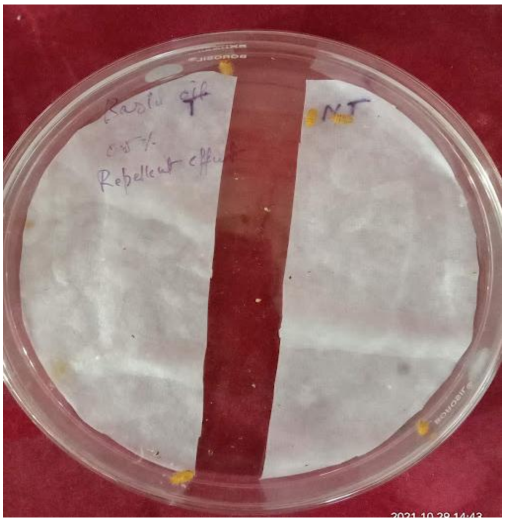
Values in parenthesis were subjected to arc sine transformation; MAT-Minutes After Treatment

Table 17. Antifeedant effect of basil and citronella oil against hadda beetle at 24 HAT.

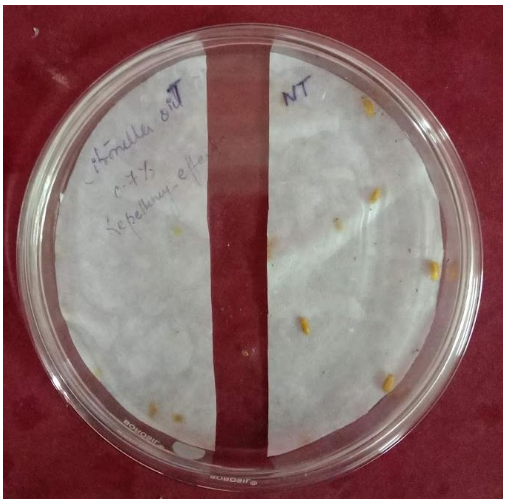
Concentration (%)	Antifeedant Index (%)	
	Basil oil	Citronella oil
0.05	1.56 (6.99)[d]	11.85 (19.92)[d]
0.10	10.31 (18.39)[c]	52.68 (46.56)[c]
0.20	43.37 (41.17)[b]	80.12 (63.64)[b]
0.50	100 (89.28)[a]	100 (89.28)[a]
0.70	100 (89.28)[a]	100 (89.28)[a]
1.00	100 (89.28)[a]	100 (89.28)[a]
Control	0.00 (0.71)[e]	0.00 (0.71)[e]
CD (0.05)	(4.06)	(4.83)

Values in parenthesis were subjected to arc sine transformation.

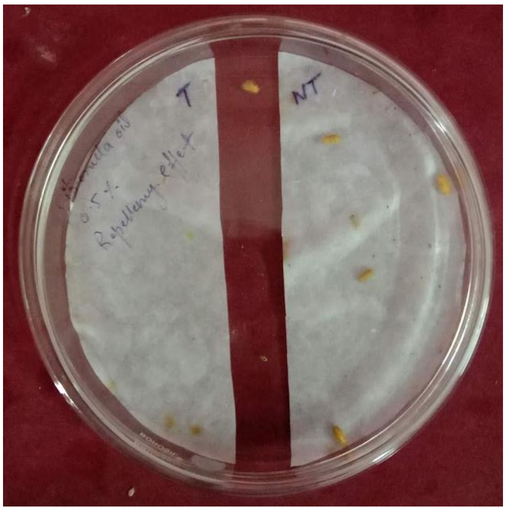
(a) Basil oil at 0.7%.



(b) Basil oil at 0.5%.

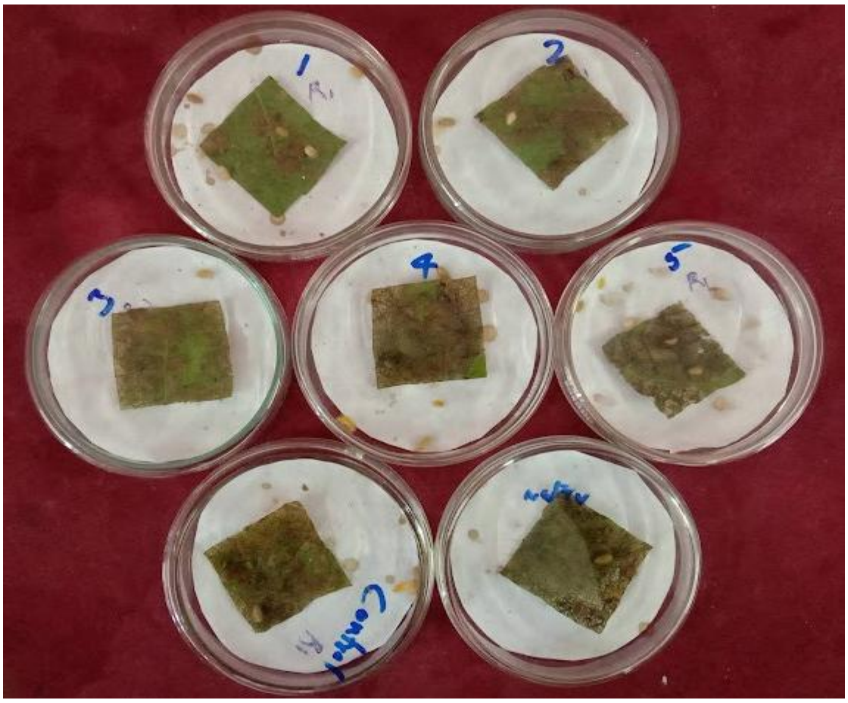


(c) Citronella oil at 0.7%.

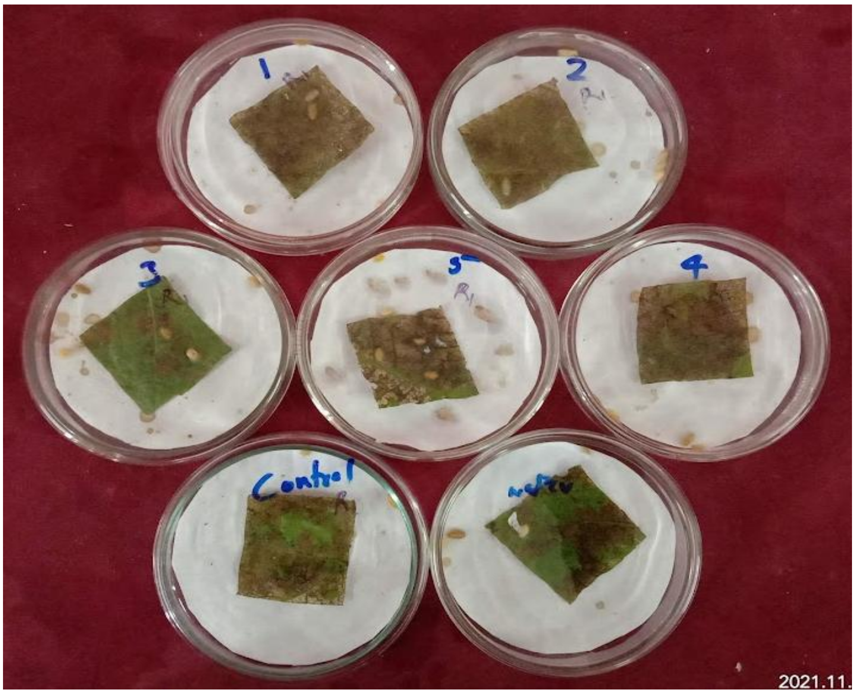


(d) Citronella oil at 0.5%.

Plate 4. Observations on repellent effect of basil oil (a & b) and citronella oil (c & d).



(a) Basil oil at 24 HAT against hadda beetle.



(b) Citronella oil at 24 HAT against hadda beetle.

Plate 5. Observations on antifeedant effect of basil oil (a) and citronella oil (b).

4.3 BLOOM TEST OF SELECTED ESSENTIAL OILS

Bloom test was performed to observe the emulsification of essential oil formulation with surfactant and water (Bessette, 2007). As per the observations, basil oil had grade 4 “Good” bloom rating with white billowing emulsion and breaking of very few droplets. While the citronella oil had grade 5 “Excellent” bloom rating with no droplets in white billowing emulsion (Plate 6).

4.4 PHYTOTOXICITY EVALUATION OF SELECTED ESSENTIAL OILS ON BRINJAL

Phytotoxicity evaluation of basil and citronella oil were conducted to identify the concentrations of essential oils that cause phytotoxic symptoms on brinjal plants. The rating/scoring of the phytotoxicity symptoms were done as per the protocol of CIBRC (Table 2).

4.4.1 Phytotoxicity Evaluation of Basil Oil on Brinjal Plant

The phytotoxic symptoms were scored and presented on table 18. Basil oil at 2% was showing necrosis (8), scorching (4), hyponasty (6) on brinjal plant. There was no symptom of epinasty and yellowing at 2 per cent concentration of basil oil. Basil oil at 1.5% was showing only necrosis (4) and other symptoms of scorching, yellowing, epinasty and hyponasty were not observed. 1% of basil oil was showing necrosis (1) and no other phytotoxic symptoms were observed (Plate 7). Basil oil at 0.7%, 0.5%, 0.3% and 0.1% were not showing any phytotoxic symptoms on brinjal plants.

As per the results of the phytotoxic symptom scoring, basil oil at 2% was showing severe phytotoxicity, whereas at 1.5% and 1% concentration, plants were showing moderate to slight phytotoxicity. At 0.1 to 0.7% concentration of basil oil, plants were devoid of any phytotoxic symptoms.

4.4.2 Phytotoxicity Evaluation of Citronella Oil on Brinjal Plant

Citronella oil at 2% was showing necrosis (9), scorching (3), hyponasty (7) and no symptoms of yellowing and epinasty were noticed on brinjal plant. 1.5 % of citronella oil was showing only necrosis (8), hyponasty (5) and no other symptoms of scorching, yellowing, epinasty and hyponasty were observed. 1% of citronella oil was showing necrosis (5), hyponasty (2) and no other phytotoxic symptoms were observed (Plate 8). 0.7%, 0.5%, 0.3% and 0.1% of citronella oil were not showing any phytotoxic symptoms on brinjal plants (Table 19).

As per the results of the phytotoxic symptom scoring, citronella oil at 2% were showing severe phytotoxic symptoms on brinjal plants. At 1% and 1.5%, plants were showing slight and moderate phytotoxicity respectively. Whereas 0.1 to 0.7 per cent concentration, plants were not showing any phytotoxic symptoms.

(a) Bloom test observations of basil oil.

(b) Bloom test observations of citronella oil

Plate 6. Bloom test of basil oil (a) and citronella oil (b).

Table 18. Phytotoxicity scoring of basil oil on brinjal plant.

Basil oil concentrations (%)	Necrosis	Scorching	Yellowing	Epinasty	Hyponasty
2.0	8	4	0	0	6
1.5	4	0	0	0	0
1.0	1	0	0	0	0
0.7	0	0	0	0	0
0.5	0	0	0	0	0
0.3	0	0	0	0	0
0.1	0	0	0	0	0
Control	0	0	0	0	0

Table 19. Phytotoxicity scoring of citronella oil on brinjal plant.

Citronella oil concentrations (%)	Necrosis	Scorching	Yellowing	Epinasty	Hyponasty
2.0	9	3	0	0	7
1.5	8	0	0	0	5
1.0	5	0	0	0	2
0.7	0	0	0	0	0
0.5	0	0	0	0	0
0.3	0	0	0	0	0
0.1	0	0	0	0	0
Control	0	0	0	0	0

4.5 FIELD EVALUATION OF ESSENTIAL OILS AGAINST INSECT PESTS OF BRINJAL AND ITS SAFETY TO NATURAL ENEMIES

Based on the laboratory and phytotoxicity evaluation, two doses of basil oil and citronella oil (0.5% and 0.7%) were selected for the pot culture experiment along with two chemical check and an untreated control. In the pot culture studies, mean population of aphid (*Aphis gossypii* Glover) per leaf, leaf webber (*Psara bipunctalis* Fb.) per plant, shoot and fruit borer (*Leucinodesor banalis* Guenee) per plant (Plate 9), natural enemies (*Cheilomenes sexmaculata* (Fabricius) & *Coccinella transversalis* (Fabricius)) per plant (Plate 10) and the biometric observations like total number of leaves, total number of damaged leaves, plant height after spray and fruit yield was observed and presented on table 20, 21, 22 and 23.

