Repellant and fumigant toxicity of essential oil from Melaleuca leucadendron as storage protectant against Callosobruchus maculatus (Fab.) (Coleoptera: Bruchidae) on Cow pea, Vigna unguiculata

Sahla A.K., E. Pushpalatha
Biopesticides & Toxicology Division
Department of Zoology, University of Calicut
Malappuram, Kerala – 673 635, India

Abstract-Repellency and fumigant toxicity bioassays were performed using essential oil extracted from the plant, Melaleuca leucadendronagainstadults of cowpea weevil Callosobruchus maculatus on cowpea (Vigna unguiculata). Repellency bioassay shows an average repellency of 75% when it is treated with a dose of 32µl using choice chamber method. Vapour toxicity bioassay performed with the treatment of essential oil of M. leucadendronproduces an LC_{50} value of 101.029 µl/l air for 24 hr exposure. GCMS analysis of the essential oil of M. leucadendronshows a spectrum of a total of 37 compounds and some unknown constituents in which viridiflorol (43.14%), α-pinene (12.89%) and limonene (8.04%) shares majority portion of the essential oils. The study reveals the potential action of the essential oil as a repellent and fumigant toxicant agents against C. maculatus.

Keywords-Callosobruchus maculatus, Melaleuca leucadendron, Essential Oil, Repellency, Fumigant toxicity

I.INTRODUCTION

Insect attack is a major reason to quality deterioration of durable food products like cereals, pulses, tubers, maizes, and roots etc.stored in humid and warm climatic conditions all over the world. Damage of stored food product is serious problem forever throughout the globe. The stored condition of crops like maize, grain and pulses are at risk of insect pest attacks. The major pests of stored items include rice weevil(*Sitophilus oryzae*)**,**red flour beetle (*Tribolium castaneum*), Angoumois grain moth (*Sitotroga cerealella*),Indian meal moth, (*Plodia interpunctell*a), Sawtoothed grain beetle (*Oryzaephilus surinamensis*),Pulse weevil (*callosobruchus spp*), Warehouse beetle (*Trogoderma variabile*) , Drugstore beetle (*Stegobium paniceum*), Warehouse moth (*Ephestia spp*), and Confused flour beetle (*Triboliumcofusum*) etc. Most of these pests infest the grain over the year under favourable conditions and results in severe damages and yield loss.

Mainly syntheticChemicals are used as the pest control measure;however, it renders undesirable effects such as health problems, environmental pollution, pesticide resistance, retention of pesticide residue, secondary pest outbreak and pest resurgence. Action of synthetic insecticides is mainly targeted to nervous system of humans and animals; it can be toxic to aquatic organisms, especially for fish. Moreover, many of these chemicalspersist for many years and make the problem of pesticide residue formation (1).Over time several insect pests have developed resistance against many of these chemical insecticides (2).

Botanical insecticides overrate chemicals for their low toxicity to environment, human being and other living things, selective action, biodegradability and low cost and availability. They resist herbivory by acting as repellents, antifeedants, attractants, growth inhibitors, chemosterilants and toxicants. Recent studies have focused on the discovery and characterization of botanicals as an alternative to other pest management methods. Various studies have demonstrated the potency of neem (*Azadirachta indica* A. Juss: Meliaceae) as a control measure against various pest species (3-5). Active ingredient of neem is azadirachtin which is chemically complexed and possess insecticidal activity through insect growth regulating action, feeding and ovipositional deterrent action. Azadirachtin structurally resembles the natural insect hormone ecdysone and inhibits production and response of ecdysone during insect growth and moulting. Thus,neem-based compounds cause insect death by blocking moulting cycle (6).
Cowpea (*Vigna unguiculata*) is a grain that is grown widely in the tropics and sub tropics, cultivated as a nutritious and highly palatable food source. It is a highly acceptable crop in various countries due to immense

tolerance to soil types, environment and low rainfall. Besides they require only few inputs of cultivation as well as their root nodules are capable of fixing atmospheric nitrogen, all of these make this plant a valuable cultivated crop and food source for many farmers and countries. It is being cultivated for its leaves, pods, beans or processed in to flour or paste used as a food ingredient (7).The present study investigated the biopesticidal efficacy of essential oils isolated from the leaves of *Melaleuca leucadendron* against adults of cowpea weevil *Callosobruchus maculatus* on cowpea (*Vigna unguiculata*).

