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Toxicity properties of Lemongrass Essential Oil against Brinjal Hadda Beetle, *Henosepilachna vigintioctapunctata* (Fabricius)

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ABSTRACT: A laboratory experiment was conducted out at the toxicology and seaweed laboratory of Department of Entomology, Annamalai University, Chidambaram during the academic year 2021-2022 to study the impacts of lemongrass essential oil against brinjal hadda beetle. Early infestation of the brinjal crop by the hadda beetle and its grub stages results in economic losses. Due to its polyphagous nature, it also treats other medicinal and solanaceous crops. Lemongrass essential oil was widely exploited for urban pest management in various formulations. In our current study, five different concentrations of lemongrass essential oil were tested with standard checks of neem oil 2 per cent, cypermethrin 25EC and distilled water. The results showed that the values of LC_{50} and mortality per cent in laboratory circumstances, the dry film residue bioassay showed substantially higher values than the leaf dip bioassay and fumigant bioassay. The bioassays of lemongrass essential oil @ 5 per cent were much effective to brinjal hadda beetle and third instar grub than neem oil @ 2 per cent and also it provided the same or more promising results of cypermethrin 25EC @ 0.04%. Therefore, it might be a promising management approach at the semi-field and field levels as well as a green pesticide for pest management.

Keywords: Pest management, Fumigation method, Leaf dip method, Dry film method, Neem oil, Laboratory Bioassay.

INTRODUCTION

Brinjal, *Solanum melongena* (Linnaeus), originated in India and is now widely farming in temperate and tropical Asian countries, as well as in the Middle East and around the Mediterranean basin (Daunay, 2008). Because of brinjal is susceptible to large number of insect pest infestations from such as shoot and fruit borer, hadda beetle, ash weevils, leafhoppers, and aphids, farmers spray at least twice a week or 48 times in six months in Simulipara, the centre of the brinjal belt, Kolkata (Singh, 2010). Hadda beetle adults and grubs were both very aggressive, feeding on the epidermal tissue of leaves, flowers and fruits, scraping the chlorophyll content and causing considerable production loss. Beetles are active throughout the crop season and begin causing harm as soon as the plant is transplanted, usually within the first week. Because of its polyphagous nature, it may survive in both on and off seasons (Ghosh and Senapathi 2001). The detection of dangerous chemical pesticide residues in food, along with greater food safety awareness, has led in to the restriction of some pesticides in agricultural production. Plant essential oils are volatile chemicals that are mostly composed of terpenoids and are utilised for their aromatic qualities (Brugger *et al*., 2021). *Cymbopogon citratus* (DC. Stapf.), a plant native to India and Sri Lanka, exhibits antifungal (Khan and Ahmad 2011), anti-infammatory (Gbenou *et al.,* 2013), and anti-protozoan (Santin *et al.*, 2009) activities. Recent research found that *C. citratus* essential oil has strong antifeedant efficacy and is poisonous to the stored yam chips pest, *Dinoderus porcellus* (Lense) (Loko *et al.*, 2021), fall armyworm, *Spodoptera frugiperda* (JE Smith) (Sombra *et al*., 2020) and cowpea weevil, *Callosobruchus maculatus* (F.) (de Souza Alves *et al.*, 2019). In light of the foregoing, this study investigates the efficiency of lemongrass essential oil against the brinjal hadda beetle using several bioassay approaches.

MATERIAL AND METHODS

Mass culturing of *H. vigintioctopunctata*

H. vigintoctopunctata adults were collected in and around Sivapuri village (N 11° 3676', E 79° 7136'), Cuddalore district, Tamil Nadu, India. To provide a constant food supply for grubs and adults, grubs and adults were maintained in potted brinjal variety Annamalai covered with fine wire mesh cages (3m × 4m × 2m) under semi-field conditions at 27 ± 2°C, 85% RH. Damaged plants were replaced with fresh plants throughout the research period. Mated female 28-spotted beetles were placed in cages covered with cylindrical nylon mesh in potted plants (60 cm height and 22.5 cm diameter). The adults were taken from the plants after oviposition. The eggs on the plants were left

alone to hatch. Using a soft camel hair brush, the grubs were transferred to fresh and healthy host plant leaves (1grub per leaf) immediately after hatching. To avoid water loss and keep the leaves fresh, the cut end of each leaf petiole was wrapped with damp cotton. Each leaf was then placed in a petridish (9 cm in diameter) and covered with a fine net. Once the grub had digested the leaf, it was replaced by a new leaf. The grub was raised to adulthood and the number of instars was calculated by the amount of skin lost (Karunaratne and Arukwatt 2008).