Basil oil and citronella oil at 0.7% recorded significant reduction in aphid population per leaf (7.13, 6.05) at 1 day after spray. At 3 DAS basil oil at 0.7%, 0.5% and citronella oil at 0.7% recorded significant reduction in aphid population (3.10, 5.48, 2.33) and were on par with chemical check thiamethoxam 25% WG @50 g a.i ha^{-1} (0.035) (3.01). At 5 DAS, basil oil 0.7% (0.88), citronella oil 0.7% (0.88), basil oil 0.5 % (1.88) recorded significantly lower population of aphids per leaf and were on par with thiamethoxam 25% WG @50 g a.i ha^{-1} (0.88). At 7 DAS, there was no aphid population on brinjal plants sprayed with basil oil 0.7%, citronella oil 0.7 % and thiamethoxam 25% WG @50 g a.i ha^{-1}. The aphid population slowly build up on the 14^{th} day and basil oil 0.7% (2.43), citronella oil 0.7% (1.88) and thiamethoxam 25% WG @50 g a.i ha^{-1} (1.86) recorded lower aphid population compared to others (Table 20).

Basil oil 0.7% and citronella oil 0.7% and 0.5% showed significant reduction in leaf webber population (7.50, 8.25, 9) at 1 DAS and they were statistically on par. At 3 DAS basil oil 0.7%, citronella oil 0.7% showed significant reduction in the pest population (2.58, 1.75) and were statistically on par with chlorantraniliprole 18.5% SC @ 40 g a.i ha^{-1} (2.33). At 5 DAS similar trend was followed where, basil oil 0.7% and citronella oil 0.7% showed significant reduction in the population (0.41, 0) and were statistically on par with chlorantraniliprole 18.5% SC @ 40 g a.i ha^{-1} (0.5). At 7 DAS, no pests were found on plots sprayed with 0.7%of basil and citronella oil and


(a) Basil oil at 1.5%.


(b) Basil oil at 1.5%.


(c) Basil oil at 1%.

Plate 7. Phytotoxicity effect of basil oil (a, b & c) on brinjal plant.

(a) Citronella oil at 2%.

(b) Citronella oil at 1.5%.

(c) Citronella oil at 1%.

Plate 8. Phytotoxicity effect of citronella oil (a, b &c) on brinjal plant.

the results were statistically on par with chlorantraniliprole 18.5% SC @ 40 g a.i ha^{-1} (0.41). After 14 days of spray, there was slight build up in population where, basil oil and citronella oil at 0.7% and chlorantraniliprole 18.5% SC @ 40 g a.i ha^{-1} recorded lower population (0.58, 0 and 0.5) compared to other treatments (Table 21).

Mean damage on shoot and fruit of plant due to shoot and fruit borer was significantly reduced in chlorantraniliprole 18.5% SC @ 40 g a.i ha^{-1} sprayed plants after 14 days of spray (1) compared to all other treatments (Table 22).

Basil oil and citronella oil at 0.7% recorded no natural enemy (*Cheilomenes sexmaculata & Coccinella transversalis*) population at 1 DAS and were statistically on par. Basil oil at 0.5%, 0.7% and citronella oil at 0.5%, 0.7% showed significant reduction in population (0, 0.41, 0, 0.66) and were statistically on par. At 5 DAS, basil oil at 0.5%, 0.7 % and citronella oil at 0.7% showed significant reduction in population (0, 1.16, 0) of ladybird beetle and were statistically on par with each other. At 7 DAS also similar trend followed where, basil oil at 0.5%, 0.7% and citronella oil at 0.7% showed lower population of natural enemies (1.58, 2.75, 2.08) and were statistically on par with each other. At 14 DAS, treatments were found to be non-significant and citronella oil 0.7% recorded lower population of natural enemies (3.58) (Table 23).

There was no significant difference between the treatments with respect to biometric observations like total number of leaves, total number of damaged leaves, plant height (cm) after spray. The results were presented on table 24. chlorantraniliprole 18.5% SC @ 40 g a.i ha^{-1} recorded higher fruit yield (515.09 g $plant^{-1}$) compared to other treatments. Among the essential oil treatments citronella 0.7% recorded maximum fruit yield (479.71 g $plant^{-1}$).

Table 20. Efficacy of different essential oils on aphid (*Aphis gossypii*) in brinjal.

Treatment	Aphids / leaf					
	1 DBS	1 DAS	3 DAS	5 DAS	7 DAS	14 DAS
Basil oil (0.7%)	112.15 (10.57)	7.13 (2.63)^{d}	3.10 (1.51)^{d}	0.88 (1.06)^{d}	0.00 (0.70)^{e}	2.43 (1.40)^{d}
Basil oil (0.5%)	102.01 (10.08)	20.75 (4.51)^{c}	5.48 (2.18)^{cd}	1.88 (1.29)^{d}	3.85 (1.90)^{d}	15.42 (3.98)^{c}
Citronella oil (0.7%)	111.15 (10.54)	6.05 (2.45)^{d}	2.33 (1.56)^{d}	0.88 (1.06)^{d}	0.00 (0.70)^{e}	1.88 (1.29)^{d}
Citronella oil (0.5%)	98.22 (9.91)	16.40 (4.04)^{c}	11.64 (3.48)^{c}	6.51 (2.64)^{c}	9.77 (3.20)^{c}	11.58 (3.45)^{c}
Chlorantraniliprole 18.5% SC @ 40 g a.i ha^{-1}	100.44 (10.01)	41.37 (6.41)^{b}	42.77 (6.57)^{b}	47.02 (6.88)^{b}	52.54 (7.28)^{b}	48.11 (6.96)^{b}
Thiamethoxam 25% WG @50 g a.i ha^{-1}	102.24 (10.10)	17.85 (4.20)^{c}	3.01 (1.72)^{d}	0.88 (1.06)^{d}	0.00 (0.70)^{e}	1.86 (1.29)^{d}
Control	103.40 (10.16)	102.89 (10.14)^{a}	118.98 (10.93)^{a}	111.97 (10.60)^{a}	111.40 (10.55)^{a}	104.34 (10.23)^{a}
CD (0.05)	NS	(0.83)	(1.52)	(1.03)	(0.87)	(1.35)

Values in parenthesis were subjected to square root transformation; DAS-Days After Spray; DBS-Days Before Spray.

Table 21. Efficacy of different essential oils on leaf webber (*Psara bipunctalis*) in brinjal.

Treatment	Leafwebbers / plant					
	1 DBS	1 DAS	3 DAS	5 DAS	7 DAS	14 DAS
Basil oil (0.7%)	23.58 (4.84)	7.50 (2.73)^{d}	2.58 (1.59)^{e}	0.41 (0.91)^{e}	0.00 (0.70)^{e}	0.58 (0.97)^{d}
Basil oil (0.5%)	21.75 (4.65)	12.66 (3.55)^{c}	8.66 (2.94)^{c}	3.50 (1.99)^{d}	2.41 (1.70)^{d}	2.33 (1.66)^{c}
Citronella oil (0.7%)	27.00 (5.19)	8.25 (2.87)^{d}	1.75 (1.31)^{e}	0.00 (0.70)^{e}	0.00 (0.70)^{e}	0.00 (0.70)^{d}
Citronella oil (0.5%)	25.41 (5.02)	9.00 (2.99)^{d}	5.83 (2.40)^{d}	6.91 (2.71)^{c}	8.08 (2.92)^{c}	3.66 (2.01)^{c}
Chlorantraniliprole 18.5% SC @ 40 g a.i ha^{-1}	24.16 (4.88)	5.25 (2.28)^{e}	2.33 (1.51)^{e}	0.50 (0.94)^{e}	0.41 (0.91)^{e}	0.50 (0.94)^{d}
Thiamethoxam 25% WG @50 g a.i ha^{-1}	25.91 (5.07)	20.33 (4.50)^{b}	17.41 (4.17)$_{b}$	22.25 (4.75)^{b}	17.33 (4.21)^{b}	20.00 (4.52)^{b}
Control	27.91 (5.28)	36.91 (6.07)^{a}	28.91 (5.36)^{a}	31.25 (5.63)^{a}	22.50 (4.78)^{a}	27.58 (5.29)^{a}
CD (0.05)	NS	(0.33)	(0.43)	(0.51)	(0.42)	(0.59)

Values in parenthesis were subjected to square root transformation; DAS-Days After Spray; DBS-Days Before Spray.

Table 22. Efficacy of different essential oils on shoot and fruit borer (*Leucinodes orbanalis*) In brinjal.

Treatment	Damage symptoms on shoot and fruit / plant	
	1 DBS	14 DAS
Basil oil (0.7%)	3.40 (1.84)	2.00 (1.59)^{c}
Basil oil (0.5%)	3.60 (1.89)	3.06 (1.75)^{b}
Citronella oil (0.7%)	3.86 (1.96)	1.93 (1.57)^{c}
Citronella oil (0.5%)	3.53 (1.87)	2.73 (1.76)^{b}
Chlorantraniliprole 18.5% SC @ 40 g a.i ha^{-1}	3.53 (1.87)	1.00 (1.09)^{d}
Thiamethoxam 25% WG @50 g a.i ha^{-1}	3.26 (1.80)	2.53 (1.69)^{bc}
Control	3.53 (1.88)	3.80 (1.94)^{a}
CD (0.05)	NS	(0.13)

Values in parenthesis were subjected to square root transformation;
DAS-Days After Spray; DBS-Days Before Spray.

Table 23. Efficacy of different essential oils on natural enemies *Cheilomenes sexmaculata* and *Coccinella transversalis* adults in brinjal.

Treatment	Natural enemies / plant					
	1 DBS	1 DAS	3 DAS	5 DAS	7 DAS	14 DAS
Basil oil (0.7%)	4.41 (2.06)	0.00 $(0.70)^{d}$	0.00 $(0.70)^{c}$	0.00 $(0.70)^{c}$	1.58 $(1.24)^{d}$	3.66 (1.91)
Basil oil (0.5%)	4.16 (2.03)	1.16 $(1.22)^{c}$	0.41 $(0.91)^{c}$	1.16 $(1.22)^{bc}$	2.75 $(1.65)^{cd}$	5.41 (2.32)
Citronella oil (0.7%)	5.16 (2.25)	0.00 $(0.70)^{d}$	0.00 $(0.70)^{c}$	0.00 $(0.70)^{c}$	2.08 $(1.44)^{cd}$	3.58 (1.86)
Citronella oil (0.5%)	6.41 (2.52)	1.50 $(1.40)^{bc}$	0.66 $(0.99)^{c}$	1.25 $(1.25)^{b}$	3.41 $(1.83)^{bc}$	5.91 (2.40)
Chlorantraniliprole 18.5% SC @ 40 g a.i ha^{-1}	4.83 (2.14)	2.25 $(1.65)^{bc}$	2.66 $(1.77)^{b}$	3.50 $(1.99)^{a}$	3.41 $(1.81)^{bc}$	4.16 (2.03)
Thiamethoxam 25% WG @50 g a.i ha^{-1}	4.91 (2.20)	3.08 $(1.86)^{ab}$	3.91 $(2.09)^{ac}$	4.16 $(2.14)^{a}$	4.66 $(2.14)^{ab}$	4.75 (2.17)
Control	4.91 (2.20)	4.83 $(2.30)^{a}$	5.66 $(2.48)^{a}$	5.33 $(2.40)^{a}$	5.91 $(2.43)^{a}$	5.16 (2.26)
CD (0.05)	NS	(0.46)	(0.46)	(0.54)	(0.42)	NS

Values in parenthesis were subjected to square root transformation; DAS-Days After Spray; DBS-Days Before Spray.

Table 24. Biometric observations and fruit yield per plant after 20 days of spraying.

Treatment	Total number of leaves	Total number of damaged leaves	Plant height (cm)	Fruit yield (g)
Basil oil (0.7%)	29.91	8.91	38.75	463.97[d]
Basil oil (0.5%)	37.25	6.08	37.33	430.76[e]
Citronella oil (0.7%)	32.08	8.59	39.58	479.71[c]
Citronella oil (0.5%)	33.41	6.25	40.00	438.47[e]
Chlorantraniliprole 18.5% SC @ 40 g a.i ha^{-1}	33.83	6.08	41.33	515.09[a]
Thiamethoxam 25% WG @50 g a.i ha^{-1}	38.25	6.25	41.83	498.99[b]
Control	34.25	7.58	41.41	387.20[f]
CD (0.05)	NS	NS	NS	14.06

(a) Pot culture experiment

(b) *Psara bipunctalis*

(c) *Aphis gossypii*

(d) *Leucinodes orbanalis*

Plate 9. Pot culture experiment (a) and major pests recorded from pot culture experiment (b, c & d)

(a) Cheilomenes sexmaculata

(b) Coccinella transversalis

(c) Immature stage of natural enemies

Plate 10. Natural enemies recorded from pot culture experiment.