II.MATERIALS AND METHODS

2.1.Test Organism-*Callosobruchus maculatus* Fabricius (Coleoptera: Bruchidae)

Pulse beetle, *Callosobruchus maculatus* is one of the cosmopolitan pests attacking cowpea seeds, *Vigna unguiculata.*These pests mainly infest stored products and come under the family bruchidae and is found in tropical and subtropical countries. This beetle cause extensive infestation in field condition, most of the damage is happening during stored conditions. In the early spring season, the adults may feed nectar of flower and start to infest the cowpea culture at the end of rainy season and found to increase their population rapidly.

Adult beetles are small reddish brown in colour with 2-3mm long, they are typical rounded in appearance compared to other insects of family the bruchidae. Eggs are oval and translucent, laid on the surface of the cow pea, emerges within 5-20 days. The first instar larva bores the seeds and rest of the life cycle completed inside the seeds. Larvae exhibit hypermetamorphosis. Larvae bore the seed and enter inside and after the adult emergence it leave a hole on the seeds. The total developmental period is around 30 days.

2.2.Maintenance of Laboratory Culture

The insect *Callosobruchus maculatus* were collected and taxonomic authentication was done from the Department of Zoology, University of Calicut, Kerala, India. The culture of parental stocks of *Callosobruchus maculatus* were maintained under appropriate laboratory conditions on the seeds of cowpea (*Vigna unguiculata*) inside a growth chamber at 30±2°C, and 70% RH. Initially, 40 pairs of 1-3 day old male and female adult insects were placed in a sealed jar containing cowpea seeds. After 7 days of mating and oviposition parent stocks were removed and cowpea seeds containing eggs were transferred to fresh cowpea seeds in the breeding jars covered with pieces of cloth fastened with rubber band to avoid contamination and escape of emerging beetles. The new progenies of beetles emerged and were used for further experimentation.

2.3.Melaleuca leucadendron (Myrtaceae)

Melaleuca leucadendron,(plate 1) commonly known as cajuput tree, grows in lowland tropics and subtropics. The fresh leaves are used for extraction of essential oil and collected during the month of December-January from the campus of the University of Calicut.

Plate 1. *Melaleuca leucadendron* (L.) L

2.4.Essential oil extraction

The fresh leaves of *Melaleuca leucadendron* was washed thoroughly in tap water, dried, chopped into small pieces and put into hydrodistillation using a modified Clevenger type apparatus (8). After the process of hydrodistillation the essential oil formed were transferred into a glass vial, dehydrated using anhydrous sodium sulphate. Thecollected oil was kept in refrigerator at 4 ^{o}C until it is used for various experiments.

2.5. GC/MS analysis of essential oils

The essential oil compound analysis was done from Indian Institute of Spices Research, Calicut, Kerala using RTX5 Shimadzu GC/MS system with column RTX5 (with carrier gas helium, flow rate 1ml/minute, temperature 250ºC, oven temperature 60 ºC, length 30 meter, thickness 0.25µm and diameter 0.25µm) and Mass spectrometry MSGP2010 with detection temperature 250 ºC.

2.6.Repellency bioassay with choice chamber method

Repellency of the selected essential oil on test insect *C. maculatus* were tested by using area preference test described (9). The entire test of repellency were carried out in a 'choice chamber'. It consists of whatman no.1 filter paper placed in the petri dishes cut in to 2 halves. Different test solutions of 10, 20, 30, 40 and 50µls from 1% of essential oil preparation were applied on the half portion of filter paper disc as uniformly as possible with micropipette. Other half is treated as control. Each half is dried to evaporate the solvents and replace in the petri dish of experimental set up. Lower part of filter paper disc was pasted in the petri dish in order to prevent the escape of insects to underneath of filter paper. Then 10 adults of mixed sex of *C. maculatus* were released at the centre of filter paper disc and closed set up by using another petri dish, where the insects were allowed to move on any direction of test half or control half. Then number of insects present in test half and control half were counted and recorded periodically after 1, 2, 4, 8 and 16 hours to find out the percentage of repellency. Each doses of treatment were replicated for 4 times. The mean value of 3 replications of each doses at above described intervals were taken and percentage of repellency (PR) was calculated using the formula adopted by Obeng-Ofori (10).

