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FUMIGANT EFFECT OF SWEET BASIL (*Ocimum basilicum* L) LEAF ESSENTIAL OIL ON THE LONGEVITY AND FECUNDITY OF ADULT COWPEA BRUCHID *Callosobruchus maculatus* (F) AND ON GERMINATION

[1]Ojumoola, A.O., [2]Adesiyun, A.A. and [3]Usman, L.A.

[1, 2]Department of Crop Protection, University of Ilorin, Ilorin, Nigeria.
[3]Department of Chemistry, Univeristy of Ilorin, Ilorin, Nigeria
[1]Corresponding author's e-mail: ojumoolaolusegun@gmail.com

ABSTRACT

Fresh pulverised leaves (600g) of Ocimum basilicum (L) were harvested in July, 2014. Essential oil yield from the hydro-distilled leaves after 3 hours was 0.3(w/w) %. GC/MS analysis revealed the predominance of oxygenated monoterpenes (41.0%) in the oil. Terpinen-4-ol was the major oil constituent accounting for 29.8%. Other significant constituents of the oil were γ-terpinene (9.6%), β – Caryophyllene (7.3%), α-Bergamotene (9.2%) and Sabinene Hydrate (5.0%). The oil was therefore of terpinen-4-ol chemotype. The essential oil (EO) vapour was used to generate an insecticidal atmosphere within airtight fumigation chambers (130ml). Each chamber contained 2 pairs of adult C. maculatus and ten seeds of Ife brown cowpea variety. The EO was tested at 10, 20, 30 and 40µl/130ml air. After 24 hours of exposure, the control had the lowest percentage mortality (0.00) while complete adult mortality was observed in both the 30µl and 40µl doses. The LD_{50} and LD_{99} of the EO within 24 hours of exposure were 13.33µl/130ml and 41.38µl/130ml, respectively. However, after 48 hours of exposure, all doses gave 100% mortality and were significantly different ($P<0.05$) from the control where no mortality was observed. At the end of both exposure periods, significantly ($P<0.05$) fewer eggs were laid on the oil treated seeds than on the control. There was however no significant difference ($P>0.05$) in the mean percentage germination of treated and control seeds after 24 and 48 hours.

Keywords: Essential oil, fumigation,Ocimum basilicum, Callosobruchus maculatus, hydrodistillation.

INTRODUCTION

Cowpea (*Vigna unguiculata* (L) Walp) is the main edible leguminous plant which is cultivated all over the West of Africa (Mondedji *et al.,* 2002). Nigeria is the largest cowpea producer in West Africa. It also has the highest level of cowpea consumption (FAOSTAT 2000).*Callosobruchus*

maculatus (F.) *is a* cosmopolitan field-to-store insect pest of cowpea that is transported via infested seeds, either from the field into storage, or in contaminated seed within and between storage facilities (Brier, 2008). Adult female *C. maculatus* lays half its total number of eggs in the first two days after copulation (Uddin II and Sanusi, *2013)*. These eggs hatch into larvae that feed inside the grain seeds and cause weight losses of up to 80% after six (6) months in storage (Aboua *et al.,* 2010).

Fumigants are especially useful for bruchid control in large-scale storage of grain legumes in airtight facilities such as silos, warehouses and other such enclosures. Methyl bromide and Phosphine are fumigants that have been widely used for this purpose in many parts of the world (Moravvej *et al.,* 2010). Unfortunately, environmental pollution, handling hazards, pesticide residues in foodstuff, development of insect resistance, and resurgence are some of the problems associated with the use of such conventional fumigants and other synthetic pesticides (Shazali *et al.,* 2003).

Currently, global research efforts now support the development of plant products with proven crop protection potentials compared to the use of chemicals which may be toxic to both the plants and the environment (Aliyu *et al.,* 2011) . Plant-derived products such as essential oils and their constituents have been shown to be a potent source of botanical pesticide (Mahfuz and Khalequzzaman, 2007).

Essential oil (E.O) extracted from the sweet basil (*Ocimum basilicum* L.) has been reported to have a range of biological activities such as insecticidal, ovicidal, antioxidant and antiviral properties (Govindarajan *et al.,* 2013; Lee *et al.,* 2005). Essential oils of *O. basilicum* have been reported by Da-Silva *et al.,* (2003) to exhibit a wide and varying array of chemical compounds, depending on variations in chemotypes, leaf and flower colours, aroma and origin of the plants.

This study was thus aimed at investigating the fumigant effect of *O. basilicum* leaf essential oil on the longevity and fecundity of adult cowpea bruchid *Callosobruchus maculatus* (F) and on the germination of cowpea seeds treated with the oil.