4.6. CHARACTERISATION OF SELECTED ESSENTIAL OILS

The effective essential were further characterised for its chemical components in GC- FID (Fig 1) and GC- MS. 22 chemical components were obtained in basil oil and the same were tabulated in table 25 along with their respective AI (Adam's Index), RRI (Relative Retention Index), chemical formula, and percentage. Methyl chavicol was found to be the most prominent component (75.73%) followed by Linalool (18.2%), (8) Cuprenene (1.58%), Geranial (0.60%), Alpha-Pinene (0.51%), Cineole (1, 8) (0.18%), (E)-p-methoxy cinnamaldehyde (0.15%), Limonene (0.12%). The minor components identified were Beta-Pinene, 6-Mehyl-5-Hepten-2-one, (E)-Beta Ocimene, Iso-Menthol, (3Z) Hexenyl Hexonoate, (alpha) Neocallitropsene, (8)Himachalene, Aciphyllene and about 5 components were unidentified.

Similarly, 41 chemical components were obtained in citronella oil and the same were tabulated in table 26 along with their respective AI, RRI, chemical formula, and percentage. Geranial (64.77%) was found most prominent component in citronella oil followed by Citronellyl acetate (7.92%), Geraniol (7.08%), (Z) Iso citral (5.29%), Neral (3.60%), Limonene (2.22%), Hepten-2-one 6-methyl-5 (1.13%), Farmesene (Z)-β (0.58%), Neryl acetate (0.37%), Linalool (0.37%), (Iso) Isopulegol (0.35%), Nerol (0.24%), Z-β-ocimene (0.18%), (α) Humulene (0.17%), E-β-ocimene (0.14%), Rosefuran epoxide (0.10%), Citronellal (0.09%), Iso citral (E) (0.08%), Isopulegol (neo) (0.08%), Verbenol (trans) (0.07%), Allyl hexanoate (0.07%), Iso eugenol (E) (0.06%), Muurolol (epi α) (0.05%), Geranyl formate (0.05%) and about 15 components were unidentified.

Table 25. Chemical components of basil oil obtained from GC-FID/GC-MS.

Sl. No.	Compound name	AI	RRI	Chemical formula	Percentage
1	Alpha-Pinene	932	928	$C_{10}H_{16}$	0.512
2	Beta-Pinene	974	973	$C_{10}H_{16}$	Minor
3	6 Mehyl-5-Hepten-2-one	981	979	$C_8H_{14}O$	Minor
4	Limonene	1024	1025	$C_{10}H_{16}$	0.121
5	(1,8)Cineole	1026	1028	$C_{10}H_{18}O$	0.186
6	(E)-Beta Ocimene	1044	1041	$C_{10}H_{16}$	Minor
7	Un identified	-	1066	-	-
8	Linalool	1095	1097	$C_{10}H_{18}O$	18.210
9	Iso Menthol	1179	1171	$C_{10}H_{20}O$	Minor
10	Methyl chavicol	1195	1196	$C_{10}H_{12}O$	75.730
11	Geranial	1264	1261	$C_{10}H_{16}O$	0.602
12	(3Z)-Hexenyl Hexonoate	1380	1378	$C_{12}H_{22}O_2$	Minor
13	Un identified	-	1245	-	-
14	Un identified	-	1433	-	-
15	Un identified	-	1447	-	-
16	(alpha) Neocallitropsene	1474	1472	$C_{15}H_{24}$	Minor
17	Un identified	-	1476	-	-
18	(8)Himachalene	1481	1487	$C_{15}H_{24}$	Minor
19	Aciphyllene	1501	1500	$C_{15}H_{24}$	Minor
20	8-Cuprenene	1532	1534	$C_{15}H_{24}$	1.580
21	(E) -p-Methoxy-Cinnamaldehyde	1562	1556	$C_{10}H_{10}0_2$	0.150

AI-Adam’s Index; RRI-Relative Retention Index.

Table 26. Chemical components of citronella oil obtained from GC-FID/GC-MS.

Sl. No.	Compound name	AI	RRI	Chemical formula	Percentage
1	Hepten-2- one 6 methyl-5	981	979	$C_8H_{14}O$	1.134
2	Limonene	1024	1025	$C_{10}H_{16}O$	2.226
3	Z-β-ocimene	1024	1031	$C_{10}H_{16}O$	0.189
4	E-β-ocimene	1044	1041	$C_{10}H_{16}O$	0.142
5	Allyl hexanoate	1079	1066	$C_9H_{16}O_2$	0.070
6	Linalool	1095	1095	$C_{10}H_{18}O$	0.370
7	Un identified	-	1123	-	-
8	Verbenol (trans)	1140	1131	$C_{10}H_{16}O$	0.075
9	Isopulegol (neo)	1144	1142	$C_{10}H_{18}O$	0.082
10	Citronellal	1148	1146	$C_{10}H_{18}O$	0.098
11	(Iso) Isopulegol	1155	1152	$C_{10}H_{18}O$	0.353
12	(Z)-Iso citral	1160	1155	$C_{10}H_{16}O$	5.294
13	Rosefuran epoxide	1173	1163	$C_{10}H_{14}O$	0.102
14	Iso citral (E)	1177	1174	$C_{10}H_{16}O$	0.088
15	Terpineol (α)	1186	1188	$C10H_{18}O$	0.093
16	Nerol	1227	1219	$C_{10}H_{18}O$	0.245
17	Citronellol	1223	1222	$C_{10}H_{20}O$	0.091
18	Neral	1235	1232	$C_{10}H_{16}O$	3.604
19	Geraniol	1249	1251	$C_{10}H_{18}O$	7.086
20	Geranial	1264	1263	$C_{10}H_{16}O$	64.773
21	Geranyl formate	1298	1293	$C_{11}H_{18}O_2$	0.058
22	Un identified	-	1306	-	-
23	Un identified	-	1332	-	-
24	Citronellyl acetate	1350	1345	$C_{12}H_{22}O_2$	7.928
25	Neryl acetate	1359	1353	$C_{12}H_{20}O_2$	0.375

26	Un identified	-	1374	-	-
27	Un identified	-	1383	-	-
28	Un identified	-	1411	-	-
29	Un identified	-	1426	-	-
30	Farnesene(Z)-β-	1440	1434	$C_{15}H_{24}$	0.581
31	Iso eugenol (E)	1448	1438	$C_{10}H_{12}O_2$	0.065
32	α-Humulene	1452	1447	$C_{15}H_{24}$	0.179
33	Un identified	-	1472	-	-
34	Un identified	-	1491	-	-
35	Un identified	-	1504	-	-
36	Un identified	-	1510	-	-
37	Un identified	-	1540	-	-
38	Un identified	-	1571	-	-
39	Un identified	-	1633	-	-
40	Muurolol (epi α)	1640	1645	$C_{15}H_{26}O$	0.059
41	Un identified	-	2088	-	-

AI-Adam's Index; RRI-Relative Retention Index.

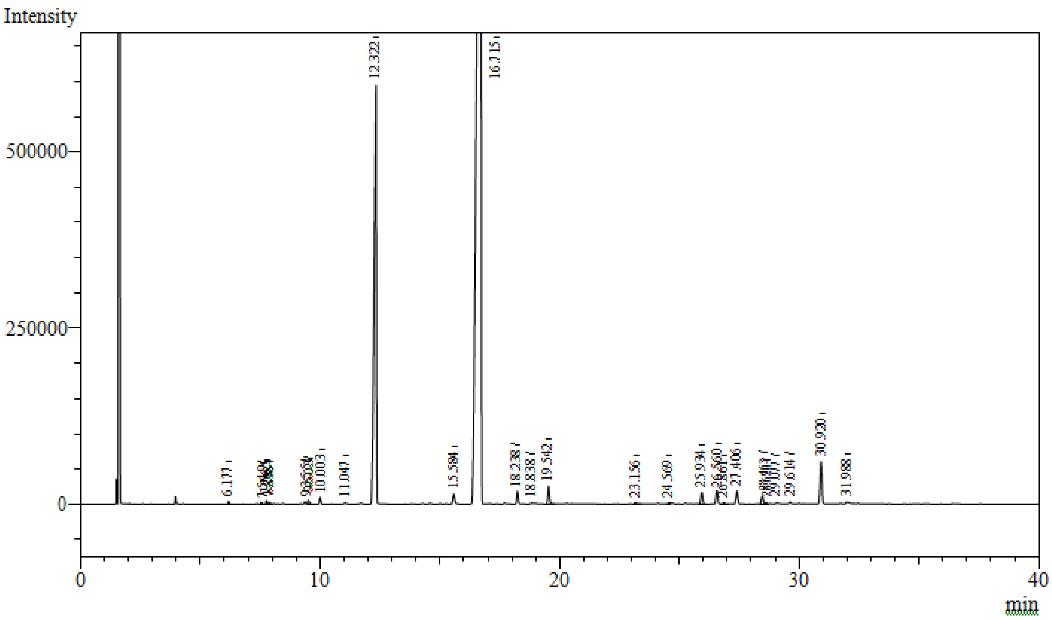


Fig. 1. GC-FID data of basil oil.

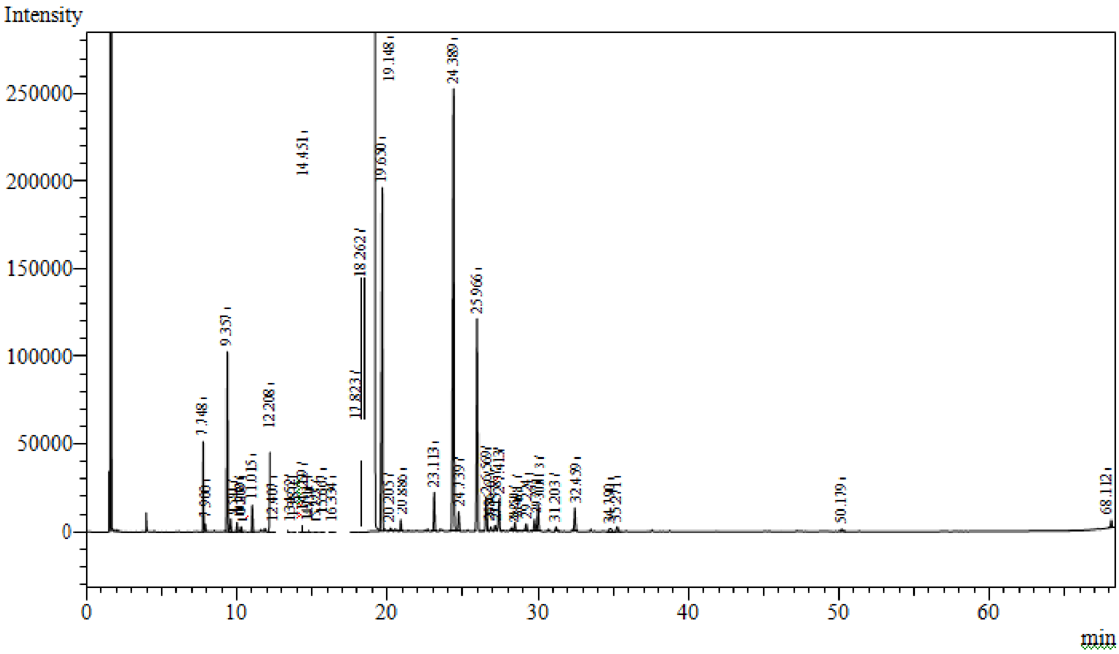


Fig. 2. GC-FID data of citronella oil.