Botanochemicals. A commercial combination of neem oil and cypermethrin 25EC was procured locally, while the study's extracted lemongrass essential oil was purchased from the National Bureau of Agricultural Insect Resources, Indian Council of Agricultural Research, Bangalore.

Bioassay methods

Fumigant bioassay method. Fumigant bioassays were carried out on third instar grubs and adults of *H. vigintioctopunctata* using concentrations of 1, 2, 3, 4 and 5% lemongrass essential oil produced with distilled water of tween 80 @ 0.1% concentration. As standard checks, 2% neem oil and 0.04% cypermethrin 25EC were included. In clean petri plates, four layers of saturated filter paper were retained (8 cm diameter). At the bottom of those filter papers, a small rectangular-sized filter paper (5 × 2 cm) was put. Lemongrass essential oil @ 5µl was applied directly on a rectangular piece of filter paper using a micropipette for each treatment. Then, to prevent direct contact of the oils with the test insects, five grubs and adults were released on the top layer of the petri dish. The entire setup was sealed by parafilm to prevent the treated oils from escaping. Distilled water was used as a control. Hadda beetle grubs and adults were killed after 24 and 48 hours was recorded (Chalise *et al.,* 2019).

Leaf dip bioassay method. The leaf dip bioassay of lemongrass essential oil was investigated using no-choice technique. The lemongrass essential oil was dissolved in distilled water to get the stock concentration. As an emulsifier, polysorbate 80 (Tween 80) was utilised at a concentration of 0.1%. Fresh brinjal leaf discs were individually dipped in concentrations of 0.25, 0.5, 1, 3, and 5% lemongrass essential oil after being punched with an iron cork borer. The leaf disc was placed in distilled water as a control. Neem oil @ 2% and cypermethrin 25EC @ 0.04% were used as standard check. After air drying, each leaf disc was placed in petridish containing wet filter paper to avoid early drying of the leaf disc and 4 hours of pre-starved *H. vigintioctopunctata* of both grubs and adults were introduced into the petridishes. Three replications were maintained per treatment and each replication had five third instar grubs and adults in each experiment. The mortality of grubs and adults were noted after 24 and 48 hours (Ahmed *et al.,* 2022).

Concentration mortality effect of lemongrass essential oil on *H. vigintioctopuntata.* The varied concentration of lemongrass essential oil was prepared and followed to work out lethal concentration (LC_{50}) by various bioassay experiments. The results of mortality of the third instar grub and adults of hadda beetle after 48 hours of treatment were further evaluated by probit analysis. The results were mentioned in discussion section (Finney, 1971).

Dry film residue bioassay method. Acute toxicity bioassays were performed on *H. vigintoctopunctata* third instar grubs and adults using 1, 2, 3, 4, and 5% concentrations of lemongrass essential oil prepared with distilled water and tween 80 @ 0.1% added to mix oil in water (Suthisut *et al.,* 2011). Standard checks included 2% neem oil and 0.04% cypermethrin 25 EC, whereas control petridishes treated just distilled water (Kodandaram *et al.,* 2014). Each treatment comprised three replications, with each replication including five third instar grubs and adults. A film of each essential oil was formed on both surfaces of the petri dish by gently swirling 1 ml of concentration and drying for 10 minutes under an electric fan. Grubs and adult beetles were placed in a petri dish and fed untreated brinjal leaves. The number of larvae and adults that died after 24 and 48 hours was recorded. Abbott's formula (Abbott, 1987) was used to adjust the original data:

$$\text{Percentage of corrected mortality} = \frac{\text{Observed mortality} - \text{Control mortality}}{100 - \text{Control mortality}} \times 100$$

Statistical analysis. According to Gomez and Gomez (1984), the data collected in the studies were subjected to analysis of variance (ANOVA) using a completely randomised block design. Data transformations were performed prior to analysis, and the CCARI-ICAR computer-based WASP programme was used for analysis (Jangam and Wadekar 2012). Duncan's Multiple Range Test (DMRT) was used to differentiate between treatments mean values (Duncan, 1955).

