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TOXICITY BIOASSAYS OF ESSENTIAL OILS AGAINST HADDA BEETLE *HENOSEPILACHNA VIGINTIOCTOPUNCTATA* (F)

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ABSTRACT

Contact, repellent and antifeedant bioassays of essential oils (EO's) viz., basil oil and citronella oil were evaluated against hadda beetle *Henosepilachna vigintioctopunctata* (F) in brinjal. Based on the results of leaf dip bioassay, the LC_{50} and LC_{90} values of basil oil and citronella oil were 1.25% and 2.85%, 0.93% and 1.52%, respectively at 24 hrs after treatment (HAT). While the corresponding LC_{50} and LC_{90} values at 48 HAT were 0.85% and 1.70%, 0.63% and 1.14%, respectively. Repellent and antifeedant effect of the chosen essential oils were also evaluated by modified preference method and no choice method, respectively. At 0.5 to 1% concentration, both basil and citronella oil showed 90-100% repellence at 30 and 60 min of treatment. 100% antifeedant effect was also observed at the same concentration (0.5 to 1%) for both the EO's at 24 HAT. GC-FID/GC-MS analysis of basil and citronella oil revealed that the predominant component of basil oil was methyl chavicol (75.73%), followed by linalool (18.21%) and cuprenene (1.58%); in citronella oil, geranial (64.77%) was the most predominant component followed by citronellyl acetate (7.92%), geraniol (7.08%), (Z) iso citral (5.29%) and neral (3.60%).

Key words: *Henosepilachna vigintioctopunctata*, essential oils, biopesticide, basil oil, citronella oil, contact bioassay, repellence, antifeedant index, LC_{50}, LC_{90}, preference and no choice method, methyl chavicol

The use of conventional insecticides even though help in reducing pest incidence, leads to many problems. The current limitations of their use are pushing for the discovery and development of less harmful products. One of the most promising solutions is the exploitation of plant-based pesticides. Currently, biopesticides comprise a value of about $3 billion worldwide, accounting for 5% of the total crop protection market (Olson, 2015). Essential oils (EOs) are an important alternative to botanical pesticides. Their safety profile is guaranteed by the fact that most of EOs 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 EOs is their lack of toxicity to 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 to human health. The biopesticidal potential of essential oils (EOs) from plant families viz., Myrtaceae, Lamiaceae, Asteraceae, Apiaceae and Rutaceae has been reviewed by many authors (Isman, 2000, Tripathi et al., 2009). Control measures against insect pests rely mostly on insecticides that leaves residues in the produce. It is important to study the prospects of effective biopesticides in managing these pests. With this background, this study evaluates the efficacy of basil oil and citronella oil. Brinjal, *Solanum melongena* is an important vegetable crop in India; it is infested by >36 insect pests from the nursery stage to harvest (Reghupathy et al., 1997); hadda beetles *Henosepilachna. vigintioctopunctata* (F) grubs and adults scrape the green matter of the upper and lower sides of the leaves leaving behind only a network of veins (Kunjwal and Srivastava, 2018). This study has used grubs as test insects for assessing the toxicity, repellence and antifeedant effects of chosen essential oils.

MATERIALS AND METHODS

The leaf dip bioassay method of Elbert and Nauen (1996) was followed to find out the lethal toxicity of essential oils. Preliminary range tests were done to fix the test dose range causing 20 to 80 % mortality approximately. Based on this, five intermediate doses along with a control were taken. The stable emulsions of essential oils prepared by mixing oil with tween 80 (1:1v/v) and double distilled water using a magnetic stirrer were used for the toxicity bioassay. Ten grubs were released over the EO treated leaf disc inside each glass Petri dish (9 cm diameter) as separate experiments. These glass petri dishes were then covered with lid to prevent insect escape. After 24 hrs and 48 hrs of exposure, the number of dead insects were recorded. The 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 (15 cm dia) with 5 cm gap between two filter paper halves and different concentrations of essential oil emulsions (1ml) were poured on one half of the filter paper, while the other half was poured with surfactant and water. Late instar grubs of hadda beetles were released into the Petri plate and observations were made at 30 and 60 MAT (minutes after treatment) and % repellence was calculated. The antifeedant properties of essential oil on hadda beetle grubs were studied by no choice method (Duraipandiyan et al., 2011). Brinjal leaves were cut into square pieces of 16 cm^2 and then dipped in various concentrations of essential oil, placed on petri plates laid with filter paper. The leaf area eaten was computed after 24 hrs using the graph and the antifeedant index (AFI) was calculated. All the above-mentioned experiments were conducted as replicated trials and control experiments were also maintained.