Discussion

5. DISCUSSION

Brinjal is attacked by more than 36 insect pests from the time it is planted to the time it is harvested (Reghupathy *et al.*, 1997). The use of chemical insecticides to combat the pest and diseases lead to the development of resistance, resurgence, residues in the food and also environmental hazards. The limitations of their use are pushing the discovery and development of less harmful products. One of the most promising solutions is the exploitation of plant-based pesticides (Jacobsen *et al.*, 2015). Monoterpenes are the most representative molecules constituting 90 % of the essential oils and allows a great variety of structures with diverse functions. Monoterpenes possess acute contact and fumigant toxicity to insects (Choi *et al*., 2006), repellent and antifeedant activity (Watanabe *et al*., 1993) as well as development and growth inhibitory activity (Karr and Coats, 1992). As such, monoterpenes provide many prototypes for the synthesis of new pesticides (Isman, 2000).

In the present investigation, laboratory assays were conducted to evaluate the toxicity of four essential oils *viz.*, basil oil, eucalyptus oil, citronella oil and orange oil on brinjal mealy bug and hadda beetle. Later probit analysis was done to calculate LC_{50} and LC_{90} of essential oils against mealy bug and hadda beetle. Two effective essential oils were selected based on results from the first experiment and their repellent and antifeedant effect was further evaluated against hadda beetle. Before field evaluation, bloom test of selected essential oil was conducted to check the emulsification of essential oil and surfactant (1:1) in distilled water. Phytotoxicity evaluation of selected essential oil was also conducted to check the development of phytotoxic symptoms on brinjal crop. Based on the laboratory evaluation, bloom test and phytotoxicity evaluation of the essential oil on brinjal crop, two doses of the two selected essential oil were evaluated in field along with two chemical insecticides (chlorantraniliprole 18.5% SC and thiamethoxam 25% WG) and a control treatment. After field evaluation, chemical characterisation of essential oils was done on GC-FID/GC-MS. The results obtained from the different experiments were discussed in this chapter.

5.1 LABORATORY LEAFDIP BIOASSAY FOR EVALUATING TOXICITY OF ESSENTIAL OILS AGAINST THE PESTS OF BRINJAL

5.1.1 Leaf dip Bioassay of Essential Oils on Mealy Bug

Since the mortality dose range of basil oil (0.3% to 1.2%), and citronella oil (0.1% to 1%) was lower than the eucalyptus oil (1%to 3%) and orange oil (0.5% to 3%), it can be concluded that basil oil and citronella oil were more toxic than eucalyptus and orange oil against mealy bug. Basil oil, eucalyptus oil, citronella oil and orange oil recorded maximum morality of mealy bug at concentration of 1%, 2.5%, 0.8% and 2.5 % respectively at 24 and 48 HAT (Fig. 3 & 4).

Based on probit analysis, at 24 HAT, basil oil recorded the lowest LC_{50} value (0.49%) followed by citronella oil (0.67%), orange oil (1.54%) and eucalyptus oil (1.86%). Corresponding LC_{90} value of basil oil, citronella oil, orange oil and eucalyptus oil were 1.33%, 2.11%, 3.53% and 4.28% respectively. After 48 hours of treatment, citronella oil recorded the lowest LC_{50} (0.28%) followed by basil oil (0.29%), eucalyptus oil (0.72%) and orange oil (1.06%). Similar trend was followed for LC_{90} values where the lowest value was recorded for basil oil (0.89%) followed by citronella oil (1.22%), eucalyptus oil (1.92%) and orange oil (2.17%). Atanasova *et al.* (2018) evaluated bioassay of eucalyptus oil on *Aphis gossyphi* and found the LC_{50-60} as 3000-6000 ppm (0.3-0.6%). Similarly, Martins *et al.* (2017) calculated the LC_{50} and LC_{95} of orange oil on mealy bug, *Dysmicoccus brevipes* as 2.21 and 3.55 per cent respectively which corroborates the present investigation.

In the present investigation, the LC_{50} and LC_{90} of basil oil and citronella oil were lower than that of eucalyptus oil and orange oil against mealy bug at 24 and 48 HAT indicating its effectiveness as a botanical insecticide against sucking pests (Table 9 and 10).

5.1.2 Leafdip Bioassay of Essential Oils on Hadda Beetle

The mortality dose range of essential oil against hadda beetle which cause 10-100 per cent mortality at 24 and 48 HAT were basil oil (0.5 to 2.2%), eucalyptus oil (2 to 4%), citronella oil (0.6 to 1.5 %) and orange oil (1 to 4.5 %). The concentration range of basil and citronella that causes mortality on hadda beetle were lower than eucalyptus and orange oil concluding that basil oil and citronella oil were more toxic than the eucalyptus and orange oil. Basil oil, eucalyptus oil, citronella oil and orange oil recorded maximum mortality of hadda beetle at 1.5, 3, 1 and 3 per cent respectively at 24 and 48 HAT (Fig. 5 & 6).

From probit analysis of essential oils against hadda beetle, citronella oil recorded lower LC_{50} value (0.93%) followed by basil oil (1.36%), eucalyptus oil (3.35%) and orange oil (3.48%). Corresponding LC_{90} values also showed similar pattern with lower LC_{90} for citronella oil (1.52%) followed by basil oil (2.72%), eucalyptus oil (5.09%) and orange oil (9.11%) against hadda at 24 HAT. Similarly at 48 HAT also, citronella oil recorded the lower LC_{50} value (0.63%) followed by basil oil (0.91%), orange oil (2.02%) and eucalyptus oil (2.09%). The corresponding LC90 values of citronella oil, basil oil, eucalyptus oil and orange oil were 1.14, 1.73, 3.51 and 4.05 per cent respectively. Similar work by Mead (2018) on *Spodoptera liuralis* using basil oil reported the LC_{50} of 1.17 per cent and LC_{90} of 9.08 per cent. Manzoor *et al.* (2012) reported LC_{50} of eucalyptus and citronella oil as 4.81% and 3.39% respectively against *Periplanata americana*. Ali *et al.* (2019) found similar results for orange oil with LC_{50} of 3.53 per cent against red palm weevil.

The results of the present study showed that basil and citronella oil were effective against hadda beetle since they recorded lower LC_{50} and LC_{90} values when compared to eucalyptus and orange oil.

The essential oils act as chemo sterilant, fumigant, ovicidal and repellent agents, altering growth, development and feeding behaviour of insects (Dubey, 2011; Isman, 2010; Koul *et al*., 2008; Nerio *et al*., 2010). The main mechanism of action is linked to the ability of EO's to interfere with the cell membrane. Their accumulation leads to the disruption of the cell wall, leakage of the cellular contents and perturbation of homeostasis (Lambert *et al*., 2001; Juven *et al*., 1994). It may also affect different targets including γ-aminobutyric acid-gated chloride channels, octopamine receptors, tyramine receptors, acetylcholine esterase, nicotinic acetylcholine receptors, and sodium channels (Tong and Coats, 2010).

Results of the above experiments concluded that both basil and citronella oil were more toxic to brinjal mealy bug and hadda beetle than eucalyptus and orange oil. Hence basil oil and citronella oil were selected for further evaluation both in laboratory and field.

5.2. EVALUATION OF THE REPPELLENT AND ANTIFFEDANT EFFECT OF SELECTED ESSENTIAL OILS AGAINST HADDA BEETLE

5.2.1. Evaluation of Repellent Action of Essential Oils against Hadda Beetle Grub

Basil oil and citronella oil at 0.7% and 1% showed 100 per cent repellence of hadda beetle grubs after 30 and 60 minutes of treatment. Whereas basil oil and citronella oil at 0.5% recorded 90 and 100 per cent repellence at 30 and 60 MAT respectively. Labinas and Crocomo (2002) reported the repellent effect of citronella oil on fall army worm, *Spodoptera fugiperda* showing higher repellence effect at 1 per cent and above. Al-Harbi *et al.* (2021) reported repellence activity of *O. basilicum* essential oil at 0.75 mg cm^{-2} with value 82.3 per cent against *Sitophilus oryzae* after 5 hours of treatment.

From the current study, basil and citronella oil at 0.5% to 1% showed maximum repellence of 90 to 100 per cent against hadda beetle grubs. At lower concentration of 0.05% and 0.2%, citronella oil was showing better repellence effect (15-70%) compared to basil oil (10-50%) (Fig. 7 & 8).

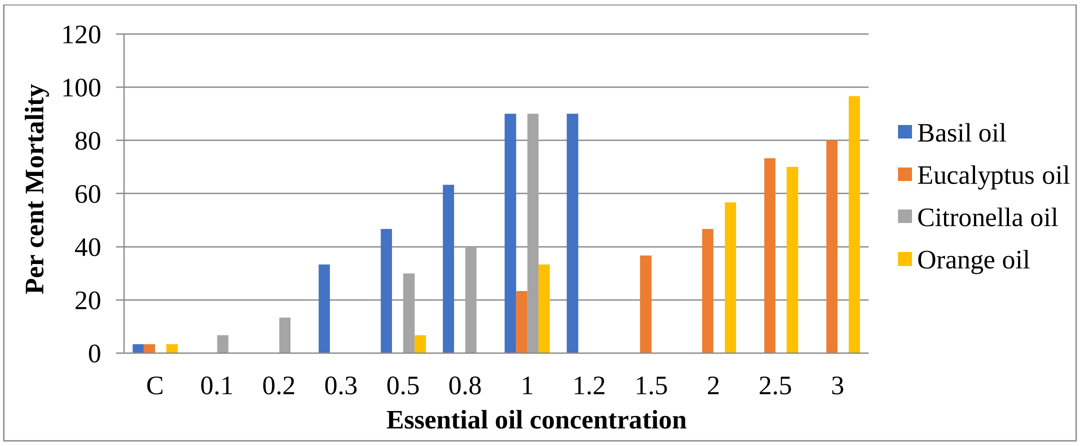


Fig. 3. Per cent mortality of mealy bug to essential oils at 24 HAT.

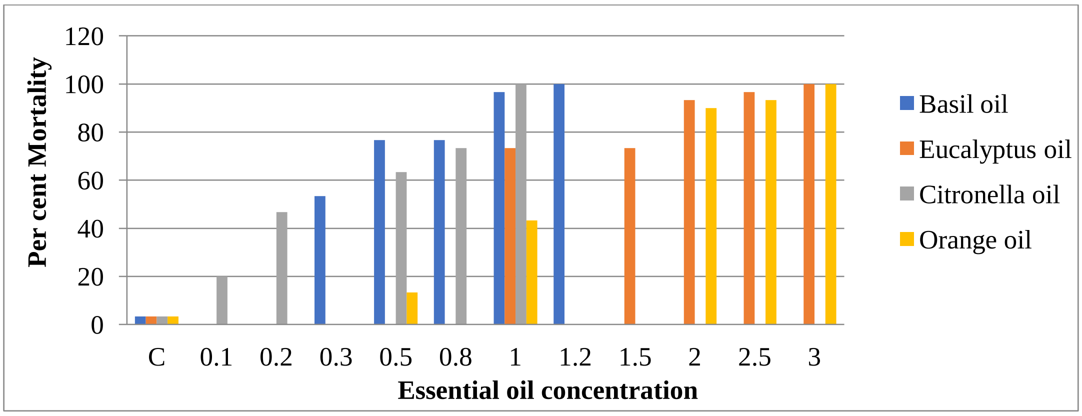


Fig. 4. Per cent mortality of mealy bug to essential oils at 48 HAT.

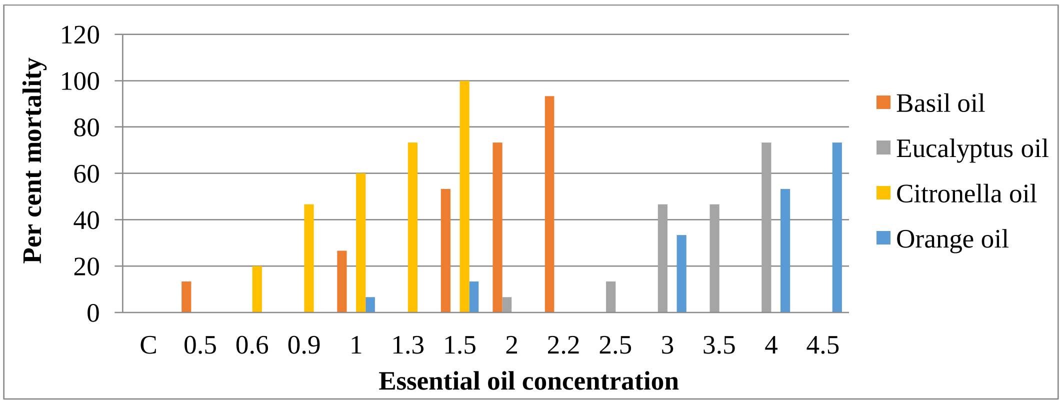


Fig. 5. Per cent mortality of hadda beetle to essential oils at 24 HAT.

