Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2018; 6(4): 185-189

Received: 29-05-2018
Accepted: 30-06-2018

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Preparation of dust formulation of essential and aromatic oils and testing the bioefficacy against pulse beetle *Callosobruchus maculatus* (Fab.) (Coleoptera: Bruchidae) in green gram storage

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Abstract
In the present study, the toxicity of seven essential and aromatic oils *viz*; neem oil, mint oil, citronella oil, mustard oil, geranium oil, coconut oil and eucalyptus oil were evaluated against pulse beetle, *Callosobruchus maculatus* Fab. in green gram seeds through contact, fumigant, repellency and settling behavior bioassay. All the treatments were found significantly superior in causing mortality of the pulse beetle compared to the untreated check. Citronella oil showed a maximum mortality for both fumigant and contact toxicity assays (100% and 93.75%) and strongest repellent (100%) against pulse beetle. The toxicity of the oils is in the order of efficacy is citronella > geranium > mint > mustard > coconut > neem > eucalyptus. Generally the oil persistency was minimum; hence in order to increase the persistency, the dust formulation of essential oils of citronella, geranium and mint oil were prepared at 5, 10 and 15 percent. The results indicated, the dust formulations of citronella and mint oil were found to be effective at 10% and geranium oil at 15%.

Keywords: green gram, *Callosobruchus maculatus*, essential oils, aromatic oils

1. Introduction
Pulses are the major source of protein in the developing world [3]. Green gram is the most important pulse crop. The 68th UN General Assembly declared, 2016 as the International Year of Pulses, which encouraging awareness on the nutritional composition of green gram as follows: 100g of green gram provides 30 calories and consist of approximately 3g proteins, 6g carbohydrates and 2g dietary fibers [3]. Green gram seeds are bound to show rapid and great losses both qualitatively and quantitatively due to the attack of bruchid insects at post-harvest stages [12]. Among the bruchids *C. maculatus* can cause damage of pulse seeds upto cent percent during storage [5]. Protecting stored pulses against *C. maculatus* is known to depend on the use of synthetic chemicals. Nowadays broad spectrum insecticids have been reported to cause development of resistance in insect populations [4].
Some products extracted from aromatic plants shows insecticidal activity against bruichid species [10] Essential and aromatic oils can be used as fumigant, contact insecticides, and as repellent in the control of stored grain pests [7, 11]. Plant material based essential oils are target specific, non-toxic to human and beneficial organisms, less prone to insect resistance and resurgence and biodegradable and also act as promising grain protectants [18]. In light of the adverse effect of insecticides on the environment, these methods of pest control are now attracting greater attention and research input [19]. Hence the present study was conducted to investigate the potential of toxicity and repellent action of selected essential and aromatic oils on pulse beetle *C. maculatus* in stored green gram.

2. Materials and methods
The present study was conducted in the Insectary, Department of Agricultural Entomology, Agricultural College and Research Institute, Madurai, Tamil Nadu during 2017 – 2018.

2.1 Mass culturing of pulse beetle, *C. maculatus*
Pulse beetles required for the study were mass reared on green gram (CO- 5) in the transparent plastic containers of 600 ml at Insectary. Each container was filled with 200g of green gram and ten pairs of matured adult beetles were released. The plastic jars were covered with muslin

cloth tightly, secured with a rubber band. Provide air passage to avoid fungal growth and placed in dark to facilitate maximum oviposition maintained at a room temperature of 30 ± 5°C and 70 ± 5% RH throughout the period of study [15]. Oviposition was completed within five days. After 25 to 30 days, adults that emerged from the culture were utilized for maintenance of sub cultures following the same procedure as described above. Freshly emerged adults were used for conducting the experiments.

2.2 Essential and aromatic oils treatment

Neem oil, eucalyptus oil, citronella oil, geranium oil, coconut oil, mustard oil and mint oil which had insecticidal and repellency action were identified for this study. The oils were made to the following concentration by using acetone as a solvent.

