

Susceptibility of the life stages of *Callosobruchus maculatus* (Fabr.) in stored cowpea grains to fumigation with essential oil vapour of *Ocimum basilicum* L. leaves

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This study was carried out to investigate the susceptibility of the life stages of *Callosobruchus maculatus* (F.) in stored cowpea to the essential oil vapour of sweet basil, *Ocimum basilicum*, L. leaves when applied as a fumigant. The extracted and analysed essential oil of *O. basilicum* leaves in this study was made up mainly of monoterpenoids and sesquiterpenoids with γ -terpinene (12.2%), and β -caryophyllene (10.4%) predominating. In separate bioassays, cowpea seeds harbouring the different life stages of *C. maculatus* were exposed to the oil vapour within a hermetic structure. The essential oil vapour had insecticidal effects on the adults of *C. maculatus*. Mortality was observed to significantly ($P < 0.05$) increase from 14.8% to 100% as dosage increased from 10 μ l/80ml air to 60 μ l/80ml air. Significantly high percentage mortality (98.48%) of *C. maculatus* eggs was observed after three days of seed fumigation with the essential oil vapour. The percentage mortality caused by the oil vapour to the larval and pupal stages within fumigated cowpea seeds was not significantly different from the control at $P = 0.05$ after three days of fumigation. It was thus concluded that the egg and adult stages of *C. maculatus* were more susceptible to the essential oil vapour of *O. basilicum* leaves than the larvae and pupae stages within stored cowpea.

Keywords: *Callosobruchus maculatus*, *ocimum basilicum*, essential oil, fumigation, cowpea

Cowpea is a major legume crop in West Africa (Langyintuo et al. 2003) and is consumed in various forms not only as a source of plant protein but also a source of vitamins and minerals (Hall et al. 2003). The pulse beetle, *Callosobruchus maculatus* (Fabr.) (Coleoptera: Bruchidae), infests stored cowpea grains from the field or infested storage structures (Ofuya 2003). The female oviposits oval, translucent eggs on the surface of cowpea seeds. The first instar larva bores directly into the seed within which it feeds and grows while decimating the cotyledon and embryo. Prior to pupation, the larva cuts a round window just below the seed coat. Pupation lasts 3-4 days depending on temperature, the adult emerges leaving the characteristic hole seen on damaged cowpea seeds (Radha and Susheela 2014; <http://www.beanbeetles.org/>). Cowpea seeds with eggs and holes are less preferred by consumers and usually have a lower market value. While several synthetic fumigants including phosphine gas, methyl bromide gas and hydrogen cyanide have been effective

against this insect pest within stored cowpea, their use has been attended by such problems as environmental pollution, mammalian toxicity, insect pest persistence hence the need to seek for more innocuous control methods of the pest (Mundhe and Pandey 1980; Abu and Muthu 1985; Koul et al. 2008).

Essential oils and their constituents have been reported to offer promising alternatives to classical fumigants against stored product pests (Papachristos and Stamopoulos 2003). Essential oils are composed mainly of terpenoids and are produced in appreciable quantities by species within such plant families as *Asteraceae*, *Lamiaceae*, *Geraniaceae*, *Zingiberaceae*, *Poaceae* (Franz and Nova 2010). While the fumigant toxicity of leaves of Sweet basil, *Ocimum basilicum* L. (Family *Lamiaceae*) essential oil to adult *C. maculatus* has been reported (Keita et al. 2001), very few reports are available on the capacity of the oil, when applied as a fumigant, to penetrate cowpea seeds and kill the immature stages within them. This study therefore seeks to

investigate the susceptibility of the egg, larval, pupal and adult stages of *C. maculatus* within cowpea seeds to the leaf essential oil of *O. basilicum* when applied as a fumigant.

Materials and Methods

Harvesting of *Ocimum basilicum* leaves

Whole *O. basilicum* plants were harvested from a local stream-side vegetable farm in Ilorin, Kwara State (8°30'N and 4°10'E) before noon in September, 2014. A second harvest was done two weeks after the first harvest. After each harvest, leaves were removed from the plant and stored in polythene bags in the refrigerator (4°C) to keep them fresh.