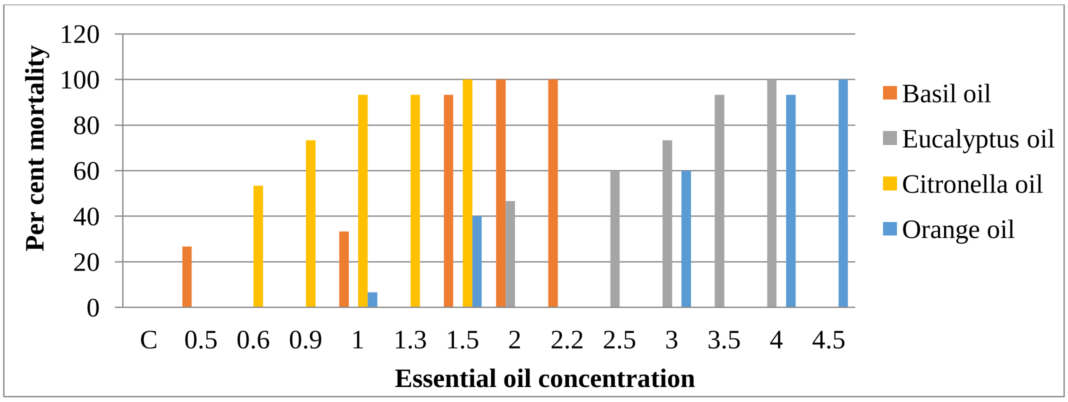


Fig. 6. Per cent mortality of hadda beetle to essential oils at 48 HAT.

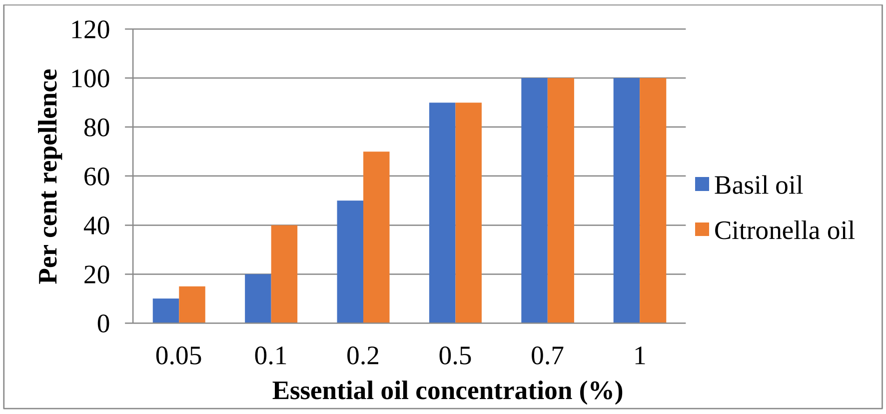


Fig. 7. Per cent repellence of basil & citronella oil at 30MAT.

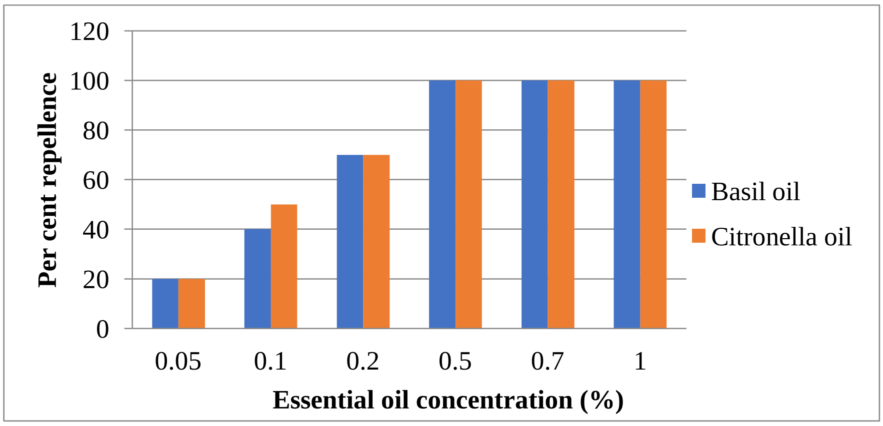


Fig. 8. Per cent repellence of basil & citronella oil at 60 MAT.

5.2.2. Evaluation of Antifeedant Effect of Essential Oil Against Hadda Beetle

Basil and citronella oil at 0.5%, 0.7% and 1% recorded maximum antifeedant index of 100 per cent after 24 hours of treatment. Kostic *et al.* (2008) reported the antifeedant effect of basil oil on *Lymantria dispar* where basil oil at 0.5% showed antifeedant effect of 60 and 85.71 per cent at 24 and 48 HAT respectively and it corroborates the results that we got in the present investigation. Saroukolai *et al.* (2014) reported that the basil oil at 16 ppm recorded Feeding Deterrence Index (FDI) of 60.28 per cent against *Leptinotarsa decemlineata* in laboratory.

In the present investigaton, 0.5% to 1% of basil and citronella oil was showing 100 per cent antifeedant index against hadda beetle grubs. Since citronella oil was showing 80 per cent antifeedant index at a lower concentration of 0.2% concentration in the present study, it may be considered as having better antifeedant properties against hadda beetle grubs.

5.3. BLOOM TEST OF SELECTED ESSENTIAL OILS

Based on bloom test, basil oil showed grade 4 “Good” bloom rate whereas, citronella showed grade 5 “Excellent” bloom rate. Citronella oil showed good emulsion where no oil droplets were found and in basil oil a few oil droplets were observed.

5.4. PHYTOTOXICITY EVALUATION OF SELECTED ESSENTIAL OILS ON BRINJAL

The results of phytotoxicity evaluation of selected essential oils on brinjal plants showed that basil oil at 2% on brinjal plants recorded severe phytotoxic symptoms (necrosis: 80-90%; scorching: 40-50%; hyponasty: 60-70%) whereas at 1% and 1.5%, slight (necrosis: 10-20%) and moderate (necrosis: 40-50%) phytotoxic symptoms were recorded respectively. Basil oil at 0.1 to 0.7% recorded no phytotoxic symptoms on brinjal (Fig. 9). Citronella oil at 2% recorded severe phytotoxic symptoms (necrosis: 90-100%; scorching: 30-40%; hyponasty: 70-80%) whereas at 1% and 1.5% slight (necrosis: 50-60%; hyponasty: 20-30%) and moderate (necrosis: 80-90%; hyponasty: 50-60%) phytotoxic symptoms were observed. Citronella oil at

0.1 to 0.7% recorded no phytotoxic symptoms on brinjal plants (Fig. 10). From the values of phytotoxicity evaluation, symptoms were much significant for citronella oil than basil oil. Hyponasty symptoms were recorded at a concentration of 1.5% and above for basil oil whereas for citronella oil hyponasty symptoms were observed at a lower concentration of 0.7% and above. It was that concluded phytotoxicity was more for citronella oil than basil oil. Phytotoxicity symptoms were recorded on brinjal plant at 0.7% and above concentrations of basil and citronella oil and hence, 0.7% is the maximum concentration of basil and citronella oil that can be sprayed on brinjal plant without any phytotoxic symptoms. Poonpaiboonpipat *et al*. (2013) found leaf wilting and reduction in chlorophyll and carotenoid content when citronella oil of 1.25%, 2.5%, 5%, and 10% was applied on leaves of *Echinochloa crusgalli* at a spray volume of 1000 litre per hectare. The essential oil caused membrane disruption and integrity loss due to electrolyte leakage. Karamaouna *et al.* (2013) reported phytotoxicity symptoms in grape vine when basil oil (4.5%) was sprayed on it.

5.5. FIELD EVALUATION OF ESSENTIAL OILS AGAINST INSECT PESTS OF BRINJAL AND ITS SAFETY TO NATURAL ENEMIES IN POT CULTURE

In the field evaluation, mean population of aphid were significantly lower for basil oil 0.7% and citronella oil 0.7% at 1 DAS and were statistically on par with thiamethoxam 25% WG @50 g a.i ha^{-1}at 3, 5 and 7 DAS. After 14 days of spraying, aphid population slowly started to build up due to the less persistence of essential oil in the field and also due to reduction in natural enemies population. The significant reduction of aphid population on plants sprayed with essential oils were mainly due to repellent and antifeedant effect along with contact toxicity (Fig. 11). Mean population of leaf webber reduced significantly for basil oil and citronella oil at 0.7% on 1, 3, 5 and 7 DAS and the treatments were statistically on par with chlorantraniliprole 18.5% SC @ 40 g a.i ha^{-1} (Fig. 12). At 14 DAS, mean damage on shoot and fruit by shoot and fruit borer was found significantly lower for chlorantraniliprole 18.5% SC @ 40 g a.i ha^{-1}followed by basil oil 0.7% and citronella oil 0.7% (Fig. 13). Mean population of natural enemies were significantly reduced in the essential oil treatments (0.7% of basil oil and citronella oil) at 1, 3, 5, 7 and 14 DAS than in the insecticidal treatments. The reduction in natural enemies mainly due to repellent and antifeedant effect and also due to the absence of insect pests which are the food for natural enemies (Fig. 114). Telaumbanua *et al*. (2021) reported that application of citronella-based pesticides can significantly reduce the insect pest population and natural enemies in rice ecosystem. Shadia *et al.* (2007) reported the effectiveness of basil oil concentration against the black cutworm, *Agrotis ipsilon* in laboratory and in a semi field trial. The field evaluation of *O. basilicum* oil at 2.5% reduced population of pod borer larvae (*Helicoverpa armigera*) and pod damage (Boulamtat *et al.*, 2021).

The results of biometric observations (total number of leaves, total number of damaged leaves, plant height) taken after the spraying were found to be non-significant. Chlorantraniliprole 18.5% SC @ 40 g a.i ha^{-1} recorded the highest fruit yield (515.09 g plant^{-1}) while among the essential oil treatments citronella oil 0.7% recorded the highest fruit yield (479.71 g plant^{-1}) (Fig. 15). Even though the insecticide treatment recorded a maximum yield, their environment hazard and other health risks on human consumption ensure that essential oil have better scope as plant protection chemicals in sustainable agriculture.

3.6. CHARACTERISATION OF SELECTED ESSENTIAL OIL

From the analysis, chemical components of basil oil obtained from GC-FID/GC-MS showed methyl chavicol as the major component with 75.73 per cent followed by Linalool (18.2%), (8) Cuprenene (1.58%), Geranial (0.60%), Alpha-Pinene (0.51%), Cineole (1,8) (0.18%), (E)-p-methoxy cinnamaldehyde (0.15%), Limonene (0.12%). Sajjadi (2006) reported chemnical components of *Ocimum basilicum* L. cv. purple and *Ocimum basilicum* L. cv. green from Iran. Methyl chavicol (52.4%), linalool (20.1%), epi-α-cadinol (5.9%) and trans-α-bergamotene (5.2%) were major constitruents of *O. basilicum* L. cv. Purple whereas, in *O. basilicum* L. cv. green methyl chavicol (40.5%), geranial (27.6%), neral (18.5%) and caryophyllene oxide (5.4%) were the major constituents. In both the essential oil methyl chavicol was the major component. Baritaux *et al.* (1992) reported methylchavicol, eugenol, linalol and 1,8-cineole were the major components of basil oil where, the methyl chavicol and eugenol content decreased after drying at 45 ^{o}C for 12 hours.

Meanwhile, geranial (64.77%) was found to be the most prominent chemical component in citronella oil followed by Citronellyl acetate (7.92%), Geraniol (7.08%), (Z) Iso citral (5.29%), Neral (3.60%), Limonene (2.22%), Hepten-2-one 6-methyl-5 (1.13%), Farmesene (Z)-β (0.58%), Neryl acetate (0.37%), Linalool (0.37%), (Iso) Isopulegol (0.35%), Nerol (0.24%), Z-β-ocimene (0.18%), (α) Humulene (0.17%), E-β-ocimene (0.14%), Rosefuran epoxide (0.10%), Citronellal (0.09%), Iso citral (E) (0.08%), Isopulegol (neo) (0.08%), Verbenol (trans) (0.07%), Allyl hexanoate (0.07%), Iso eugenol (E) (0.06%), Muurolol (epi α) (0.05%), Geranyl formate (0.05%) from current study. Pontes*et al.* (2018) reported major chemical component in *Cymbopogan nardus* as monoterpenes geraniol (33.88%), citronellal (27.55%) and citronellol (14.40%). Wany *et al.* (2014) classified citronella oil chemotype as Ceylon citronella oil (*Cymbopogon nardus* (inferior type)) and Java citronella oil (*Cymbopogon winterianus* (superior type)). Geraniol (18–20 %), limonene (9–11 %), methyl isoeugenol (7–11 %), citronellol (6–8 %), and citronellal (5–15 %) were found to be the major component of Ceylon citronella oil whereas, java citronella oil was containing citronellal (32–45 %), geraniol (11–13 %), geranyl acetate (3–8 %), and limonene (1–4 %) as major component.