RESULTS AND DISCUSSION

Dry film residue method. In the dry film residue bioassay, the mortality of the third instar grubs and adults of *H. vigintioctopunctata* were tabulated (Table 1). In the third instar grubs, after 24 hours of treatment the mortality rate for lemongrass essential oil at 5% was greatest with 80.00 per cent and was followed by rates of 4, 3 and 2 percent, which were 73.33, 53.33 and 40.00 per cent, respectively. Cypermethrin 25EC @ 0.04% and neem oil @ 2%, however caused 46.67 and 33.33 per cent mortality rate, respectively. After 48 hours of treatment, cypermethrin 25EC @ 0.04% and lemongrass essential oil @ 5% both had maximum mortality rate of 93.33 per cent. Neem oil @ 2% (66.67%), lemongrass essential oil @ 4% (80.00%), and lemongrass essential oil 2% (60.00%) were next in line. The 1% concentration of lemongrass essential oil had the lowest fatality rate, which was 46.67 per cent. Similar trend was recorded against both the third instar grubs and adults of *H. vigintioctopunctata*. In adult stage, after 24 hours of treatment, lemongrass essential oil at 5% had the highest mortality rate of 86.67% and lemongrass essential oil at 1% had the lowest mortality rate of 46.67%. Neem oil at 2% and cypermethrin 25 EC at 0.04% had mortality rates that were statistically

equivalent to each other at 60.00 per cent. After 48 hours of treatment, 5% of lemongrass essential oil was found to be 100 per cent fatal, followed by 3% and 4% of lemongrass essential oil, and cypermethrin 25 EC @ 0.04% with 93.33 per cent, 86.7% of lemongrass essential oil @ 2% and 2% of neem oil, which was shown to be 80 per cent fatal. Lemongrass essential oil was shown to have the lowest per cent mortality rate of 73.33, at 1%.

The lemongrass essential oil proved efficient insecticide against both third instar grubs and adults of *H. vigintioctopunctata* when used in this method. It was confirmed that the percentage mortality of grubs and adults of *H. vigintioctopunctata* was directly related to the concentration of lemongrass essential oil and the period of exposure. For third instar grubs and adults of *H. vigintioctopunctata*, the LC_{50} of lemongrass essential oil was 1.28% and 0.38%, respectively. The contact toxicity bioassay technique of lemongrass, betel vine, myrtle grass, and clove essential oils against the stored product mite, *Tyrophagus* sp., likewise produced similar results (Pumnuan *et al.,* 2014). After 48 hours of exposure, *Spodoptera frugiperda* larvae (Sombra *et al.*, 2020), *Sitophilus granarius* adults (Plata-Rueda *et al.*, 2020b), *Drosophila melanogaster* adults (Aljedani, 2021), *Nilaparvata lugens* nymphs and adults (Mardiningsih and Ma'mun 2021), and *Trichoplusia ni* larvae (Ghosh and Senapati 2001) all shown similar trends in insecticidal activity of lemongrass essential oil. Lemongrass essential oil's citral (neral + geraniol) combination demonstrated a strong insecticidal action on *T. urticae* (Mead and Hala 2012). Hadda beetle adult mortality suggested a neurotoxic impact through the aberrant elongation of elytra. Adult *Ulomides dermestoides* (Plata-Rueda *et al.,* 2020a) and *Bemisia tabaci* showed similar outcomes after being exposed to lemongrass essential oil, which caused paralysis that was irreversible (Kim *et al.,* 2011).