Percentage repellency (PR) = $[(Nc - Nt) / (Nc)] \times 100$
Where, Nc=number of insects present in control half, Nt=number of insects present in test half

2.7. Fumigation toxicity assay and calculation of LC50

Toxicity of *Melaleuca leucadendron* essential oil vapours were tested against *Callosobruchus maculatus*adults by using a modified toxicity assay described by Huang *et al*. (11). The entire experiment was carried out in the glass bottle of 100 ml. The essential oil concentrations 40,80,120,160 and 200 µl of *M.leucadendron* was applied on the Whatman no.1 filter paper pieces and attached to the under surface of screw caps of test glass bottle. The inner sides of bottle neck were painted with Vaseline to prevent direct contact of insects with essential oil applied. Caps were screwed tightly on to the bottle containing 5 pairs of adult *C.maculatus* of mixed sex. Control experiments were set with same conditions without any application of essential oil. Three replications of each doses of treatment were set for experiment and control. The number of dead insects were counted after 24 hours of treatment and LC_{50} were calculated by SPSS analysis.

III.RESULTS

The essential oilextraction from the fresh leaves of *Melaleuca leucadendron* wasdone by hydrodistillation method and the essential oil formed were concentrated, hydrophobic and yellow coloured with strong odour. In order to calculate the yield of essential oil extracted from the selected plants, the volume of oil formed as layer is measured directly from graduated reservoir and the percentage of yield was calculated. The data is reported as the average volume of 4 extractions of essential oil and yield as the ratio of volume to weight of the plant material. The percentage of yield of essential oil obtained for *M. leucadendron* is 1.45 %.

Repellency bioassay of *M. leucadendron* shows considerable effect, and the data obtained are presented in table 1. The result shows therate of percentage repellency at different doses of 8, 16, 24 and 32 µl of *M. leucadendron* at different durations of time at 1, 2, 4, 8 and 16 hrs against *C. maculatus*. The lowest dose of 8µl provide percentage repellency of 24 ± 8.43% and 30 ± 10.54 during initial exposure of 1 and 2 hrs respectively.

Table 1: Repellency of various doses of essential oil extraction of *M. leucadendron* on*Callosobruchus maculatus* (Choice chamber method)

Dose (µl)	Percentage repellency at varying durations of exposure (h)					Overall average of % repellency	Repellency class
	1	**2**	**4**	**8**	**16**		
8	24±8.43	30±10.54	46±9.66	34±9.66	22±6.32	31.2	II
16	42±6.32	50±10.54	68±10.32	44±8.43	28±10.32	46.4	III
24	66±9.66	72±10.32	84±12.64	62±11.35	32±10.32	60	IV
32	76±8.43	88±10.32	92±10.32	74±9.66	48±9.66	75.6	IV

Values are expressed as mean ± SD (n=4)

Sample =10 insects × 4 doses × 4 replication = 160 insects

Repellency classes assigned according to scale described by Mc Govern *et al.*(12),

[Class I - range of % repellency 0.1-20 Class II - range of % repellency 20.1- 40

Class III = range of % repellency 40.1 - 60 Class IV - range of % repellency 60.1- 80

Class V- range of repellency 80.1-100]