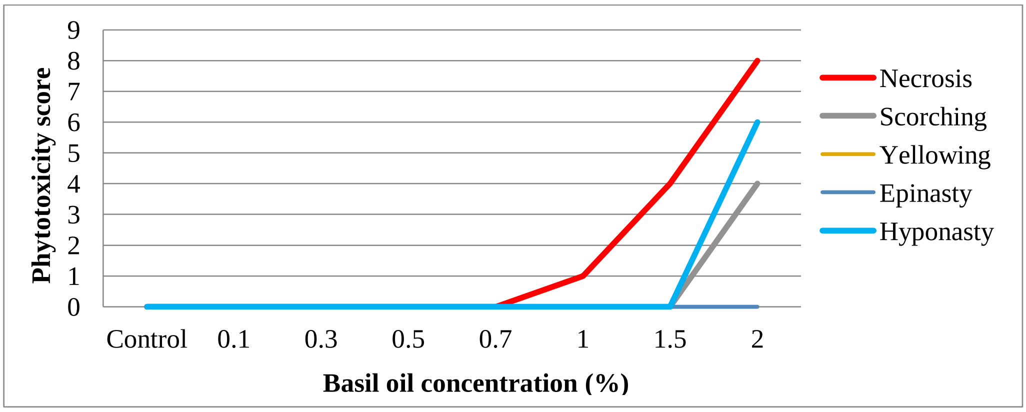


Fig. 9. Phytotoxicity scoring of basil oil on brinjal plant.

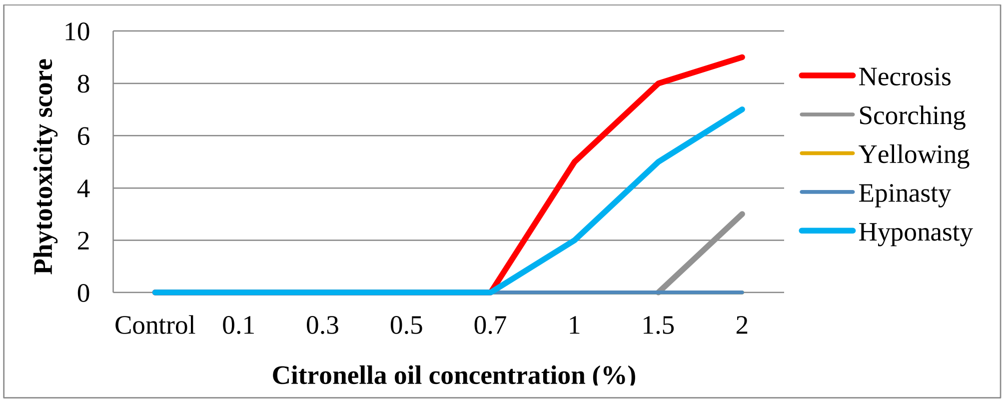


Fig. 10. Phytotoxicity scoring of citronella oil on brinjal plant.

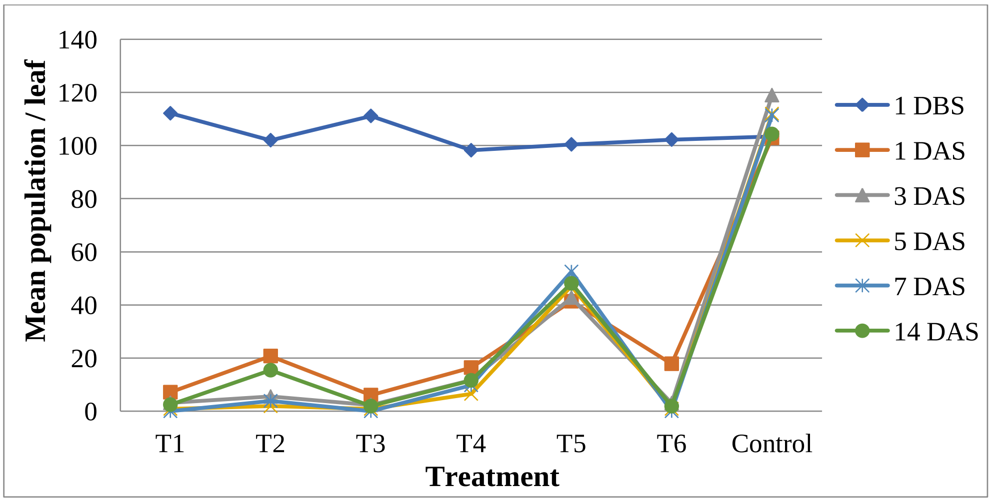


Fig. 11. Mean population of aphid (*Aphis gossypii*) per leaf before and after spray.

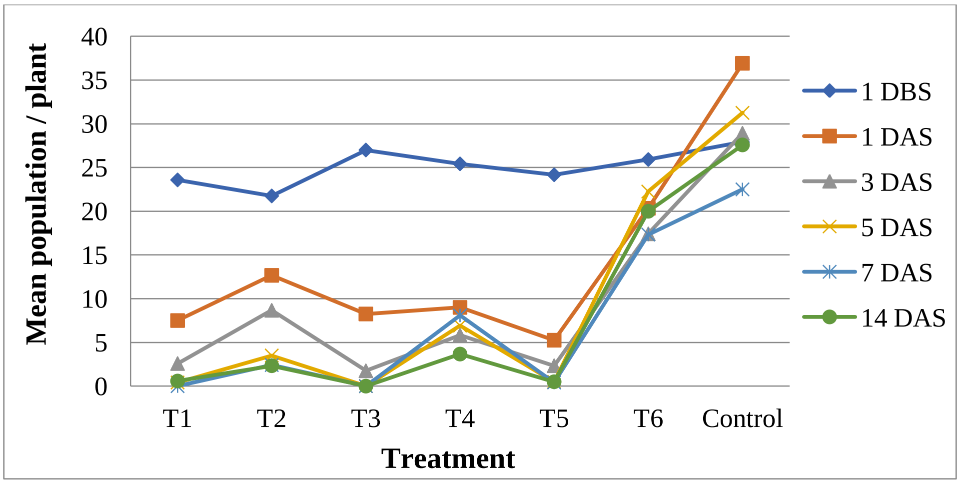


Fig. 12. Mean population of leaf webber (*Psara bipunctalis*) per plant before and after spray.

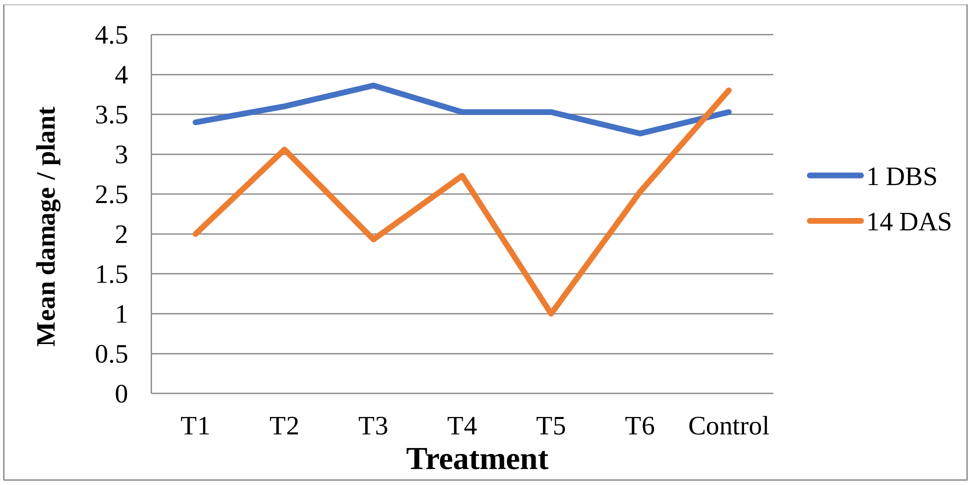


Fig. 13. Mean damage symptoms of shoot and fruit borer (*Leucinodes orbanalis*) on shoot and fruit per plant before and after spray.

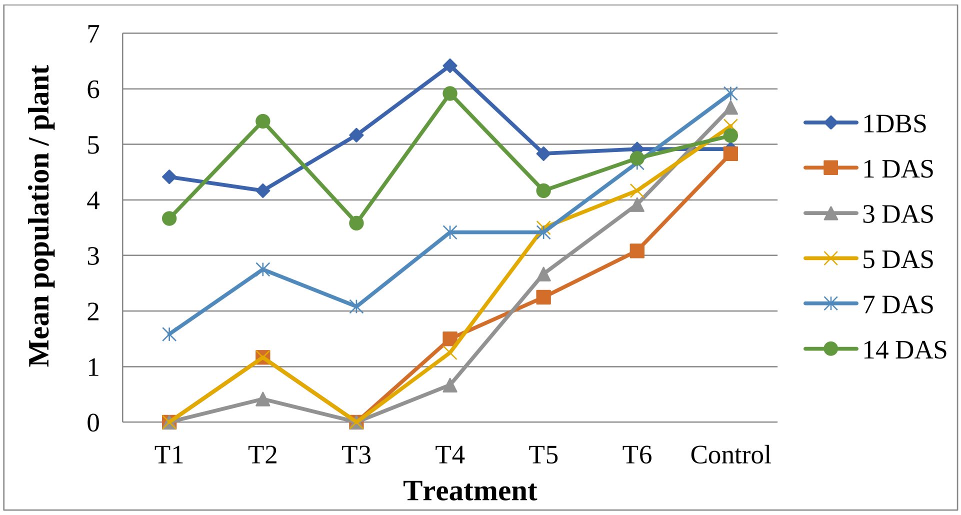


Fig. 14. Mean population of natural enemies *Cheilomenes sexmaculata* and *Coccinella transversalis* adults per plant before and after spray.

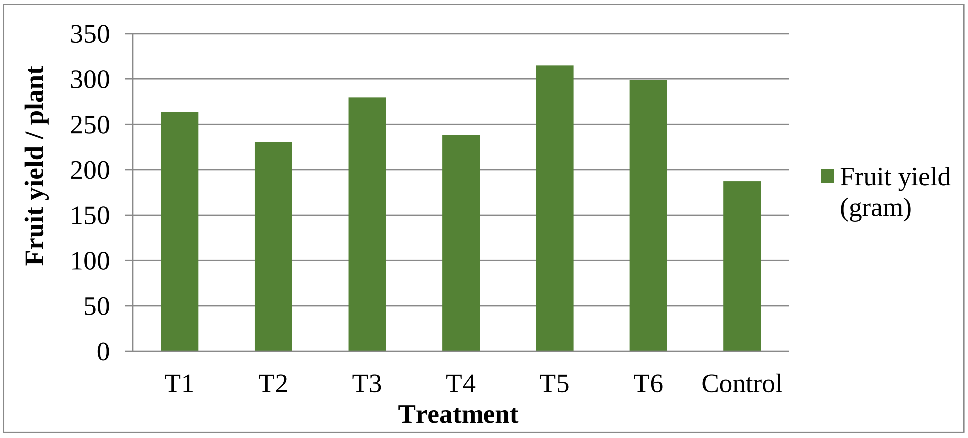


Fig. 15. Fruit yield per plant recorded after 20 days of spray.

Summary

6. SUMMARY

Brinjal, *Solanum melongena* L. is the most widely cultivating vegetable crop in the country. Due to its higher production and productivity, it is known as the "King of Vegetables" and "Common Man's Vegetable". However, significant infestations of major insect and non-insect pests severely reduce the crop's output. In order to control these pests, farmers are using harmful synthetic pesticides on a regular basis at various stages of the crop. The use of these pesticides on a regular basis resulted in residue, reduction of natural enemies, secondary pest outbreaks, health risk and contamination of many environmental components. The best option for minimising these ill effects is to maximise the use of botanicals in pest management.