MATERIALS AND METHODS

Insect rearing

The initial culture of *C. maculatus* was obtained from the Entomology Unit of the Nigerian Stored Products Research Institute (NSPRI), Ilorin, Nigeria. The insects were then reared in Kilner jars for three generations on untreated cowpea seeds. Rearing was done in the laboratory under ambient

temperature of 30-35°C, 75-80% relative humidity and 10:14h (Light: Dark) photoperiod. Adults of less than 2 days old were used in the bioassay. These were sexed by examination of the elytral pattern (Halstead, 1963).

Sterilization of cowpea seeds

The cowpea seeds (Ife brown variety) used for the experiment were sourced from the International Institute for Tropical Agriculture (IITA), Ibadan. The variety is medium sized, with a brown testa and white hilium. It is also known to be susceptible to bruchid infestation. The seeds were kept in air-tight plastic bags at -4°C for 7days to disinfest them. The disinfested seeds were then preserved in airtight plastic jars (1L capacity) in order to impede further infestation. Only pristine, eggless seeds were used in the bioassays.

Extraction of essential oil

Fresh leaves of *O. basilicum* were harvested in July, 2014 from a farmer's farm in Ilorin, Kwara state. The leaves were pulverized in the laboratory using mortar and pestle and subsequently weighed using a sensitive balance (S. Mettler 0.001g-310g). Essential oil was extracted from a sample (600g) of the ground leaves by hydrodistillation technique in an all-glass Clevenger-type apparatus (Hassanpouraghdam *et al.,* 2009) for 3 hours. An oil yield of 0.3 % (w/w) was obtained at the end of the extraction. The oil was stored in a hermetically sealed glass vial having a rubber lid and covered with aluminium foil to protect the contents from photo-conversion. The oil was kept under refrigeration at 4° C until it was needed for chemical analysis and bioassay studies.

GC/MS analysis of essential oil

Analysis of the extracted essential oil was done using the Gas Chromatography-Mass Spectrometry (GC/MS) technique. An Agilent 19091S-433HP-5MS instrument with a DB-5 coated fused phenylmethylsilox capillary column (30m length x 0.25mm *i.d* x 0.25µm film thickness) was used. The injector temperature was set at 250 °C and the detector temperature was 200 °C. The column temperature program was 35 °C for 5min then at 4°C /min to 150 °C for 2min then 20°C /min to 250 °C for 5min. Helium (1.5ml/ min) was used as the carrier gas at 11.604 P.S.I. inlet pressure. For MS, the electron impact was 70 eV. Compounds were identified by their GC retention time, expressed by Kovat's index, and by comparison of test compound's mass spectra with those present in the National Institute for Standard Technology computer data bank (NIST) and published spectra (Adams, 2001). Quantification of the relative amounts of the individual constituents was

performed according to the area percentage method (Telci *et al*., 2006).

Bioassays

A bioassay was designed to determine the toxicity of the essential oil vapour of *O. basilicum* leaves to adults of *C. maculatus* as well as the oil dose that will cause mortality of 50% (LD_{50}) and 99% (LD_{99}) of test insects 24 and 48 hours after treatment. The oviposition deterrent potential of the oil during the exposure period was also assessed. In addition, the viability of fumigated cowpea seeds after exposure to the oil vapour for 48 hours was investigated in a germination test.

The toxicity of the essential oil to adults of *C. maculatus* and its potential to deter oviposition in the female bruchid was carried out simultaneously in small fumigation chambers. The chamber was made up of a 100ml plastic container (the lid of which had a 2.6 diameter hole covered with a fine muslin mesh from inside) and a transparent V- shaped 200ml plastic container. The smaller container (insect cage) was inserted headlong into the larger one until an airtight space (30ml) was obtained between the narrow base of the larger container and the lid of the smaller one. The experimental doses of essential oil were deposited on a cut piece of Whatman No 1 (4cm in diameter) filter paper fixed at the base of the larger container just above the meshed lid of the insect cage. Polystyrene material was used to stuff the base of the setup to further improve its air tightness.

Two pairs of adult *C. maculatus* on ten seeds of cowpea were exposed to four different experimental doses (10, 20, 30 and 40 µl/130ml air) of the essential oil vapour for 24 and 48 hours. No oil served as control. Tests for the different exposure periods were done separately. Each tested dose and control was replicated three times. The number of dead adults and the number of eggs laid per seed were counted at the end of each exposure period. Beetles were considered dead when they failed to crawl after being prodded with forceps. Furthermore, for each treatment, five seeds were randomly selected from the fumigated lot. The seeds were planted on moist Whatman No 1 filter paper in Petri-dishes. Each treatment was replicated three times. A control experiment was also set up and percentage germination was assessed based on the number of seeds with radicles and primary leaves 4 and 5 days after planting (DAP).