Fumigant toxicity. The fumigant toxicity of lemongrass essential oil against third instar grubs and adults of *H. vigintioctopunctata* were tabulated (Table 2). After 24 hours of treatment in third instar hadda beetle grub, cypermethrin 25EC @ 0.04% reported the highest death rate of 33.33 per cent. While lemongrass essential oil @ 2 and 3% both had the lowest mortality rate of 6.67 per cent. Neem oil @ 2% and lemongrass essential oil @ 5%, however, both showed 20 per cent mortality. The maximum mortality, 80.00 per cent, was seen in lemongrass essential oil @ 5% after 48 hours of treatment. The mortality rate that was the lowest, 13.33 per cent, was found in lemongrass essential oil @ 1%. Fumigant bioassay of lemongrass essential oil against the adults of *H. vigintioctopunctata* exhibited similar trend as recorded against the third instar grubs of *H. vigintioctopunctata.* The highest mortality of 26.67 per cent was recorded after 24 hours of treatment with cypermethrin 25EC @ 0.04% and lemongrass essential oil @ 5%. With mortality rate of 6.67 percent, lemongrass essential oil @ 2% was shown to have the lowest death rate. Neem oil @ 2% and lemongrass essential oil @ 3 and 4 percent mortality rates were statistically equivalent to each other with 13.33 per cent. The two treatments with the highest death rates lemongrass essential oil @ 5% and cypermethrin 25 EC @ 0.04% of 53.33 per cent after 48 hours of treatment. Lemongrass essential oil @ 1% had the lowest mortality of 20 per cent. Neem oil @ 2% and lemongrass essential oil @ 3% had mortality rates of 40%, which were statistically equivalent.

For the third instar grubs and adults of *H. vigintioctopunctata*, the percent mortality for lemongrass essential oil was recorded as ranging from 13.33 to 80.00 and from 20.00 to 53.33, respectively. Lemongrass essential oil had LC_{50} values for both grubs and adults of 3.24% and 4.49%, respectively. Similar outcomes of the fumigant action of lemongrass essential oil were seen against lesser grain borer, *Rhyzopertha dominica* (Michaelraj *et al.,* 2007), *Sitophilus oryzae*, and cowpea weevil, *Callosobruchus maculates* (de Souza Alves *et al*., 2019). The fumigant action of lemongrass essential oil against thrips, *Frankliniella schultzei*, mealybugs, *Pseudococcus jackbeardsleyi* (Pumnuan and Insung 2016) and cabbage looper, *Trichoplusia ni* (Tak *et al.,* 2016) was significantly influenced by citral (*trans-citral* and *cis-citral*) compound, a blend of citral and geranial (Tamer *et al.,* 2019). Dose and mortality rate had positive correlation for lemongrass essential oil and thyme oil in tomato pinworm, *Tuta absoluta* (Ngongang *et al.,* 2022); cabbage aphid, *Brevicoryne brassicae* and black bean aphid, *Aphis fabae* under fumigation studies (Chalise *et al*., 2019). The lemongrass essential oil proved potent fumigant insecticidal property and similar trend was noted in the fumigant study of lemongrass essential oil with other 97 essential oils against cecidomyiid gall midge, *Camptomyia corticalis* (Kim *et al*., 2012). The presence of monoterpene aldehydes of citral a (geranial), citral b (neral), linalool, and linalyl acetate against *Sitophilus oryzae* was responsible for the lemongrass essential oil's fumigant insecticidal ability (Paranagama *et al.,* 2004).

Leaf dip bioassay. The third instar grubs of *H. vigintioctopunctata* were subjected to a leaf dip assay using various doses of lemongrass essential oil. The findings are listed in Table 3. The highest mortality after 24 hours of treatment of 46.67 per cent was cypermethrin 25EC @ 0.04%. Neem oil @ 2% and lemongrass essential oil @ 3% both had a 20 per cent mortality rate. Cypermethrin 25 EC @ 0.04% showed the greatest mortality of 86.67 per cent after 48 hours of treatment. The mortality rate that was reported at the lowest level was 6.67 percent for lemongrass essential oil @ 0.25%. Neem oil @ 2% and lemongrass essential oil @ 3% both statistically equaled each other in terms of mortality at 46.67 per cent. Lemongrass essential oil's leaf dip bioassay against adults of *H. vigintioctopunctata* showed a similar pattern to that seen against the third instar grubs of *H. vigintioctopunctata.* Cypermethrin 25EC 0.04% was shown to be the most effective treatment after 24 hours, with a death rate of 53.33 per cent. Lemongrass essential oil at a concentration of 0.5% had the lowest death rate, which was 6.67 per cent. After 48 hours of treatment, cypermethrin 25EC @ 0.04% achieved a