The current study titled "Bioactivity of essential oils against insect pests of brinjal" was conducted from 2019 to 2021 at Department of Agricultural Entomology, College of Agriculture, Vellayani and Jawaharlal Nehru Tropical Botanical Garden and Research Institute, Palode, with an objective of evaluation of bioactivity of essential oils *viz.* basil oil, citronella oil, eucalyptus oil and orange oil against insect pests of brinjal and characterization of the most effective essential oil

The following are the results of the present investigations

- Basil oil in the concentration range of 0.3 to 1.2% caused 10 to 100 percent mortality of brinjal mealy bug. The LC_{50} and LC_{90} values of basil oil against mealy bug were 0.49%, 1.33% and 0.29%, 0.89% at 24 and 48 HAT respectively. Basil oil in the range of 0.5 to 2% caused 10 to 100 per cent mortality of hadda beetle in brinjal. The same recorded the LC_{50} and LC_{90} values of 1.25% and 2.85% at 24 HAT and 0.85% and 1.70% at 48 HAT against hadda beetle.

- Eucalyptus oil in the concentration range of 1 to 3% recorded mortality in the range of 10 to 100 per cent against brinjal mealy bug. Eucalyptus oil recorded the LC_{50} and LC_{90} values of 1.86 % and 4.28% at 24 HAT and 0.72% and 1.92% respectively at 48 HAT. Eucalyptus oil recorded 10 to100 per cent mortality of hadda beetle at a concentration range of 2 to 4 % and the same recorded the LC_{50} and LC_{90} values of 3.35%, 5.09% and 2.09%, 3.51% against hadda beetle at 24 and 48 HAT.

- Citronella oil in the concentration range of 0.1 to 1% recorded 10 to 100 per cent mortality of brinjal mealy bug. The LC_{50} and LC_{90} values of citronella oil against mealy bug were 0.64% and 2.39% at 24 HAT and 0.28% and 1.22% at 48 HAT respectively. Citronella oil recorded 10 to100 per cent mortality of hadda beetle at a concentration range of 0.6 to 1.5%. Against hadda beetle, the LC_{50} and LC_{90} values were 0.93%, 1.52% and 0.63%, 1.14% at 24 and 48 HAT respectively.

- Orange oil in the concentration range of 0.5 to 3% caused 10 to100 per cent mortality of brinjal mealy bugs. Orange oil recorded the LC_{50} 1.54%, 1.06% and LC_{90} 3.53%, 2.17% values respectively at 24 HAT and 48 HAT against brinjal mealy bug. Against hadda beetle, orange oil recorded 10 to 100 per cent mortality at a concentration range of 1 to 4.5%. The LC_{50} and LC_{90} values of orange oil against hadda beetle were 3.48% and 9.11% at 24 HAT and 2.02% and 4.05% at 48 HAT.

- In the laboratory bioassay, basil oil and citronella oil recorded lower LC_{50} and LC_{90} values than eucalyptus and orange oil, indicating its toxicity and effectiveness against brinjal mealy bug and hadda beetle.

- Basil and citronella oil at 0.5 to 1% recorded 90 to 100 % repellence against hadda beetle grubs. Basil and citronella oil at 0.5 to 1% recorded highest antifeedant index of 100 per cent.
- In the bloom test conducted to check the emulsification of essential oil and surfactant formulation (1:1) in distilled water, basil oil was showing grade 4 “Good” bloom rate whereas citronella oil showed grade 5 “Excellent” bloom rating.
- Phytotoxicity evaluation of basil oil recorded no phytotoxic symptoms at 0 to 0.7% concentration. Slight (necrosis: 10-20%), moderate (necrosis: 40-50%) and severe phytotoxic symptoms (necrosis: 80-90%, scorching: 40-50%, hyponasty: 60-70%) were recorded at 1%, 1.5% and 2% respectively.

Citronella oil showed no phytotoxic symptoms at concentration o 0 to 7%. Slight (necrosis: 50-60%, hyponasty: 20-30%)), moderate (necrosis: 80-90%, hyponasty: 50-60%) and severe phytotoxic symptoms (necrosis: 90-100%, scorching: 30-40%, hyponasty: 70-80%) were recorded at 1%, 1.5% and 2 % respectively.

- Based on laboratory screening and phytotoxicity evaluation, 0.5 and 0.7% of basil and citronella oil were selected for field study along with two conventional chemical insecticide check (chlorantraniliprole 18.5% SC @ 40 g a.i ha^{-1} and thiamethoxam 25% WG @ 50 g a.i ha^{-1}). Basil oil and citronella oil at 0.7% showed significant reduction in aphid population and it was statistically on par with thiamethoxam 25% WG @ 50 g a.i ha^{-1} even at 14 DAS. The leaf webber population was also significantly reduced on plants sprayed with basil and citronella oil at 0.7% and it was statistically on par with chlorantraniliprole 18.5% SC @ 40 g a.i ha^{-1} even after 14 days of spraying. chlorantraniliprole 18.5% SC @ 40 g a.i ha^{-1} showed significant reduction in the shoot and fruit borer damage after 14 days of spraying. Spraying of basil oil and citronella oil at 0.7 per cent showed significant reduction of lady bird beetle population. In biomteric observations (total number of leaves, total number of damaged leaves, plant height in cm) recorded after spray, treatments were found to be statistically non-significant. Fruit yield recorded maximum for the insecticidal treatment, chlorantraniliprole 18.5% SC @ 40 g a.i ha^{-1} (515.09 g $plant^{-1}$).
- GC-FID/GC-MS characterisation identified 24 chemical components in basil oil and 28 components in citronella oil. In basil oil, methyl chavicol (75.73%) was found to be the most prominent compound followed by Linalool (18.2%), (8) Cuprenene (1.58%), Geranial (0.60%), Alpha-Pinene (0.51%), Cineole (1,8) (0.18%), (E)-p-methoxy cinnamaldehyde (0.15%), Limonene (0.12%). The minor components identified were Beta-Pinene, 6-Mehyl-5-Hepten-2-one, (E)-Beta Ocimene, Iso-Menthol, (3Z) Hexenyl Hexonoate, (alpha) Neocallitropsene, (8) Himachalene, Aciphyllene and about 5 components were unidentified. In citronella oil, geranial (64.77%), was the most abundant component, followed by Citronellyl acetate (7.92%), Geraniol (7.08%), (Z) Iso

citral (5.29%), Neral (3.60%), Limonene (2.22%), Hepten-2-one 6-methyl-5 (1.13%), Farmesene (Z)-β (0.58%), Neryl acetate (0.37%), Linalool (0.37%), (Iso) Isopulegol (0.35%), Nerol (0.24%), Z-β-ocimene (0.18%), (α) Humulene (0.17%), E-β-ocimene (0.14%), Rosefuran epoxide (0.10%), Citronellal (0.09%), Iso citral (E) (0.08%), Isopulegol (neo) (0.08%), Verbenol (trans) (0.07%), Allyl hexanoate (0.07%), Iso eugenol (E) (0.06%), Muurolol (epi α) (0.05%), Geranyl formate (0.05%) and about 15 components were unidentified.

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BIOACTIVITY OF ESSENTIAL OILS AGAINST INSECT PESTS OF BRINJAL.

by

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ABSTRACT

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ABSTRACT

The study entitled "Bioactivity of essential oils against insect pests of brinjal" was undertaken in the Department of Agricultural Entomology at College of Agriculture, Vellayani during the period 2019-2021 with an objective of evaluating the bioactivity of essential oils *viz.,* basil oil, citronella oil, eucalyptus oil and orange oil against insect pests of brinjal and characterization of the most effective essential oils.

Laboratory screening of essential oils *viz.,* basil oil, eucalyptus oil, citronella oil and orange oil was conducted against one sucking pest (mealy bug, *Coccidohystrix insolita* (Green)) and one chewing pest (Hadda beetle, *Henosepilachna vigintioctopunctata* (Fabricius)) in brinjal. Acute toxicity of the essential oils against the test insects was determined by leaf dip bioassay. Preliminary test dose range causing 10 to 100 per cent mortality was fixed and based on these 6 doses including a control treatment was taken.

Based on the results of leaf dip bioassay of essential oils against mealy bug, LC_{50} and LC_{90} of basil oil, eucalyptus oil, citronella oil and orange oil were (0.49 and 1.33), (1.86 and 4.28), (0.64 and 2.39) and (1.54 and 3.53) per cent respectively at 24 hours after treatment (HAT). The corresponding LC_{50} and LC_{90} values of the above four oils at 48 HAT were (0.29 and 0.89), (0.72 and 1.92), (0.28 and 1.22) and (1.06 and 2.17) per cent respectively. Against hadda beetle the LC_{50} and LC_{90} values of basil oil, eucalyptus oil, citronella oil and orange oil were (1.25 and 2.85), (3.35 and 5.09), (0.93 and 1.52) and (3.48 and 9.11) per cent respectively at 24 HAT. While the corresponding LC_{50} and LC_{90} values at 48 HAT were (0.85 and 1.70), (2.09 and 3.51), (0.63 and 1.14) and (2.02 and 4.05) per cent respectively.

Based on toxicity bioassays, basil and citronella oil had lower LC_{50} and LC_{90} values than eucalyptus and orange oil against both mealy bug and hadda beetle at both 24 and 48 HAT and these two were selected for further lab and field evaluation.

Repellent and antifeedant effect of the selected essential oils from the first experiments *viz.,* basil oil, citronella oil were evaluated against hadda beetle by modified preference method and no choice method respectively. At 0.5 to 1 per cent

concentration, both basil and citronella oil showed 90-100 per cent repellence at 30 and 60 minutes of treatment. On other hand, 0.5 to 1 per cent concentration of basil and citronella oil showed antifeedant effect of 100 per cent after 24 hours of treatment.

Before conducting field evaluation of essential oils, bloom test and phytotoxicity evaluation on brinjal plants were done. Bloom test was conducted to check the emulsification of essential oil and surfactant formulation in distilled water. Results were showing “Good” bloom rating for basil oil and “Excellent” bloom rating for citronella oil.

Phytotoxicity evaluation of basil and citronella oil was carried on brinjal plant as per the protocols of CIBRC (Central Insecticide Board and Registration Committee). Basil oil and citronella oil at 0 to 0.7% were not showing any phytotoxic symptoms on brinjal plants while the higher doses of 1%, 1.5% and 2% were showing slight moderate and severe phytotoxic symptoms.

Based on the laboratory and phytotoxicity evaluation, two doses of basil oil and citronella oil (0.5% and 0.7%) were selected for the pot culture experiment along with two chemical checks and an untreated control. The experiments were laid in completely Randomized design with three replications.

In field study, spraying of basil oil and citronella oil at 0.7% showed significant reduction in aphid population and it was statistically on par with thiamethoxam 25% WG even at 14 DAT. The leaf webber population was also significantly reduced for basil and citronella oil at 0.7% and it was statistically on par with chlorantraniliprole 18.5% SC even at 14 DAT. Basil oil and citronella oil at 0.7% showed significant reduction in the shoot and fruit borer damage after 14 days of treatment and it was statistically on par with chlorantraniliprole 18.5% WG at 14 days after treatment. Basil oil and citronella oil at 0.7 per cent showed significant reduction in the lady bird beetle population.

There was no statistical difference among the different treatments with regard to the biometric observations *viz.*, total number of leaves, damaged leaves, plant height after the field spraying, however the fruit yield was recorded significantly higher in chlorantraniliprole 18.5% WG.

GC-FID/GC-MS studies on basil and citronella oil revealed that the predominant component of basil oil is Methyl chavicol (75.73%), followed by Linalool (18.21%) and (8) Cuprenene (1.58%). In citronella oil, Geranial (64.77%) was the most abundant component followed by Citronellyl acetate (7.92%), Geraniol (7.08%), (Z) Iso citral (5.29%) and Neral (3.60%).

സംഗ്രഹം

വഴുതനയിലെ കീടങ്ങൾക്കെതിരെ സിട്രോനെല്ല ഓയിൽ, യൂക്കാലിപ്റ്റസ് ഓയിൽ, ഓറഞ്ച് ഓയിൽ എന്നിവ ഉപയോഗിച്ചുകൊണ്ട് ഏറ്റവും ഫലപ്രദമായ അവശ്യ എണ്ണകളുടെ സ്വഭാവവും ജൈവ പ്രവർത്തനക്ഷമതയും വിലയിരുത്തുക എന്ന ലക്ഷ്യത്തോടെ 2019-2021 കാലയളവിൽ വെള്ളായണിയിലെ കാർഷിക കോളേജിലെ അഗ്രികൾച്ചറൽ എന്റമോളജി വിഭാഗത്തിൽ "വഴുതനയിലെ കീടങ്ങൾക്കെതിരെ അവശ്യ എണ്ണകളുടെ ബയോ ആക്ടിവിറ്റി" എന്ന വിഷയത്തിൽ പഠനം നടത്തുകയുണ്ടായി..