The essential oil was analysed by gas chromatography-flame ionization detector (GC-FID) and gas chromatography-mass spectrometry (GC-MS). 1 µl of diluted essential oil (30 µl of basil oil and citronella oil diluted in 1.5 ml acetone) were injected into 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). Relative % of individual components in basil and citronella oils were obtained from the peak area-percent report of volatiles in the GC-FID data. GC-MS analyses were performed by splitless injection of 1 µl (each) of the diluted basil oil and citronella oil (30 µl of oil diluted to 1.5 ml with acetone, each) on GC -MS- QP2020 NX (Shimadzu, Japan) fitted with a Rxi-5 Sil MS capillary column (5% diphenyl 95% dimethyl polysiloxane, non-polar, 30 m x 0.32 mm i.d., 0.25 µm film thickness; Crossbond, USA). Individual components were identified by WILEY12 database matching, comparison of LRIs and comparison of mass spectra with published data (Adams, 2007). Data on % 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 % repellence and Antifeedant Index were analysed by ANOVA after arc sine transformation.

RESULTS AND DISCUSSION

When leaf dip bioassays were conducted to prove the contact toxicity of basil and citronella oil, it was found that citronella oil was more toxic to hadda beetles than basil oil. The LC_{50} and LC_{90} values of citronella oil at 24 HAT were 0.93 and 1.52% and of basil oil were 1.31 and 2.62%, respectively. At 48 HAT, the LC_{50} and LC_{90} values followed the same pattern in which citronella oil recorded lowest values of 0.63 and 1.14% whereas basil oil recorded 0.98 and 1.75% (Table 1). In a similar study using basil oil against the lepidopteran polyphagous pest, *Spodoptera littoralis* it was found that the LC_{50} and LC_{90} values were 1.17 and 9.08%, respectively (Mead, 2018). Manzoor et al. (2012) reported the LC_{50} of eucalyptus and citronella oil as 4.81 and 3.39%, respectively against *Periplanata americana*. In the present investigation, much lower dose of essential oil was used.

The repellence of these essential oils seems to be statistically similar and both the essential oils showed 100% repellence at 0.5% concentration and above. When the data was examined closely, it was observed that citronella oil showed more repellence at lower concentration than basil oil. At lower concentration of 0.05 and 0.2%, citronella oil was showing better repellence effect (15-70%) compared to basil oil (10-50%) (Table 1). Basil oil and citronella oil showed 100% repellence of hadda beetle grubs after 30 and 60 min of treatment at a concentration of 0.7% and above. Labinas and Crocomo (2002) reported the repellent effect of citronella oil on fall army worm, *Spodoptera fugiperda* and reported that the oil showed repellence effect at 1% and above. Al-Harbi et al. (2021) reported repellence activity (82.3%) of *O. basilicum* essential oil against *Sitophilus oryzae* after 5 hr of treatment. The essential oils act as chemosterilant, fumigant, ovicidal and repellent agents, altering the growth, development and feeding behaviour of insects (Dubey, 2011; Isman, 2010; Nerio et al., 2010). The main mechanism of action is linked to the ability of EOs 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

Table 1. Efficacy of basil and citronella oil against *H. vigintioctopunctata*

Essential oil	χ^2	d.f.	LC_{50} (%) (95% CL)	LC_{90} (%) (95% CL)	Slope± SE
24 HAT					
Basil oil	3.25	4	1.25 (0.90-1.74)	2.85 (2.04-3.97)	3.5± (-0.35)
Citronella oil	1.96	4	0.93 (0.77-1.14)	1.52 (1.24-1.85)	6.11± 1.34
48 HAT					
Basil oil	7.44	4	0.85 (0.62-1.17)	1.70 (1.24-2.35)	4.27± 0.28
Citronella oil	1.47	4	0.63 (0.48-0.83)	1.14 (0.87-1.50)	4.98± 1.38