Extraction and analysis of essential oils

The collected leaves of *O. basilicum* were subjected to 3 hours of hydro-distillation using a Clevenger type apparatus. 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. Compounds were identified by their GC retention time, expressed by Kovat's index and by comparison of test compound's mass spectra with published spectral data (Adams 2001).

Cowpea seed variety

The cowpea seed variety, IT89KD-391(now SAMPEA-12) from the International Institute for Tropical Agriculture (IITA) Ibadan, Nigeria was used in this experiment. The cowpea seeds were sterilized by freezing (-4°C) for 7 days. The sterilized seeds were stored in airtight plastic containers and kept in the refrigerator until needed.

Insects

The parent insect culture of *C. maculatus* used

for the experiment was obtained from the Nigerian Stored Products Research Institute (NSPRI), Ilorin, Kwara state, Nigeria. This culture was maintained in the laboratory at 30-35°C, 70-75% R.H. and 14:10 (light: dark) photoperiod. Under these conditions, eclosion of the 1st instar larvae occurred within 3 and 4 days after oviposition (DAO). The last larval instar was found in the seeds between the 11th and 14th DAO. Pupation lasted for about 3days (15th DAO - 18th DAO). Emergence of the adult started to occur by the 19th DAO. Only beetles of no older than 2 days were used in the experiments.

Fumigation apparatus

The oil was tested in small fumigation structures which consisted of an insect cage (a 100 ml plastic container with a 2.6 cm diameter hole cut into its lid and covered with a fine muslin mesh from inside with glue) and a 4 cm diameter filter paper unto which the essential oil was introduced using a 1000 µl capacity micropipette. After putting the oil on the filter paper, it was immediately placed over the meshed area of the insect cage both of which were then inserted headlong and hermetically into another larger transparent V- shaped 200ml plastic container. The total volume of space within the empty airtight fumigation chamber was approximately 130ml.

Fumigant toxicity bioassay on adult *C. maculatus*

Five dosages of the oil, 10µl, 20µl, 30ul, 40µl and 60µl, were tested in this bioassay. Forty grams (approx. 240 seeds) of whole clean cowpea seeds and ten adult beetles were introduced into the insect cages. Thereafter, the total volume of empty space left in each chamber was approximately 80ml. A control experiment was also set up in which no oil was introduced into the fumigation chambers. The set up was left for 24 hours at room temperature after which the percentage of dead

adults was determined. Adults were considered dead when they failed to crawl after been repeatedly prodded with laboratory forceps.

Fumigant toxicity bioassay on immature stages of *C. maculatus*

Approximately 120 whole, eggless seeds of cowpea were infested in a Petri dish with 10 pairs of 2 day old adult *C. maculatus* for 20 minutes. Thereafter, the beetles were removed but not discarded. The seeds were sorted into groups based on the number of eggs they had on them. Eggless seeds were set aside and re-infested with the pairs of beetle for another 20 minutes. The infestation and sorting of seeds was continued until all the seeds had one or more eggs. Only seeds bearing single eggs were used. The procedure was repeated 2 and 4 days after the first one was done. This ensured the availability of beetles at different stages of development. A predetermined oil dosage of 30ul was used for all bioassays involving immature stages.

Fumigant toxicity bioassay on egg

Ten seeds of cowpea each bearing an egg of about 2½ days old were exposed to the vapour of *O. basilicum* leaf essential oil within the fumigation structures. A control experiment was also set up. The setup was left for 3 days (i.e. approx. 6 days after the eggs were laid (6 DAEL) at the end of which the fumigated and control seeds were aerated and left in labelled Petri dishes for another 7 days (approx. 13 DAEL) after which dissection was done. Dissection was not done immediately after the experiment to avoid crushing the very tiny first instar larvae. The number of dead larvae was expressed as a percentage of the total number of larvae per replicate. A larva was considered dead if it failed to wriggle and or if it appeared brown in colour.