Treatment	Common name	Scientific name	Dose
T1	Neem oil	*Azadirachta indica*	1%
T2	Eucalyptus oil	*Eucalyptus globules*	0.5%
T3	Citronella oil	*Cymbopogon nardus*	1%
T4	Geranium oil	*Pelargonium graveolens*	1%
T5	Coconut oil	*Cocos nucifera*	1%
T6	Mustard oil	*Brassica juncea*	1%
T7	Mint oil	*Mentha longifolia*	1%

2.3 Contact toxicity

The insecticidal activity of essential and aromatic oils against *C. maculatus* was studied by direct contact application of various treatments of essential oils on filter paper [14]. The prepared solvents were uniformly sprayed in the filter paper and were allowed to evaporate for 20 min. The filter paper was then placed in the petriplate and followed by the release of three day old 20 numbers of unsexed adults separately into each petriplate. Each treatment had four replications. Insect mortality was recorded after 24h exposure.

$$\text{Per cent control} = \frac{X - Y}{X} \times 100 \quad [1]$$

Where,
X = Per cent living in the check
Y = Per cent living in the treatment
X – Y = Per cent killed by the treatment

2.4 Fumigant toxicity

Filter paper disc of 4 cm diameter were impregnated with different solvents of essential oils, to give equivalent fumigant concentration of 26.5µl/ 250ml in air [8]. The impregnated filter paper was then attached to the inner surface of the screw cap of the glass bottle (250ml). Twenty adults of known age were released in each glass bottles before the cap were screwed tightly. Number of dead insects was determined at 3, 6, 12, and 24 h after exposure [14] and the per cent mortality was recorded.

2.5 Repellency test

Filter papers (Whatman No.40) of 9 cm in diameter were cut in to two halves. One ml of 10 percent solution prepared in acetone and applied to one half of the filter paper (treated half) and on the other half, 1 ml of acetone was applied as uniformly as possible with a pipette. The treated filter paper was then air dried. Full discs were then re-made by attaching treated halves to untreated halves of the same dimensions with cello tape. Ten newly emerged adults were released at the centre of each petridish and subsequently covered. The seven treatments were replicated for 3 times. Insects that settled on each half of the filter paper disc were counted after a time lapse of 1, 2 and 3 hour. The data were expressed as percent repellency [2]. The percentage repellency using the following formula.

$$\text{Percent repellency} = \frac{A - B}{A} \times 100$$

Where,
A = Average number of insect present on untreated portion
B = Average number of insect present on the treated portion

Repellency rate	Class
>0.01-0.1%	0
0.1-20%	I
20.1-40%	II
40.1-60%	III
60.1-80%	IV
80.1-100%	V

2.6 Studies on settling behavior

The one per cent oil solutions were sprayed outside the stored bags (15×10cm) kept in the experimental cages. Fifty newly emerged adults were released in each cage (25×25×35cm). The seven treatments were sprayed in each cage using hand sprayer. The treatments were replicated for three times. Untreated check was also maintained simultaneously. The seven treatments were replicated for three times. Adult beetles that settled on each bags were counted 48 and 72 hour after the release.

2.7 Dust (D) formulation of essential oils

Dusts were made up of a finely ground mixture of low concentration of active ingredient (10% or less by weight) combined with very fine and dry inert carrier made from talc in powder form [9]. The dust formulation of essential oils was prepared at 5% (0.52 ml/10g), 10% (1ml/10g) and 15% (1.7ml/10g) and the dust was applied along with green gram seeds in plastic containers. For testing the persistency, the dust formulation was applied immediately after preparation and which was compared with the same formulation after one month. Released 12 pairs of 3 days old unsexed pulse beetle adults and recorded the percentage mortality after 24hr. Three replications of treatments and untreated checks were maintained.

3. Results and Discussion

Considering the public demand for wholesome and pest free food products, there is a need for developing the biorational pest management strategies by reducing the use of contact chemical insecticides and fumigants in stored products.

3.1 Contact and fumigant toxicity

The results clearly demonstrated that the selected essential and aromatic oils showed potent fumigant and contact activity against the pulse beetle, *C. maculatus.* All the treatments were found significantly superior in mortality of the pulse beetle. Among the contact toxicity assay, citronella oil 1% and mint oil 1% showed cent percent mortality with in 24hr (Table 1)

followed by geranium oil 1% (96%); coconut oil 1% (35%), eucalyptus oil 0.5% (20%) and neem oil 1% (11.25%). The lowest mean mortality was in mustard oil 1% (8.75%).

Among the fumigant toxicity assay, highest mortality percent (93.75%) was showed by citronella oil after 24 hours (Table 1). This was on par with geranium oil (87.5%), mint oil (79.5%) and eucalyptus oil (79.5%) followed by coconut oil (16.37%) mustard oil (9.65%) and neem oil (8.05%).