Repellent and antifeedant effect

Concentration	% Repellence (%)				Antifeedant index (%)	
	Basil oil		Citronella oil		Basil oil	Citronella oil
	30 MAT	60 MAT	30 MAT	60 MAT		
0.05	10 (13.92)[c]	20(26.56)[c]	15 (20.24)[c]	20 (26.56)[d]	1.56 (6.99)[d]	11.85 (19.92)[d]
0.10	20 (26.56)[c]	40 (38.66)[c]	40 (38.66)[c]	50 (44.71)[c]	10.31 (18.39)[c]	52.68 (46.56)[c]
0.20	50 (44.71)[b]	70 (60.25)[b]	70 (60.25)[b]	70 (60.25)[b]	43.37 (41.17)[b]	80.12 (63.64)[b]
0.50	90 (79.23)[a]	100 (88.71)[a]	90 (79.23)[ab]	100 (88.71)[a]	100 (89.28)[a]	100 (89.28)[a]
0.70	100 (88.71)[a]	100 (88.71)[a]	100 (88.71)[a]	100 (88.71)[a]	100 (89.28)[a]	100 (89.28)[a]
1.00	100 (88.71)[a]	100 (88.71)[a]	100 (88.71)[a]	100 (88.71)[a]	100 (89.28)[a]	100 (89.28)[a]
CD (p=0.05)	(16.27)	(14.29)	(19.88)	(13.65)	(4.06)	(4.83)

Table value of χ^2 at 4 df= 9.48, χ^2 non-significant at: $p< 0.05$; LC_{50}-Lethal Concentration causing 50% mortality; LC_{90}-Lethal Concentration causing 90 % mortality; CL-Confidence Limit; SE-Standard Error. Values in parentheses subjected to arc sine transformation; MAT-Minutes After Treatment.

including γ-aminobutyric acid-gated chloride channels, octopamine receptors, tyramine receptors, acetylcholine esterase, nicotinic acetylcholine receptors, and sodium channels (Tong and Coats, 2010; Ali et al., 2019).

Citronella and basil oil showed Antifeedant Index (AFI) of 100% at 0.5, 0.7 and 1% concentration of EO. At lower concentrations, citronella oil showed significantly more antifeedant effects than basil oil. At 0.2% concentration, citronella oil showed 80% AFI, while basil oil showed only 40%. It can be concluded that citronella oil had slightly higher antifeedant properties than basil oil (Table 1). 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% at 24 and 48 HAT, respectively, and it corroborates the present results. Saroukolai et al. (2014) reported that the basil oil at 16 ppm recorded Feeding Deterrence Index (FDI) of 60.28% against *Leptinotarsa decemlineata* in laboratory. EOs extracted from plants tend to disrupt the substrate recognition site therefore relics the coordination and locomotion activity of insects. After the EOs have entered the insect body, due to their lipophilic chemical structure and good penetrance, they switch on different signalling pathways and insecticidal mechanisms, causing biochemical, physiological, developmental dysfunction, and eventually mortality (War et al., 2012). Qualitative analysis of essential oils for their chemical components was done in GC- FID and GC- MS. About 21 chemical components were obtained in basil oil and 41 in citronella oil of which 14 components were unidentified (Table 2). Methyl chavicol was found to be the most prominent component (75.73%) in basil oil followed by others; geranial (64.77%) was found to be the most prominent component in citronella oil followed by others. There are many reports on the insecticidal activity of the major components of the above essential oils (de Menezes et al., 2020). The components of these essential oils may act singly or synergistically (Wang et al., 2021). Monoterpenes, a major constituent of essential oils, 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). From the above experiments on lethal and sublethal toxicity of essential oils, it can be concluded that the sublethal effects (repellent and antifeedant effects) of essential oils are more pronounced even at a lower concentration of less than 1%.