An increase to a rate of 46 ± 9.66%of repellency of this dose attained at 4 hrs of duration, followed by decrease in rate of repellency of 34 ± 9.66 and 22 ± 6.32% during 8 and 16 hrs. respectively (fig.1). A dose of 16 µl provide repellency rate of 42 ± 6.32% and 50 ± 10.54% during initial exposure of 1 and 2 hrs respectively followed by 68 ± 10.32%, 44 ± 8.43% and 28 ± 10.32% during 4, 8 and 16 hrs. With 24 µl percentage repellency exerted was 66 ± 9.66% and 72 ± 10.32% at 1 and 2 hrs of exposure, followed by 84 ± 12.64%, 62 ± 11.35% and 32 ± 10.32% during 4, 8 and 16 hrs of exposure respectively. Highest dose of 32 µl provide best effect with 72±8.43% - 92±10.32% during 1 to 4 hrs of exposure followed by a decrease in rate of repellency up to 74 ± 9.66% and 48 ± 9.66% during 8 and 16 hrs of exposures (fig.1). The doses of 24 µl and 32 µl can be included in repellency class of IV according to Mc Governs *et al* 1977 (12).

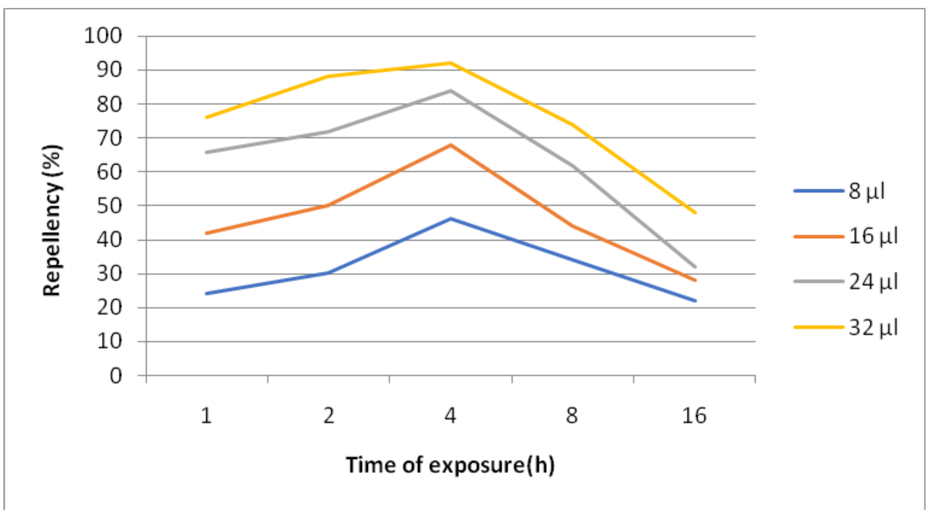


Figure 1: Repellent activities of different doses *M. leucadendron* essential against C. maculatus during different durations of time

3.1.Fumigant toxicity bioassay

The test insect, *Callosobruchus maculatus* were subjected to vapour toxicity bioassay of 24 hrs treatment with essential oil of *M. leucadendron* with 10 insects for each dose to find out the adult insect mortality. Percentage mortality towards different concentrations of essential oils is given in the table 2. The vapour toxicity of *M. leucadendron* produced percentage mortalities of 13.33, 26.66, 46.66, 76.66 and 100 for 40, 80, 120, 160 and 200 µls of doses respectively. The result indicates that the *M. leucadendron* essential oil is significantly toxic against the test insect *C. maculatus* and exerts strong insecticidal activity with LC_{50} value of 101.029 µl/l air (table 2).

Table 2: Adult percentage mortality of *C. maculatus* with different doses of *M. leucadendron* at 24 hrs of treatment

Essential oil	**Dose (µl)**	**Percentage mortality**	24 hr LC_{50}**(µl)** (Upper limit -Lower limit)	24 hr LC_{90}**(µl)** (Upper limit - Lower limit)
Melaleuca leucadendron	40	13.33	101.029 (205.22-31.75)	217.227 (4100.7-135.49)
	80	26.66		
	120	46.66		
	160	76.66		
	200	100		
Control	0	0.0		