Fumigant toxicity bioassay on larva

After 11 DAEL, 10 seeds (each bore an egg), were exposed to the essential oil vapour for 3 days (i.e. 14 DAEL). After the time lapsed, the seeds were removed, aerated and left in labelled Petri dishes for a day. Dissection was done at 15 DAEL because it was expected that pupation of the last instar larvae would have commenced. The number of pupating or pupated larvae was expressed as a percentage of the total number of pupae found per replicate. Thereafter, the percentage of dead larvae per replicate was obtained by subtraction.

Fumigant toxicity bioassay on pupa

Ten seeds, each bearing an egg that was laid 14 days earlier, were fumigated for 3 day (i.e. 14 - 17 DAEL). After the time lapsed, the seeds were removed, aerated and left in labelled Petri dishes for a day after which dissection was done. The number of dead adults within the seeds was expressed as a percentage of the total number of adults found in each replicate. Pupae were counted as living if they completed their development *in vitro* two days after dissection.

Data analysis

The experiment was laid out in a completely randomized design (CRD). Percentage adult mortality data were subjected to 1-way ANOVA. Separation of means was done using Duncan Multiple Range Test (DMRT) at 5% level of significance. Data of percentage mortality of immature stages was however subjected to the independent sample T-test at 5% level of significance. Abbott's (1925) formula was used to correct mortality rates when there was mortality in the control. The analysis of the dose-mortality response was obtained by Probit analysis (Finney, 1971). All data analyses were done using IBM SPSS statistics version 21.

Results

Phytochemical analysis

Thirty constituents, representing 90.40% of the total extracted *O. basilicum* leaves essential oil, were identified by GC-MS analysis (Table 1). The predominant hydrocarbon

monoterpenes were γ -terpinene (12.2%), terpinolene (8.6%), m-cymene (6.2%) and cyclofenchene (5.0%). The oxygenated monoterpenes was predominated by terpinen-4-ol (8.7%). β -caryophyllene (10.4%) and α -ylangene (7.6%) were the most abundant hydrocarbon sesquiterpenes.

Table 1: Chemical composition of essential oil extracted from *O. basilicum* leaves

S/N	Compound ^a	Class ^b	Percentage Composition
1	γ-Terpinene	HM	12.2
2	Cyclofenchene	HM	5.0
3	Camphene	HM	1.3
4	β -Pinene	HM	0.8
5	1-Octen-3-ol	NT	1.5
6	α -Thujene	HM	0.9
7	Terpinolene	HM	8.6
8	m-cymene	HM	6.2
9	Sabinene Hydrate	OM	1.2
10	Linalool	OM	4.2
11	Octenyl Acetate	NT	2.8
12	Cis-p-Ment-2-en-1-ol	OM	1.0
13	Camphor	OM	2.3
14	Terpinen-4-ol	OM	8.7
15	α -Terpineol	OM	1.7
16	Fenchyl Acetate	OM	1.2
17	Bornyl Acetate	OM	1.5
18	Myrtenyl Acetate	OM	0.7
19	β-Caryophyllene	HS	10.4
20	α-ylangene	HS	7.6
21	α -Caryophyllene	HS	1.0
22	Germacrene D	HS	0.7
23	E- β -farnesene	HS	1.3
24	E- α -farnesene	HS	1.5
25	δ -Cadinene	HS	1.6
26	Trans-Nerolidol	OS	1.3
27	Caryophyllene oxide	OS	0.6
28	Epiglobulol	OS	1.2
29	β -Eudesmol	OS	0.6
30	Alloaromadendrene oxide	OS	0.8
TOTAL			90.40%

^a Most predominant constituents are in bold

^b HM: Hydrocarbon Monoterpenoids, OM: Oxygenated Monoterpenoids, HS: Hydrocarbon Sesquiterpenoids, OS: Oxygenated Sesquiterpenoids, NT: Non- Terpenic constituents

Adult toxicity test

The percentage mortality of adult *C. maculatus* exposed to different dosages of the leaf essential oil vapour of *O. basilicum* is presented in Table 2. As dosage increased from 10 µl/80ml air to 60 µl/ 80ml air, the percentage mortality increased from 14.8 to 100. There was no significant difference ($P>0.05$) in the mortality of adults when exposed to 30, 40 and 60 µl/80ml air dosages of the essential oil (E.O). The dosage required to kill 50% of a population of adult *C. maculatus* (LD_{50}) was 22.38 µl/ 80ml air (Table 3).