Table 2. Chemical components of basil oil and citronella oil (GC-FID/GC-MS analysis)

Basil oil		Citronella oil	
Component	%	Component	%
Alpha-pinene	0.512	Hepten-2- one 6 methyl-5	1.134
Beta-pinene	Minor	Limonene	2.226
6 Mehyl-5-hepten-2-one	Minor	Z-β-ocimene	0.189
Limonene	0.121	E-β-ocimene	0.142
(1,8) Cineole	0.186	Allyl hexanoate	0.070
(E)-Beta ocimene	Minor	Linalool	0.370
Un identified	-	Un identified	-
Linalool	18.210	Verbenol (trans)	0.075
Iso menthol	Minor	Isopulegol (neo)	0.082
Methyl chavicol	75.730	Citronellal	0.098
Geranial	0.602	(Iso) Isopulegol	0.353
(3Z)-Hexenyl hexonoate	Minor	(Z)-Iso citral	5.294
Unidentified	-	Rosefuran epoxide	0.102
Unidentified	-	Iso citral (E)	0.088
Unidentified	-	Terpineol (α)	0.093
(alpha) Neocallitropsene	Minor	Nerol	0.245
Unidentified	-	Citronellol	0.091
(8) Himachalene	Minor	Neral	3.604
Aciphyllene	Minor	Geraniol	7.086
8-Cuprenene	1.580	Geranial	64.773
(E) -p-Methoxy-cinnamaldehyde	0.150	Geranyl formate	0.058
		Citronellyl acetate	7.928
		Neryl acetate	0.375
		Farnesene(Z)-β-	0.581
		Iso eugenol (E)	0.065
		α-Humulene	0.179
		Muurolol (epi α)	0.059

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AUTHOR CONTRIBUTION STATEMENT

TSV conceived and designed the research. KM conducted the experiments and analysed the data. TSV wrote the manuscript. NA, MSN and VG edited the manuscript. All authors read and approved the manuscript.

CONFLICT OF INTEREST

No conflict of interest.

REFERENCES

Adams R P. 2007. Identification of essential oil components by gas chromatography / mass spectrometry (Vol. 456). Carol Stream, IL: Allured publishing corporation. 804 pp.

Al-Harbi N A, Al Attar N M, Hikal D M, Mohamed S E, Latef A H A, Ibrahim A A, Abdein, M A. 2021. Evaluation of insecticidal effects of plants essential oils extracted from basil, black seeds and lavender against *Sitophilus oryzae*. Plants 10(829): 1-24.

Ali M A, Mohanny K M, Mohamed G S, Allam R O H. 2019. Efficacy of some promising plant essential oils to control the red palm weevil *Rhynchophorus ferrugineus* Olivier (Coleoptera: Curculionidae) under laboratory conditions. SVU- International Journal of Agricultural Science 1(2): 12-45.

Brut S. 2004. Essential oils: Their antibacterial properties and potential applications in foods-A review. International Journal of Food Microbiology 94: 223-253.

Choi W S, Park B S, Lee Y H, Jang D Y, Yoon H Y, Lee S E. 2006. Fumigant toxicities of essential oils and monoterpenes against *Lycoriella mali* adults. Crop Protection 25: 398-401.

de Menezes C W G, Carvalho G A, Alves D S, de Carvalho A A, Aazza S, de Oliveira Ramos V, Pinto J E B P, Bertolucci S K V. 2020. Biocontrol potential of methyl chavicol for managing *Spodoptera frugiperda* (Lepidoptera: Noctuidae), an important corn pest. Environmental Science and Pollution Research 27: 5030-5041.

Dubey N K. 2011. Natural products in plant pest management. CABI, Wallingford, UK. 293 pp.

Duraipandiyan V, Ignacimuthu S, Paulraj M G. 2011. Antifeedant and larvicidal activities of Rhein isolated from the flowers of *Cassia fistula* L. Saudi Journal of Biological Sciences 18(2): 129-133.

Elbert A, Nauen R. 1996. Bioassays for imidacloprid for resistance monitoring against the whitefly *Bemisia tabaci*. Proceedings. Brighton crop protection conference pests and diseases (vol. 2), 1996. pp. 731-738.

Finney D J. 1971. Probit analysis (3rd ed.). University Press, Cambridge, UK, 333 pp.

Isman M B. 2000. Plant essential oils for pest and disease management. Crop Protection 19(8-10): 603-608.

Isman M B. 2010. Botanical insecticides, deterrents, repellents and oils. Industrial crops and uses; ISBN: 978-1-84593-616-7 510. pp. 433-445.

Juven B J, Kanner J, Schved F, Weisslowicz H. 1994. Factors that interact with the antibacterial action of thyme essential oil and its active constituents. Journal of Applied Bacteriology 76(6): 626-631.