Values are expressed as means of 4 observations

3.2.GC/MS analysis of essential oil of M. leucadendron

The essential oil of *M. leucadendron* was subjected to GC/MS analysis for the identification and categorization of compounds present in it. Relative proportions of the constituents were analyzed based on the percentage of area of each constituent (figure 3). Vast spectrum volatile chemical compounds were identified from essential oil from the leaves of *M. leucadendron*. A total of 37 compounds and some unknown constituents were identified from the *M. leucadendron*, of these Viridiflorol takes the major portion (43.14%). Other major compounds are Alpha-pinene (12.89%), Limonene (8.04%), Eucalyptol (6.26%), Ledol (4.74%) Alpha-terpineol (3.14%) and Beta-caryophyllene epoxide (2.24%). Minor quantities of various other compounds like Beta-pinene, O-Cymene, Gamma-terpene, 4-Terpineol, Caryophyllene, Ledene and Tau-cardinol etc (table 3).

Figure 3: Chromatogram of GC/MS analysis of essential oil from *Melaleuca leucadendron*

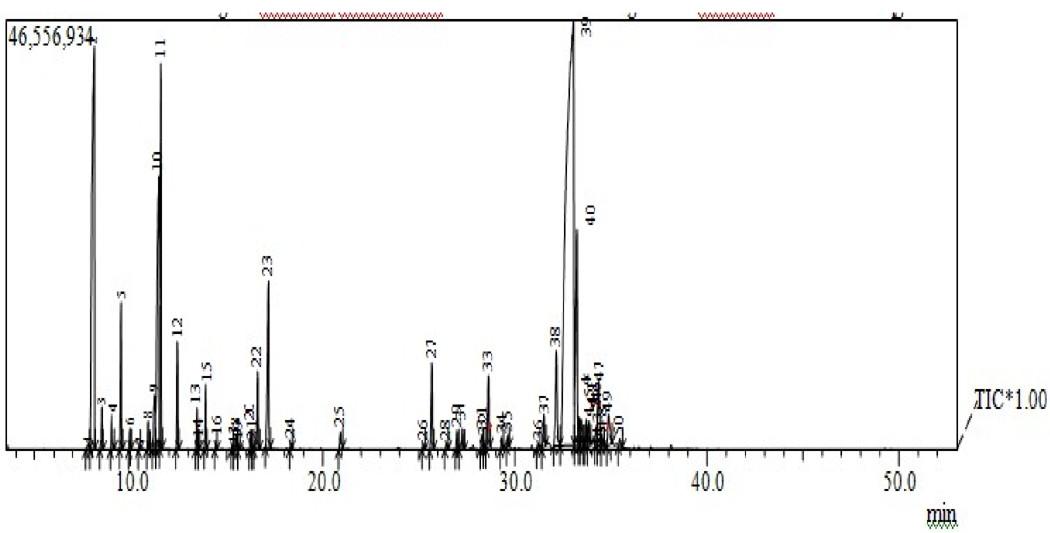


Table 3: List of volatile compounds identified from GC-MS analysis of Melaleuca leucadendron essential oil

Sl.No.	**Peak**	**Retention time(min)**	**Area %**	**Height %**	**Compound**
1	1	7.33	0.17	0.13	Alpha-thujen
2	2	8.088	12.89	12.54	Alpha-pinene
3	3	8.489	0.52	1.34	Camphene
4	4	9.002	0.45	1.08	Benzaldehyde
5	5	9.497	1.75	4.58	Beta-pinene