Data Analysis

The experiment was set up in completely randomised design. Data were transformed with the formula sqrt (x + 1), and subsequently subjected to analysis of variance (ANOVA). Where there

was significant treatment effect, Duncan's Multiple Range test at 5%probability level was used to separate the means. Means of untransformed data were reported. The 50% and 99% lethal dose (LD_{50} and LD_{99}) values and their corresponding Confidence Interval (CI) were assessed by Probit analysis (Finney, 1971). All statistical analyses were carried out using IBM SPSS Statistics 21.

RESULTS

Table 1 shows the identities, Kovat indices, percentage composition and mass spectra data of the constituents of the essential oil of *O. basilicum* leaves. In the Table, fifteen compounds are represented. 90.7% of the oil constituents were identified from their mass spectra. Hydrocarbon monoterpenes (HM) and oxygenated monoterpenes (OM) constituted 30.0 and 41.0% of the oil respectively. Percentage composition of hydrocarbon sesquiterpenes in the oil was 17.6%. The major hydrocarbon monoterpenes in the oil was ϒ-Terpenene (9.6%) while Terpinen-4-ol (29.8%) and Sabinene Hydrate (5.0%) were the major oxygenated monoterpenes. Other monoterpenes in the oil existed in appreciable amounts. Hydrocarbon sequiterpenes made up just 17.6% of the total oil constituent. α-Bergamotene (9.2%) and β-Caryophyllene (7.3%) were the major hydrocarbon sequiterpenes in the oil. A non-terpenic (NT) compound, Octenyl Acetate made up 2.1% of the total oil. 9.3% of the oil constituents could not be accounted for.

Adult toxicity test

After 24 hours of exposure an increase in percentage adult mortality was observed as the dose increased from 10µl/130ml to 20µl/130ml air (Table 2). However, at 30µl/130ml air, the highest adult mortality of 100% was attained and maintained even when the oil vapour dose increased to 40µl/130ml air. All oil vapour doses, within 48 hours of exposure, resulted into complete (100%) mortality of exposed adults. No mortality was recorded in the control during the 48 hours exposure period. Probit analysis results in Table 3 show that the 50% and 99% lethal doses of the essential oil vapour were 13.33µl/130ml air and 41.38µl/130ml air respectively.

Oviposition deterrence test

Few eggs were laid by the female bruchids on all seeds exposed within 24 hours to varying doses of the essential oil vapour (Table 4). The control had the highest mean number (29.3) of eggs laid within this period and was significantly ($P<0.05$) different from all the other treatments. The same

trend was observed after 48 hours exposure period. There was however no significant difference (P>0.05) in the mean number of eggs laid on seeds exposed to the 10, 20, 30 and 40 µl/130ml air doses of the EO at the end of both exposure periods.

Germination test

The mean percentage germination of seeds fumigated for 48 hours (assessed by the number of seeds with radicles and primary leaves 4 and 5 DAP) are presented in Table 5. At the end of both exposure periods, there was no significant difference (P>0.05) in the germination of treated seeds exposed to 10, 20, 30 and 40 µl/130mlair doses of the EO. Also, the germination of control seeds did not differ significantly (P>0.05) from those of fumigated seeds after 4 and 5 DAP.

Table 1: Composition of essential oil of *O. basilicum* leaves (L) by Gas-Chromatography/Mass Spectroscopy

S/N	Compound	Class	Kovat Index	% composition	Mass Spectra Data
1	α-Thujene	HM	931	2.8	136,105,91,77,53
2	α-Pinene	HM	937	3.8	136,121,91,77,53
3	β-Pinene	HM	980	3	136,107,93,69,53
4	α-Terpinene	HM	1018	3.7	136,121,105,93,58
5	o-cymene	HM	1020	3.8	134,119,91,65,51
6	ϒ-Terpenene	HM	1062	9.6	136,121,105,91,65
7	Terpinolene	HM	1001	3.3	136,115,93,67,53
8	Linalool	OM	1098	4.6	154,121,93,71,53
9	Sabinene Hydrate	OM	1068	5	154,121,93,71,55
10	Camphor	OM	1143	1.6	152,108,95,77,55
11	Terpinen-4-ol	OM	1177	29.8	154,111,93, 71,53
12	Octenyl Acetate	NT	1110	2.1	128,99,86,72,54
13	β-Caryophyllene	HS	1418	7.3	204,161,133,93,69
14	α-Bergamotene	HS	1436	9.2	204,175,133,93,69
15	δ-Cadinene	HS	1524	1.1	204,161,119,91,55
TOTAL				90.70%	

Base peak values are in bold under the column for Mass Spectra Data

Table 2: Mortality of adults after exposure to different doses of vapour from E.O of *O. basilicum* leaves

	Mean percentage mortality	
Dose (µl/130ml air)	24 hours	48 hours
10	33.3ab	100.0a
20	66.8ab	100.0a
30	100.0a	100.0a
40	100.0a	100.0a
Control	0.0b	0.0b

Means in a column followed by the same letter (s) are not significantly different at P=0.05.