Fumigant toxicity to immature stages

A significantly ($P<0.05$) higher mean percentage (98.48) number of egg mortality was observed in fumigated seeds (Table 3). The mean percentage number of larvae and pupae mortalities recorded in fumigated seeds was also higher but not significantly different when compared to the control at $P=0.05$.

Table 2a: Mortality of adult *C. maculatus* exposed to the leaf essential oil vapour of *O. basilicum* for 24 hours

Essential oil dosage (µl/80ml of air)	Mean percentage adult mortality 24 hours after treatment
10	14.8±14.8bc
20	40.7±9.8b
30	85.1±7.4a
40	85.1±9.8a
60	100.0±0.0a
Control	0.0±0.0c

Values in a column followed by the same letter(s) are not significantly different at $P=0.05$ by the Duncan Multiple Range Test

Table 2b: Probit analysis of the toxicity of the leaf essential oil vapour of *O. basilicum* to adult *C. maculatus*

Exposure period	LD_{50} (µl/80ml)	CI (95%) (µl/80ml)	Slope	χ^2 df=3
24 hours	22.38	13.19 - 25.91	3.43	13.78

CI: Confidence Interval; LD_{50} : 50% lethal dosage

Table 3: Percentage mortality of the immature stages of *C. maculatus* in cowpea seeds fumigated with the leaf essential oil vapour (30 µl/130ml) of *O. basilicum*

Treatments	Mean percentage mortality 3 days after treatment		
	Eggs	Larvae	Pupae
Fumigated Seeds	98.48±1.5a	26.67±17.6	30.16±16.6
Control Seeds	65.14±9.2b	16.67±16.7	13.10±7.2
		NS	NS

NS: not significant

Discussion

In this study, the oil vapour caused significant adult mortality after 24h and thus had fumigant toxicity effect against the adult bruchid. This is in agreement with Keita et al. (2000) who recorded 99% mortality of *C. maculatus* adults in fumigation treatments with *O. basilicum* pure essential oil after 24h exposure. Rajendran and Sriranjini (2008) reported that the toxicity of essential oils to stored-product insects is influenced by the chemical composition of the oil. In this study, adults exposed to the essential oil vapour of *O. basilicum* exhibited uncoordinated movements of their metathoracic limbs while females that died from exposure to the vapour exhibited extruded ovipositors. These physiological effects can be attributed to the terpenic constituents of the oil (Ismail 2006). The essential oils of *O. basilicum* have however been reported 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 (Da-Silva et al. 2003).

The essential oil of *O. basilicum* caused significant *C. maculatus* egg mortality. This ovicidal activity of the oil may also be attributed to its terpenic constituents (Rice and Coats 1994). Findings in this study further showed that the larval and pupal stages of the insect were the most resistant stages to the essential oil vapour of *O. basilicum*. Some authors, (eg. Don-Pedro 1996; Erler 2005 and Eliopoulos et al. 2015), who worked on *C. maculatus*, *P. interpunctella* and *T. confusum* respectively have also reported that the larval and pupal stages were highly tolerant to vapours of various essential oils or their constituents unlike the egg or adult stages. In this study, the sedentary egg stage of *C. maculatus* was susceptible to the essential oil vapour of *O. basilicum* leaves like the active adult. On the other hand, the pupa which is a sedentary stage was as tolerant to the fumigant as the actively feeding larvae. This is contrary

to Rajendran and Sriranjini (2008) who stated that for fumigants, the active stages of insects are more susceptible than the sedentary stages because they differ in their rate of respiration. The cowpea seed coat, amongst other functions, exerts seed coat dormancy on the seed and by so doing makes it impermeable to water and gases (<http://www.ext.colostate.edu/>). Additionally, the seed hilum is an important structure that controls embryo-external environment relationships (Souza and Marcos-Filho, 2001). Since the larva and pupa respire at different rates, then their observed tolerance in this study may have been facilitated by the functional roles of these specialized structures of the cowpea seed within which the two stages are confined and not by their rate of respiration.

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