6	6	9.952	0.22	0.66	Beta-myrcene
7	7	10.445	0.04	0.09	Beta-phellandren
8	8	10.900	0.34	0.88	Alpha-terpinene
9	9	11.243	1.22	1.66	O-Cymene
10	10	11.455	8.04	8.49	Limonene
11	11	11.567	6.26	11.99	Eucalyptol
12	12	12.428	1.25	3.34	Gamma-terpene
13	13	13.444	0.47	1.29	Alpha-terpinolen
14	15	13.893	0.74	2.00	Linalol
15	16	14.419	0.10	0.28	Fenchol
16	17	15.279	0.05	0.13	Pinocarveol
17	18	15.476	0.18	0.45	Isopulegol
18	22	16.607	1.05	2.42	4-Terpineol
19	23	17.162	3.14	5.20	Alpha-terpineol
20	24	18.326	0.09	0.20	Citronellol
21	26	25.234	0.08	0.16	Alpha-gurjunene
22	27	25.626	1.44	2.69	Caryophyllene
23	28	26.441	0.12	0.19	Aromadendrene
24	29	26.983	0.31	0.59	Alpha-caryophyllene
25	30	27.269	0.34	0.65	Alloaromadendrene
26	31	28.276	0.26	0.45	Beta-eudesmene
27	33	28.622	1.29	2.22	Ledene
28	34	29.315	0.18	0.36	Gamma-cardinene
29	35	29.640	0.17	0.35	Delta-cardinene
30	36	31.187	0.10	0.16	Epiglobulol
31	37	31.506	0.57	1.00	Palustrol
32	38	32.160	2.24	2.95	Beta-caryophyllene epoxide
33	39	33.053	43.14	13.25	Viridiflorol
34	40	33.218	4.74	6.71	Ledol
35	47	34.354	1.58	2.01	Tau-cardinol
36	48	34.520	0.20	0.37	Delta-cardinol
37	49	34.874	0.94	0.94	Alpha-cardinol

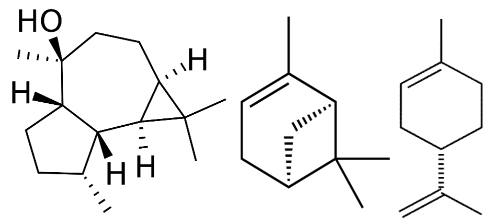
IV.DISCUSION

Essential oils exhibiting insecticidal activities are extensively used as botanical pesticides as an alternative to chemical pesticides. In order to assess the pesticidal properties of essential oils from various plants, several studies were conducted and suggested varieties of plant essential oils which confer the pesticide/insecticidal properties.

An increased level of repellency was observed in the case of the essential oil of *M. leucadendron*during the 1-4 hours followed by decrease during 8-16 hours. It provides an overall percentage repellency rate from 31 to 75.6 % with 8 - 32 µl of concentration. *M. leucadendron* cause commendable efficacy against *C. maculatus* with 92% of maximum repellency at highest treated dose of 32 µl. The repellency rate of *M. leucadendron* start with 24% up to maximum of 92% with 8 - 32 µl of concentration treated against *C. maculatus*.

Don-Pedro,1987 (13) reported the efficacy of tangerine peel oil and lime peel oil against *C. maculatus* with higher repellent, fumigant activity and exert reduction in acetylcholine esterase activity. Another related findings of *M. leucadendron* investigated by Adjalian (14) shows that oils were highly effective against Angoumois grain moth with 61% of average percent repellency and reduction in egg laying for different percentage of concentration of the essential oil. The essential oils from *Melaleuca quinquenervia* exhibit repellency with developmental inhibitory and adulticidal activity against *Aedes aegypti, Culex quinquefasciatus and Aedes albopictus* (15).

Essential oils are the volatile aromatic compounds obtained from natural sources, usually from plant parts as secondary metabolites. They are characterized by strong odour, and the chemical constituents present in the essential oil impart some special characteristic properties that, it is used as fungicidal, bactericidal, insecticidal and parasiticidal agents. In the field of entomology essential oils are extensively used as insecticidal agents. A total of 37 compounds and some unknown constituents were identified from *M. leucadendron*, and from among these compounds, Viridiflorol , a sesquiterpene compound takes the major portion of 43.14%. Other major compounds are Alpha-pinene(12.89%), Limonene (8.04%), Eucalyptol (6.26%), Ledol (4.74%) Alpha-terpineol (3.14%) and Beta-caryophyllene epoxide (2.24%). Minor quantities of various other compounds like Beta-pinene, O-Cymene, Gamma-terpene, 4-Terpineol, Caryophyllene, Ledene and Tau-cardinol etc. are also observed.