Karr L L and Coats J R. 1992. Effect of four monoterpenoids on growth and reproduction of the German cockroach (Blattodea: Blattellidae). Journal of Economic Entomology 85: 424-429.

Kostic M, Popovic Z, Brkic D, Milanovic S, Sivcev I, Stankovic S. 2008. Larvicidal and antifeedant activity of some plant-derived compounds to *Lymantria dispar* L. (Lepidoptera: Lymantridae). Bioresource Technology 99(16): 7897-7901.

Kunjwal N, Srivastava R M. 2018. Insect pests of vegetables. Pests and their Management. Springer, Singapore. pp. 163-221.

Labinas A M, Crocomo W B. 2002. Effect of Java grass (*Cymbopogon winterianus* Jowitt) essential oil on fall army worm *Spodoptera frugiperda* (J E Smith, 1797) (Lepidoptera, Noctuidae). Maringa 24(5): 1401-1405.

Lambert R J W, Skandamis P N, Coote P J, Nychas G J E. 2001. A study of the minimum inhibitory concentration and mode of action of oregano essential oil, thymol and carvacrol. Journal of Applied Microbiology 91(3): 453-462.

Manzoor F, Munir N, Ambreen A, Naz S. 2012. Efficacy of some essential oils against American cockroach *Periplaneta americana* (L.). Journal of Medicinal Plant Resources 6(6): 1065-1069.

Mead H M. 2018. Composition and larvicidal action of *Ocimum basilicum* L. essential oil against *Spodoptera littoralis* (Boisd.). Journal of Plant Protection and Pathology 9(2): 139-143.

Nerio L S, Olivero-Verbel J, Stashenko E. 2010. Repellent activity of essential oils: A review. Bioresource Technology. 101(1): 372-378.

Olson, S. 2015. An analysis of the biopesticide market now and where is going. Outlooks Pest Management 26: 203-206.

Pavela R, Benelli G. 2016. Essential oils as ecofriendly biopesticides? challenges and constraints. Trends Plant Science 21: 1000-1007.

Reghupathy A, Palanisamy S, Chandramohan N, Gunathilagaraj K. 1997. A Guide on crop pests. Sooriya Desktop Publishers, Coimbatore. 264 pp.

Saroukolai A T, Nouri-Ganbalani G, Hadian J, Rafiee-Dastjerdi H. 2014. Antifeedant activity and toxicity of some plant essential oils to Colorado potato beetle, *Leptinotarsa decemlineata* Say (Coleoptera: Chrysomelidae). Plant Protection Science 50(4): 207-216.

Tong F, Coats J R. 2010. Effects of monoterpenoid insecticides on [3H]-TBOB binding in house fly GABA receptor and 36Cl - uptake in American cockroach ventral nerve cord. Pesticide Biochemistry and Physiology 98: 317-324.

Tripathi A K, Upadhayay S, Bhulyan M. and Bhattacharya P R. 2009. A review on prospects of essential oils as biopesticide in insect pest management. Journal of Pharmacognosy and Phytotherapy 1(5): 52-63.

Wang Z, Xie Y, Sabier M, Zhang T, Deng J, Song X, Liao Z, Li Q, Yang S, Cao Y, Liu X, Zhou G, 2021. Trans-anethole, a potent toxic fumigant that partially inhibits rusty grain beetle (*Cryptolestes ferrugineus*) acetylcholinesterase activity. Industrial Crops Production 161, 113207 https://doi.org/10.1016/j. indcrop.2020.113207.

War A R, Paulraj M G, Ahmad T, Buhroo A A, Hussain B, Ignaicimuthu S, Sharma H C. 2012. Mechanisms of plant defence against insect herbivores. Plant Signalling and Behaviour 7(10): 1306-1320.

Watanabe K, Shono Y, Kakimizu A, Okada A, Matsuo N, Satoh A, Nishimura H. 1993. New mosquito repellent from *Eucalyptus camaldulensis*. Journal of Agricultural and Food Chemistry 41: 2164-2166.

Wei X M, Guo S S, Yan H, Cheng X L, Wei F, Du S S. 2018. Contact toxicity and repellency of the essential oil from *Bupleurum bicaule* helm against two stored product insects. Journal of Chemistry 2018: 1-9.

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