3.2 Repellency assay

Among the essential oils tested citronella oil 1% (100%) (Class V) was the strongest repellent against *C. maculatus* by registering cent percent repellency (Class V) (Table 2). Followed by geranium oil 1% (97.7%) (Class IV) which was on par with citronella oil. The other oils significant repellent activity with neem oil 1% (57.7%) (Class III) and followed by coconut oil (51.1%) (Class III) on par with each other. The lowest repellency was recorded with the eucalyptus oil 0.5% (20) (Class II). Similar results were also reported by [16], when essential oil and botanicals tested against pulse beetle, *C. maculatus* in green gram, citronella oil showed highest repellency (100%), toxicity effects, reduced oviposition and no adult emergence (0.0%) at 14,15,16 and 17 days after treatment.

3.3 Settling behavior

After four days pulse beetle showed no settling behavior (0%) to citronella oil 1% (Table 3) which was on par with geranium oil (2%). followed by mustard oil (6.1%), mint oil (9.1%) neem oil (13.2%) and coconut oil (21.3%). The high settling behavior was shown by eucalyptus oil with 76%. [17] Have also proven that the tested essential and aromatic oils showed minimum settling behavior, oviposition and seed damage (7.7%) when green gram was treated with citronella oil at five per cent when compared with untreated check (27.35%).

3.4 Dust (D) formulation of essential and aromatic oils

Among the essential and aromatic oils, geranium, citronella and mint oil showed promising result in repellency, contact and fumigant toxicity and settling behavior assays. The effective essential and aromatic oils namely geranium, citronella and mint oil were prepared as dust formulations and tested for repellent effect settling behavior of *C. maculatus.* Dust formulation of 10 and 15% Citronella oil recorded cent percent mortality on the day of preparation of dust formulation and also 30 days after preparation the 15% dust formulation of mint oil effected 100% mortality on the day of preparation of the dust and its effects reduce slightly after a period of 30 days thus effecting only 97.2% mortality of *C. maculatus* in 24hr (Table 4). geranium 15% dust was effected only 63.8% and 51.3% mortality after 1 and 30 day after preparation of the formulation.

Increasing the concentration of the tested oil increased the rate of insect mortality. Higher concentrations (15%) of geranium, mint and citronella oils induced the highest mortality rate against *C. maculatus* (57.5%, 98.6%, and 100%), respectively. On the other hand, the lower concentrations of 5 and 10 percent of geranium, mint and citronella oils induced a less mortality against *C. maculatus* (22.85%, 36.8%, 81.8%, 22.8%, 93% and 100%) respectively. The effect of dust formulation was higher when the dust applied immediately after preparation when compared with one month. Dust formulation of geranium oil showed the higher mortality rate (27.7%, 29.03% and 63.8%) of *C. maculatus* when compared with the dust formulation after one month (18%, 16.6% and 51.3%) respectively. Similarly mint oil had the highest mortality rate (37.5%, 95.8% and 100%) of *C. maculatus* when compared with the dust formulation prepared after one month (36.1%, 90.2% and 97.2%) respectively. [6] Also reported that citronella at 2.5 ml/kg of seed effectively controlled *C. maculatus* population by reducing oviposition rate and these treatments also recorded the least seed damage and weight loss due to pulse beetle infestation, as well as the highest percentage of gram seed germination.

4. Conclusion

The results clearly showed that the tested essential and aromatic oils in this study have affected the settling, oviposition and development of the pulse beetle. Thus they have high value for utility as the most promising essential and aromatic oils for the control of pulse beetle damage in stored green gram. Among all the essential and aromatic oil treatments, dust formulation of citronella oil at 10 per cent can be recommended for the management of the pulse beetle in storage.

Table 1: Insecticidal activity of essential oils against *Callosobruchus* spp. in contact and fumigant toxicity assay.

Treatment	Dose	Contact toxicity mg/cm^2	Fumigant toxicity mg/L
T1- Neem oil	1%	11.25 (19.4)d	8.05 (13.5)b
T2- Eucalyptus oil	0.5%	20 (26.0)d	78.75 (70.1)a
T3- Citronella oil	1%	100 (89.5)a	93.75 (82.1)a
T4- Geranium oil	1%	96 (81.8)b	87.5 (78.3)a
T5- Coconut oil	1%	35 (36.1)c	16.37 (12.6)b
T6- Mustard oil	1%	8.75 (16.7)d	9.65 (12.3)b
T7- Mint oil	1%	100 (89.5)a	79.5 (70.2)a
SEd		3.5182	14.4
CD(.05)		7.3164	30.1
CV		9.68	42.20

Letters followed by mean values are significantly different from each other by LSD. Values are average of four replications. Original data were corrected by Abbott's formula and then transformed into arcsine percentage values during ANOVA test.