maximum mortality of 90.00%, whereas lemongrass essential oil @ 0.25% recorded a minimum mortality of 6.67 per cent. Neem oil @ 2% and lemongrass essential oil @ 3% were statistically equivalent with regard to mortality at 53.33 per cent each. In this bioassay, lemongrass essential oil caused mortality rates of 6.67 to 53.33 percent and 6.67 to 66.67 percent in *H. vigintioctopunctata* of the third instar grubs and adults, respectively. Lemongrass essential oil had a lethal concentration (LC_{50}) against third-instar grubs and adults of 3.52% and 2.68%, respectively. Lemongrass essential oil residue on filter paper discs against adults of *Tribolium castaneum* showed a similar mortality trend (Olivero-Verbel *et al.*, 2010). Both the cabbage aphid, *Brevicoryne brassicae*, and the black bean aphid, *Aphis fabae*, were impacted by lemongrass essential oil @ 1% with the presence of limonene and citronellal, resulting in 100% death (Chalise *et al.*, 2019). Contrarily, cabbage looper, *T. ni*, only had a 40% death rate when exposed to 1% lemongrass essential oil (Jiang *et al*., 2010). *Aedes aegypti*, the mosquito that transmits yellow fever, was rendered larvicidal by the chemical diethyl phthalate, which was present in honeysuckle essential oil (Muturi *et al*., 2019). A leaf disc study against the citrus red mite, *Panonychus citri*, confirms the potential of lemongrass essential oil as a natural pesticide (Zhu *et al*., 2023).

Table 1: Dry film residue bioassay of lemongrass essential oil against the third instar grubs and adults of *H. vigintioctopunctata.*

Treatments	Concentration	*Grub Mortality (%)		* Adult Mortality (%)	
		24 HAT	48 HAT	24 HAT	48 HAT
T_1	Lemongrass essential oil @ 1%	33.33(35.23)^{f}	46.67(43.09)^{f}	46.67(43.09)^{e}	73.33(58.93)^{e}
T_2	Lemongrass essential oil @ 2%	40.00(39.22)^{e}	60.00(50.78)^{e}	73.33(58.94)^{c}	86.67(68.60)^{c}
T_3	Lemongrass essential oil @ 3%	53.33(46.91)^{c}	73.33(58.91)^{c}	73.33(58.93)^{c}	93.33(75.34)^{b}
T_4	Lemongrass essential oil @ 4%	73.33(58.95)^{b}	80.00(63.48)^{b}	80.00(63.45)^{b}	93.33(75.34)^{b}
T_5	Lemongrass essential oil @ 5%	80.00(63.49)^{a}	93.33(75.13)^{a}	86.67(68.61)^{a}	100.00(89.71)^{a}
T_6	Neem oil @ 2%	33.33(35.24)^{f}	66.67(54.74)^{d}	60.00(50.77)^{d}	80.00(63.48)^{d}
T_7	Cypermethrin 25 EC @ 0.04%	46.67(43.08)^{d}	93.33(75.07)^{a}	60.00(50.77)^{d}	93.33(75.13)^{b}
T_8	Control	0.00(0.28)^{g}	0.00(0.28)^{g}	0.00(0.28)^{f}	0.00(0.28)^{f}
C.D. (p=0.05)		3.60	2.81	2.74	4.12
SED		1.70	1.32	1.29	1.94

HAT: Hours after Treatment; *Mean of three replications; Values in parenthesis are sine arc transformed; In a column means followed by the same letter(s) are not significantly different (p=0.05) by DMRT.

Table 2: Fumigation bioassay of lemongrass essential oil against the third instar grubs and adults of *H. vigintioctopunctata*