Viridiflorol α-pinene Limonene

Viridiflorol is the major chemical constituent of *M. leucadendron* essential oil which may be the major reason for the activity of this essential oil. Alpha-pinene detected from *M. leucadendron* are the examples for the monoterpene category of chemical constituent and limonene is a monoterpene group of compounds. Other major chemical compounds such as eucalyptol, ledol, alpha-terpineol andbeta-caryophyllene epoxide and minor quantities of beta-pinene, O-Cymene, gamma-terpene, 4-terpineol, caryophyllene, ledene and Tau-cardinol etc, are the cause of insecticidal activities of *M. leucadendron* essential oil. Study of phytochemical properties and chemical composition of essential oil from *M. leucadendron* reported the major compound as 1,8-Cineole (54.24%), α-Terpineol (7.14%), γ-Terpineol (2.06%), β-Caryophyllene (4.46%), Globulol (2.70%)and Eugenol (2.91%) (Pujiarti *et al* in 2011). Analysis of chemical composition of 7 *Melaleuca* spp by Silva (2007) revealed that the major compounds identified were terpinene 4-ol (53.7 % in *M. alternifolia*), 1,8-cineole (79.5 % in *M. ericifolia*, 80.2 % in *M. armillaris*, 41 % in *M. cajuputi* subsp. Platyphylla, 43.7 % in *M. cajuputi* sub spp. Cajuputi, methyl eugenol (96.6 % in *M. leucadendra*) and viridiflorol (71% in *M. quinquenervia*). Present findings revealed viridiflorol (43.14%) as the major compound in *M. leucadendron.*

Botanical insecticides have an immemorial history of insect repellency and are used as an alternative to chemical pesticides. Essential oils as plant secondary metabolites impart characteristic insect repellent properties; it has been exploited in various fields of pest control. Insecticidal activities of monoterpenes are reported by several researchers. Reis *et al,*2016 (16) reported repellent and insecticidal activity of 5 monoterpenes (citronellol,geraniol, geranial, eugenol and citronellal) against 2 stored grain pests,*Sitophilus zeamais* and *Callosobruchus maculatus* and confirms the result with pronounced effect on these pests. Important evidences are existing in the case of sesquiterpene also that ensure insecticidal activity. Elguea-Culebras *et al,* 2017 (17) reported the antifeedant effect of 24 terpenes against *Leptinotarsa decemlineata*and confirms the insect control activity of sesquiterpene, strengthen the findings of the present study.

V.CONCLUSION

Use of essential oils of plants and plant-based pesticides for protecting stored grains against pests is going to be one of the best methods in storage pest management especially in small store rooms. Present study is an inquiry to find out an alternative method of pest control in order to reduce the ever-existing problem of chemical mode of pest control. Essential oil of *Melaleuca leucadendron* shows considerable insecticidal activity against *C. maculatus.* Fumigation test provided insect mortality with higher percentage of mortality for *M. leucadendron*. Hence as a botanical insecticide, the essential oil from *Melaleuca leucadendron* can be used as alternative to chemical insecticides against *Callosobruchus maculatus* that ensure effective pest management in an eco-friendly way for buildingup and maintaining a better tomorrow.

ACKNOWLEDGMENT

We are thankful to University Grants Commission, New Delhi for their technical support through Major Research Project and also acknowledge the University of Calicut for their financial support.