Table 3: Probit analysis of mortality of adult *C. maculatus* exposed to different doses of vapour from E.O of *O. basilicum* leaves

Exposure period(hours)	LD_{50}(µl/130ml air) (95%) CI	LD_{99}(µl/130ml air) (95%) CI	Slope	χ^2df=2
24	13.33 (9.07-16.87)	41.38 (28.57-116.71)	2.86	2.21
48	-	-	-	-

CI: Confidence Interval (minimum to maximum lethal dose)

LD_{50} and LD_{99} values for 48 hours are not stated because all doses gave 100% mortality

Table 4: Number of eggs laid after exposure to different doses of vapour from E.O of *O. basilicum* leaves

Dose (µl/130ml air)	Mean number of eggs laid	
	24 hours	48 hours
10	2.0b	1.3b
20	2.3b	4.0ab
30	0.0b	6.3ab
40	2.7b	0.0b
control	29.3a	20.0a

Means in a column followed by the same letter (s) are not significantly different at P=0.05

Table 5: Mean percentage germination of seeds after 48 hours exposure to different doses of vapour from E.O of *O. basilicum* leaves

Dose (µl/130ml)	Percentage Germination			
	4 DAP		5 DAP	
	percentage of seeds with radicles	percentage of seeds with primary leaves	percentage of seeds with radicles	percentage of seeds with primary leaves
10	100.0	13.3	100.0	53.3
20	93.3	60.0	100.0	66.7
30	93.3	26.7	93.3	60.0
40	100.0	53.3	100.0	80.0
control	93.3	46.7	100.0	73.3

Means in a column followed by the same letter (s) are not significantly different at P=0.05

DISCUSSION

In this study, *O. basilicum* essential oil vapour was toxic to adult *Callosobruchus maculatus* at the different doses used. This is in agreement with Keita *et al.,* (2000) who recorded 99% mortality in *C. maculatus* adults in fumigation treatments with *O. basilicum* pure essential oil after 24 hours

exposure. Similar to the observations of Ojiako (2007) who worked on *O. gratissimum*, the toxicity of the *O. basilicum* vapour was also observed to be dose related. However, the combination of exposure period with dose further helped to accentuate the toxic effect of the oil on the beetles showing that the toxicity of the oil was dependent on both dose and time of exposure. This confirms the statement of Ogendo et al.,(2010) that the fumigant toxicity of plant essential oil constituents against adult insects were significantly influenced by time after application, concentration, insect species and corresponding factor interactions. Rajendran and Sriranjini (2008) reported that the toxicity of essential oils to stored-product insects is influenced by the chemical composition of the oil. Monoterpenoids have been reported to have strong toxicity to insects due to their high volatility and lipophilic properties that enable them penetrate rapidly into insects and also interfere with physiological functions in them (Bamphitlhi *et al*., 2015).

Fecundity (expressed by the number of eggs laid), was significantly reduced in *C. maculatus* females exposed to the oil vapour compared with the control. This observation is validated by Ketoh *et al.,* (2002) who stated that exposure of the *C. maculatus* females to 0.5 µ/L concentration of *Ocimum basilicum* caused significant reduction in their fecundity when compared with the control. However, with respect to treated seeds, there was no significant difference in the number of eggs laid by females exposed to the different dose of the oil. This study also showed that fumigating cowpea seeds with the essential oils vapour for 24 and 48 hours will not affect the germination of the seeds. This agrees with Keita *et al.,* (2001), who also reported that cowpea seeds treated with essential oils of basil did not lose their viability.

CONCLUSION

Results from this study have shown that the vapour from essential oil of *O. basilicum* leaves, when applied as a fumigant, reduces the life span and fecundity of adult *C. maculatus*. It also has no adverse effect on the germination of cowpea seeds fumigated for 48 hours. It may thus be used as an eco-friendly alternative to synthetic fumigants in the control of this insect pest. However for practical purposes, further investigations are needed on the toxicity of this essential oil's vapour to human health, its residual effect on treated cowpea seeds exposed to it for longer periods as well as its behavior under practical fumigation conditions.

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