Treatments	Concentration	*Grub mortality (%)		*Adult mortality (%)	
		24 HAT	48 HAT	24 HAT	48 HAT
T_1	Lemongrass essential oil@1%	0.00 (0.28)^{e}	20.00 (26.50)^{e}	0.00 (0.28)^{d}	20.00 (26.50)^{e}
T_2	Lemongrass essential oil@2%	6.67 (14.95)^{d}	33.33 (35.26)^{d}	6.67 (14.95)^{c}	33.33 (35.26)^{d}
T_3	Lemongrass essential oil @3%	6.67 (14.95)^{d}	40.00 (39.23)^{c}	13.33 (21.40)^{b}	40.00 (39.23)^{c}
T_4	Lemongrass essential oil@4%	13.33 (21.39)^{c}	46.67 (43.08)^{b}	13.33 (21.40)^{b}	46.67 (43.08)^{b}
T_5	Lemongrass essential oil@5%	20.00 (26.53)^{b}	53.33 (46.91)^{a}	26.67 (31.09)^{a}	53.33 (46.91)^{a}
T_6	Neem oil @ 2%	20.00 (26.53)^{b}	40.00 (39.23)^{c}	13.33 (21.39)^{b}	40.00 (39.23)^{c}
T_7	Cypermethrin 25 EC @ 0.04%	33.33 (35.26)^{a}	53.33 (46.91)^{a}	26.67 (31.09)^{a}	53.33 (46.91)^{a}
T_8	Control	0.00 (0.28)^{e}	0.00 (0.28)^{f}	0.00 (0.28)^{d}	0.00 (0.28)^{f}
C.D. (p=0.05)		2.08	2.42	1.54	2.42
SED		0.98	1.14	0.72	1.14

HAT: Hours after Treatment; *Mean of three replications; Values in parenthesis are sine arc transformed; In a column means followed by the same letter (s) are not significantly different (p=0.05) by DMRT.

Table 3: Leaf dip bioassay of lemongrass essential oil against the third instar grubs and adults of *H. vigintioctopunctata.*

Treatments	Concentration	Grub Mortality (%)		Adult Mortality (%)	
		24 HAT	48 HAT	24 HAT	48 HAT
T_1	Lemongrass essential oil @ 0.25%	0.00 (0.28)^{e}	6.67 (14.95)^{f}	0.00 (0.28)^{g}	6.67 (14.95)^{f}
T_2	Lemongrass essential oil @ 0.5%	0.00 (0.28)^{e}	13.33 (21.36)^{e}	6.67 (14.95)^{f}	26.66 (31.07)^{e}
T_3	Lemongrass essential oil @ 1%	6.67 (14.95)^{d}	33.33 (35.25)^{d}	13.33 (21.36)^{e}	40.00 (39.21)^{d}
T_4	Lemongrass essential oil @ 3%	20.00 (26.54)^{c}	46.67 (43.07)^{c}	20.00(26.54)^{d}	53.33 (46.89)^{c}
T_5	Lemongrass essential oil @ 5%	33.33 (35.25)^{b}	53.33 (46.89)^{b}	40.00 (39.21)^{b}	66.66 (54.72)^{b}
T_6	Neem oil @ 2%	20.00 (26.54)^{c}	46.67 (43.07)^{c}	26.67 (31.08)^{c}	53.33 (46.89)^{c}
T_7	Cypermethrin 25 EC@ 0.04%	46.67 (43.07)^{a}	86.67 (68.60)^{a}	53.33 (46.89)^{a}	90.00 (71.66)^{a}
T_8	Control	0.00 (0.28)^{e}	0.00 (0.28)^{g}	0.00 (0.28)^{g}	0.00 (0.28)^{g}
C.D. (p=0.05)		1.31	2.00	1.61	2.12
SED		0.61	0.93	0.75	0.99

HAT: Hours after Treatment; *Mean of three replications; Values in parenthesis are sine arc transformed; In a column means followed by the same letter (s) are not significantly different (p=0.05) by DMRT.

CONCLUSIONS

Lemongrass essential oil also has antifeedant and repelling qualities, in addition to exhibiting insecticidal qualities against hadda beetle grubs and adults. It had a number of secondary metabolites that affected these characteristics. According to the statistical analysis, lemongrass essential oil at 5 @ is more effective as a repellent and a protector of the leaf area. The insecticidal properties of 5 per cent lemongrass essential oil and cypermethrin 25EC were identical in action but took more time than chemical.

FUTURE SCOPE

To determine effectiveness of hadda beetle pest management, field-level experiments would also be conducted, which also fix helps to fix the right dosage of lemongrass essential oil. It also helps to manage others field level insects for that laboratory studies have to carry out. By incorporating with nano particles, it might be a promising pesticide for crop pest management.

Conflict of Interest. None.

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