വഴുതനയിലെ ഒരു നീരുറ്റികുടിക്കുന്ന കീടത്തിനെതിരെയും (മീലി ബഗ്, കോക്സിഡോഹിസ്ട്രിക്സ് ഇൻസോളിറ്റ (പച്ച) ഒരു ചവച്ചരച്ചു കഴിക്കുന്ന കീടത്തിനെതിരെയും (ഹഡ്ഡ വണ്ട്, ഹെനോസെപിലാച്ന വിജിൻറ്റിയോക്റ്റോപങ്ക്) അവശ്യ എണ്ണകളായ ബേസിൽ ഓയിൽ, യൂക്കാലിപ്റ്റസ് ഓയിൽ, സിട്രോനെല്ല ഓയിൽ, ഓറഞ്ച് ഓയിൽ എന്നിവയുടെ ലബോറട്ടറി സ്ക്രീനിംഗ് നടത്തി. ലീഫ് ഡിപ്പ് ബയോഅസെയിലൂടെ പരീക്ഷണ പ്രാണികൾക്കെതിരായ അവശ്യ എണ്ണകളുടെ രൂക്ഷമായ വിഷാംശം നിർണ്ണയിക്കപ്പെട്ടു. 10 മുതൽ 100 ശതമാനം വരെ മരണത്തിന് കാരണമാകുന്ന പ്രാഥമിക പരിശോധന ഡോസ് റേഞ്ച് നിശ്ചയിക്കുകയും ഈ 6 ഡോസുകളുടെ അടിസ്ഥാനത്തിൽ ഒരു നിയന്ത്രണവും ഉൾപ്പെടുത്തി..

അവശ്യ എണ്ണകളുടെ ലീഫ് ഡിപ്പ് ബയോഅസേ ഫലങ്ങളെ അടിസ്ഥാനമാക്കി മീലി ബഗ് നു എതിരെ ബേസിൽ ഓയിൽ, യൂക്കാലിപ്റ്റസ് ഓയിൽ, സിട്രോനെല്ല ഓയിൽ, ഓറഞ്ച് ഓയിൽ എന്നിവയ്ക്ക് വേണ്ട LC_{50}, LC_{90} മൂല്യം യഥാക്രമം (0.49, 1.33), (1.86,

4.28), (0.64, 2.39) കൂടാതെ ചികിത്സ കഴിഞ്ഞ് 24 മണിക്കൂറിൽ (HAT) (1.54, 3.53) ശതമാനവും, 48 HAT-ൽ മുകളിലുള്ള നാല് എണ്ണകളുടെ അനുബന്ധ LC50, LC90 മൂല്യങ്ങൾ യഥാക്രമം (0.29, 0.89), (0.72, 1.92), (0.28, 1.22), (1.06, 2.17) ശതമാനം ആയിരുന്നു. ഹഡ വണ്ടിനെതിരെ ബാസിൽ ഓയിൽ, യൂക്കാലിപ്റ്റസ് ഓയിൽ, സിട്രോനെല്ല ഓയിൽ, ഓറഞ്ച് ഓയിൽ എന്നിവയുടെ LC50, LC90 മൂല്യങ്ങൾ (1.25, 2.85), (3.35, 5.09), (0.93, 1.52), (3.48, 9.11) എന്നിവ യഥാക്രമം 2 ശതമാനം HAT ആണ്. . 48 HAT-ലെ LC50, LC90 മൂല്യങ്ങൾ യഥാക്രമം (0.85, 1.70), (2.09, 3.51), (0.63, 1.14), (2.02, 4.05) എന്നിങ്ങനെയാണ്.

ടോക്സിസിറ്റി ബയോഅസെയുടെ അടിസ്ഥാനത്തിൽ, 24, 48 HAT എന്നിവയിൽ ബേസിൽ, സിട്രോനെല്ല ഓയിൽ എന്നിവയ്ക്ക് യൂക്കാല്ിപ്റ്റസ്, ഓറഞ്ച് ഓയിൽ എന്നിവയേക്കാൾ LC50, LC90 മൂല്യങ്ങൾ കുറവാണ്.

പരിഷ്കരിച്ച മുൻഗണനാ രീതിയിലൂടെയും തിരഞ്ഞെടുക്കൽ രീതിയില്ലാതെയും ആദ്യ പരീക്ഷണങ്ങളിൽ നിന്ന് തിരഞ്ഞെടുത്ത അവശ്യ എണ്ണകളുടെ വികർഷണവും ആന്റിഫീഡന്റ് ഫലവും ഹഡ വണ്ടിനെതിരെ യഥാക്രമം ബേസിൽ ഓയിൽ, സിട്രോനെല്ല ഓയിൽ എന്നിവ വിലയിരുത്തി. 0.5 മുതൽ 1 ശതമാനം വരെ സാന്ദ്രതയിൽ, 30, 60 മിനിറ്റ് ചികിത്സയിൽ ബേസിൽ, സിട്രോനെല്ല എണ്ണ എന്നിവ 90-100 ശതമാനം പ്രതിരോധം കാണിച്ചു. മറുവശത്ത്, 0.5 മുതൽ 1 ശതമാനം വരെ ബേസിൽ, സിട്രോനെല്ല എണ്ണ എന്നിവയുടെ സാന്ദ്രത 24 മണിക്കൂർ ചികിത്സയ്ക്ക് ശേഷം 100 ശതമാനം ആന്റിഫീഡന്റ് പ്രഭാവം കാണിച്ചു.

അവശ്യ എണ്ണകളുടെ ഫീൽഡ് മൂല്യനിർണ്ണയം നടത്തുന്നതിന് മുമ്പ്, വഴുതന ചെടികളിലെ ബ്ലൂം ടെസ്റ്റ്, ഫൈറ്റോടോക്സിസിറ്റി

വിലയിരുത്തൽ എന്നിവ നടത്തി. വാറ്റിയെടുത്ത വെള്ളത്തിൽ അവശ്യ എണ്ണയുടെ എമൽസിഫിക്കേഷനും സർഫക്ടന്റ് ഫോർമുലേഷനും പരിശോധിക്കാൻ ബ്ലൂം ടെസ്റ്റ് നടത്തി. ബേസിൽ ഓയിലിന് "നല്ല" ബ്ലൂം റേറ്റിംഗും സിട്രോനെല്ല ഓയിലിന് "മികച്ച" ബ്ലൂം റേറ്റിംഗും ഫലങ്ങൾ കാണിക്കുന്നു.

സിഐബിആർസിയുടെ (സെൻട്രൽ ഇൻസെക്ടിസൈഡ് ബോർഡും രജിസ്ട്രേഷൻ കമ്മിറ്റിയും) പ്രോട്ടോക്കോളുകൾ അനുസരിച്ച് തുളസിയുടെയും സിട്രോനെല്ല ഓയിലിന്റെയും ഫൈറ്റോടോക്സിസിറ്റി വിലയിരുത്തൽ വഴുതന ചെടിയിൽ നടത്തി. ബേസിൽ ഓയിലും സിട്രോനെല്ല എണ്ണയും 0 മുതൽ 0.7% വരെ വഴുതന ചെടികളിൽ ഫൈറ്റോടോക്സിക് ലക്ഷണങ്ങളൊന്നും കാണിക്കുന്നില്ല, 1%, 1.5%, 2% എന്നിവയുടെ ഉയർന്ന ഡോസുകൾ നേരിയ മിതമായതും കഠിനവുമായ ഫൈറ്റോടോക്സിക് ലക്ഷണങ്ങൾ കാണിക്കുന്നു.

ലബോറട്ടറിയിലെ ഫൈറ്റോടോക്സിസിറ്റി വിലയിരുത്തലിന്റെ അടിസ്ഥാനത്തിൽ, രണ്ട് ഡോസ് ബേസിൽ ഓയിലും സിട്രോനെല്ല ഓയിലും (0.5%, 0.7%) പോട്ട് കൾച്ചർ പരീക്ഷണത്തിനായി തിരഞ്ഞെടുത്തു, കൂടാതെ രണ്ട് രാസ പരിശോധനകളും നിയന്ത്രണവും. മൂന്ന് പകർപ്പുകളോടെ പൂർണ്ണമായും ക്രമരഹിതമായ രൂപകൽപ്പനയിലാണ് പരീക്ഷണങ്ങൾ നടത്തിയത്.

ഫീൽഡ് പഠനത്തിൽ, ബേസിൽ ഓയിലും സിട്രോനെല്ല ഓയിലും 0.7% സ്പ്രേ ചെയ്യുന്നത് മുഞ്ഞയുടെ എണ്ണത്തിൽ ഗണ്യമായ കുറവുണ്ടാക്കി, ഇത് സ്ഥിതിവിവരക്കണക്കനുസരിച്ച് 14 DAT-ൽ പോലും തയോമേത്ഒകസം 25% WG-ന് തുല്യമാണ്. ബേസിൽ, സിട്രോനെല്ല ഓയിൽ എന്നിവയുടെ ലീഫ് വെബർ

ജനസംഖ്യ 0.7% ആയി ഗണ്യമായി കുറഞ്ഞു, ഇത് സ്ഥിതിവിവരക്കണക്കനുസരിച്ച് 14 DAT-ൽ പോലും ക്ലോറൻട്രാനിലിപ്രോളിന്റെ 18.5% SC തുല്യമാണ്. ബേസിൽ ഓയിലും 0.7% സിട്രോനെല്ല എണ്ണയും 14 ദിവസത്തെ ചികിത്സയ്ക്ക് ശേഷം ചിനപ്പുപൊട്ടൽ, കായ് തുരപ്പൻ കേടുപാടുകൾ എന്നിവയിൽ ഗണ്യമായ കുറവ് കാണിച്ചു, ചികിത്സയ്ക്ക് ശേഷം 14 ദിവസങ്ങളിൽ ഇത് ക്ലോറൻട്രാനിലിപ്രോളിന്റെ 18.5% WG-ന് തുല്യമാണ്. ബേസിൽ ഓയിലും സിട്രോനെല്ല ഓയിലും 0.7 ശതമാനവും ലേഡി ബേർഡ് വണ്ടുകളുടെ എണ്ണത്തിൽ ഗണ്യമായ കുറവ് കാണിച്ചു.

ബയോമെട്രിക് നിരീക്ഷണങ്ങളുമായി ബന്ധപ്പെട്ട് വ്യത്യസ്ത ചികിത്സകൾക്കിടയിൽ സ്ഥിതിവിവരക്കണക്കുകൾ വ്യത്യാസമില്ല, അതായത്, മൊത്തം ഇലകളുടെ എണ്ണം, കേടായ ഇലകൾ, ഫീൽഡ് സ്പ്രേ ചെയ്തതിന് ശേഷമുള്ള ചെടികളുടെ ഉയരം, എന്നിരുന്നാലും ക്ലോറൻട്രാനിലിപ്രോളിൽ 18.5% WG-ൽ ഫലങ്ങളുടെ വിളവ് ഗണ്യമായി ഉയർന്നതായി രേഖപ്പെടുത്തിയിട്ടുണ്ട്.

തുളസിയിലേയും സിട്രോനെല്ല ഓയിലിനേയും കുറിച്ചുള്ള GC-FID/GC-MS പഠനങ്ങൾ ബേസിൽ ഓയിലിന്റെ പ്രധാന ഘടകം മീഥൈൽ ചാവിക്കോൾ (75.73%) ആണെന്ന് വെളിപ്പെടുത്തി, തുടർന്ന് ലിനലൂൾ (18.21%), (8) കുപ്രെനീൻ (1.58%). സിട്രോനെല്ല ഓയിലിൽ, ജെറേനിയൽ (64.77%) ആണ് ഏറ്റവും സമൃദ്ധമായ ഘടകം, അതിനുശേഷം സിട്രോനെല്ലിൽ അസറ്റേറ്റ് (7.92%), ജെരാനിയോൾ (7.08%), (Z) ഐസോ സിട്രൽ (5.29%), നെറൽ (3.60%) എന്നിവയും .