Table 2: Average repellency of essential oils to Pulse beetle adults using treated filter paper assay

Treatments	Average % Repellency rate at hours after insect release			Mean repellency (%)	Repellency class
	1	2	3		
T1- Neem oil @ 1%	60 (50.7)	53.3 (46.8)	60 (50.7)	57.7 (49.4)	III
T2- Eucalyptus oil @ 0.5%	26.7 (31.1)	13.3 (21.3)	20 (26.5)	20 (26.3)	I
T3- Citronella oil @ 1%	100 (89.5)	100 (89.5)	100 (89.5)	100 (89.5)	V
T4- Geranium oil @ 1%	86.7 (68.6)	73.3 (58.8)	66.67 (54.7)	97.7 (60.7)	IV
T5- Coconut oil @ 1%	46.6 (43.0)	53.3 (46.8)	53.3 (46.8)	51.1 (45.5)	III
T6- Mustard oil @ 1%	40 (39.2)	46.6 (43.0)	33.3 (35.2)	39.9 (39.1)	II
T7- Mint oil @ 1%	53.3 (46.8)	40 (39.2)	33.3 (35.2)	30.2 (40.4)	II
SEd	9.1873	10.3603	10.3603	3.9273	
CD(.05)	19.4765	21.9631	21.9631	8.3255	
CV%	26.8352	23.07	31.11	10.11	

The average values were classified based on the rate of repellency (%) as given below; 0= >0.01 - <0.1, I= 0.1 - 20, II= 20.1 – 40.0, III= 40.1 – 60.0, IV= 60.1 – 80.0, V= 80.1 – 100.0. Original data were corrected by Abbott's formula and then transformed into arcsine percentage values during ANOVA test.

Table 3: Mean settling behavior of Pulse beetle adults on green gram stored bags treated with various essential oils

Treatment	Dose	Mean settling behavior
T1- Neem oil	1%	13.2 (21.2)b
T2- Eucalyptus oil	0.5%	76 (60.6)d
T3- Citronella oil	1%	0 (0.4)a
T4- Geranium oil	1%	2 (8.1)a
T5- Coconut oil	1%	21.3 (27.4)c
T6- Mustard oil	1%	6.1 (14.0)a
T7- Mint oil	1%	9.1 (17.5)b
SEd		2.8390
CD(.05)		6.7135
CV %		15.56

Values are average of thee replications. Original data were corrected by Abbott's formula and transformed into arcsine percentage values during ANOVA test. Letters followed by mean values are significantly different from each other by LSD.

Table 4: Efficacy of the dust formulation of selected essential oils

Treatment	Dose (%)	Percent mortality rate at 24hr after insect release		Percent mean mortality
		Immediately after preparation of dust	1 month after preparation of dust	
Geranium oil	5	27.7 (31.7)e	18 (25.1)e	22.85 (28.4)e
	10	29.03 (32.5)e	16.6 (24.0)e	22.8 (28.2)e
	15	63.8 (53.0)d	51.3 (45.7)d	57.5 (49.3)d
mint oil	5	37.5 (37.7)e	36.1 (36.8)e	36.8 (37.2)e
	10	95.8 (78.1)b	90.2 (71.7)b	93 (75.0)b
	15	100 (89.5)a	97.2 (80.3)a	98.6 (85.0)a
Citronella oil	5	86.08 (68.2)c	77.6 (61.8)c	81.8 (65)c
	10	100 (89.5)a	100 (89.5)a	100 (89.5)a
	15	100(89.5)a	100 (89.5)a	100 (89.5)a
SEd		2.7	4.8	4.3
CD(.05)		5.8	10.2	9.7
CV%		5.3	10.1	7.1

Values are average of thee replications. Transformed into arcsine percentage values during ANOVA test. Letters followed by mean values are significantly different from each other by LSD.

5. Acknowledgement

I sincerely thankful to Tamil Nadu State Council for Science and Technology (Students Project Scheme Scholarship) Dote Campus, Chennai for providing the financial support in conducting this research work.

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