REFERENCES

[1] Coats, J.R. (1994). Risk from natural versus synthetic insecticides. Annual Rev. Entomol. 39.489-515
[2] Roush, R.T. (1989) Managing resistance to insecticides: A key for the future of crop protection. *IPM Laboratories Quartely*, Locke, NY. 1: 2–3.
[3] Makanjuola, W.A. (1989) Evaluation of extracts of neem (*Azadirachta indica* A. Juss.) for the control of some stored product pests. *Journal of Stored Products Research*, **25**(4): 231–237.
[4] Lale, S.E.S. and Abdurahman, H.T. (1999) Evaluation of neem (*Azadirachta indica* A. Juss.) seed oil obtained by different methods and neem powder for the management of *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae) in storage cowpea. *Journal of Stored Products Research*, **35**(2): 135–143.
[5] Golob, P. and Gudrups, I. (1999) The use of spices and medicinals as bioactive protectants for grains. *FAO Agricultural Services Bulletin, No. 137, FAO, Rome, Italy*.
[6] Mordue, A.J. and Blackwell, A. (1993) Review of the activity of azadirachtin. *Journal of Insect Physiology,* **39**(11): 903–924.
[7] Gonçalves, A., Goufo, P., Barros, A., Domínguez-Perles, R., Trindade, H., Rosa, E.A.S., Ferreira, L. and Rodrigues, M. (2016) Cowpea (*Vigna unguiculata* L.Walp), a renewed multipurpose crop for a more sustainable agri-food system: nutritional advantages and constraints. *Journal of the Science of Food and Agriculture,* **96**(9): 2941–2951.
[8] Cheng, S.S., Lin, H.Y. and Chang, S.T. (2005) Chemical composition and antifungal activity of essential oils from different tissues of Japanes cedar (*Cryptomeria japonica*). *Journal of Agricultural and Food Chemistry*, **53**: 614-619Coats, J.R. (1994) Risks from natural versus synthetic insecticides. *Annual Review of Entomology,* **39**: 489–515.
[9] Mc Donald, L.L., Guy, R.H. and Speirs, R.D. (1970) Prelminary evaluation of new candidate materials as toxicants, repellents and attractants against stored product insects. Marketing Research Report of Agriculture, **822**: 8p.
[10] Obeng-Ofori, D. (1995) Plant oils as grain protectants against infestations of *Cryptolestes pussilus* and *Rhyzopertha dominica* in stored grain. *Entomologia Experimentalis et Applicata*, **77**(2): 133–139.
[11] Huang, Y., Lam, S. L. & Ho, S. H. (2000). Bio-activities of essential oil from *Elletaria cardamomum* (L.) Maton. to *Sitophilus zeamais* Motschulsky and *Tribolium castaneum* (Herbst). *Journal of Stored Product Research*, **36** (2): 107-117.
[12] Mc Govern, T.P., Gillenwater, H.B. and Mc Donald, L.L. (1977) Repellents for adults *Tribolium confusum*: mandelates.*Journal of the Georgia Entomological Society*, **12**: 79-89.Don-Pedro (1987).
[13] Don-Pedro, K.N. (1987). Insecticidal activity of plant oils against stored product pests, Ph D thesis, Department of Pure and Applied Biology Imperial College at Silwood Park Ascot, Berkshire.
[14] Adjalian, E., Sessou, P., Yehouenou, B., Fifa, T., Bothon, D., Noudogbessi, J., Kossou, D., Menut, C. and Sohounhloue, D. (2015) Anti-Oviposition and Repellent Activity of Essential Oil from *Melaleuca leucadendron* Leaf Acclimated in Bénin Against the Angoumois Grain Moth.*International Journal of Biology, Pharmacy and Allied Science*, **4**(2): 797–806.
[15] Leyva, M., French-Pacheco, L., Quintana, F., Montada, D., Castex, M., Hernandez, A., Marquetti, M.(2016) Melaleuca quinquenervia (Cav.) S.T. Blake (Myrtales: Myrtaceae): Natural alternative for mosquito control. *Asian Pacific Journal of Tropical Medicine*, **9** (10): 979–984
[16] Reis, S.L., Mantello, A.G., Macedo, J. M., Gelfuso, E.A., da Silva, C.P., Fachin, A.L., Cardoso, A.M. and Beleboni, R..O. (2016) Typical Monoterpenes as Insecticides and Repellents against Stored Grain Pests.*Molecules*, **21**(3): 258p.
[17] Elguea-Culebras, G.O., Sánchez-Vioque, R., Berruga1,M.I., Herraiz-Peñalver, D. and Santana-Méridas, O. (2017) Antifeedant effects of common terpenes from Mediterranean aromatic plants on *Leptinotarsa decemlineata*. *Journal of Soil Science and Plant Nutrition*, **17** (2), 475–485.