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COMPARATIVE CHEMICAL COMPOSITIONS AND INSECTICIDAL ACTIVITIES OF THE VOLATILE OILS OF *ROSMARINUS OFFICINALIS* AND *CALLISTEMON VIRIDIS* AGAINST *CALLOSOBRUCHUS MACULATUS* F.

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ABSTRACT

Background and aim: Use of synthetic chemicals in controlling destructive activities of cowpea weevil, *Callosobruchus maculatus* is marred by shortcomings which necessitate the search for safer, cheaper alternatives like volatile oils. This work examined the constituents and effects of *Rosmarinus officinalis* and *Callistemon viridis* volatile oils on *Callosobruchus maculatus*.

Method: Hydro-distilled volatile oils obtained from fresh *R. officinalis* and *C. viridis* leaves were evaluated for insecticidal activity using seed-dressing method at 2.5, 5 and 20 µL per 50 cowpea seeds measured into Petri-dishes. Acetone (300 µL) was added and shaken to ensure uniform seed coating. Controls were treated with acetone only. Treated seeds were transferred into 250 mL plastic vials with tight covers and inoculated with five pairs of male and female adult *Callosobruchus maculatus*. On day 14, insects were removed and eggs laid on each seed counted. Adult insects' emergence was determined between days 23 and 40. Chemical constituents of the oils were determined by GC-MS.

Results: Both volatile oils significantly *($p<0.05$)* reduced oviposition, adult emergence and other parameters with increase in concentrations. Anti-oviposition effects of 2.5, 5.0 and 20 µL of *R. officinalis* (represented by 81.70, 90.79 and 91.26 % reductions in fecundity compared to control), surpassed that of *C. viridis* (69.88 and 91.40 % reductions). While 1,8-cineole (31.72%) and camphor (30.11%) formed major constituents of *R. officinalis*, 1,8-cineole (72.98%) and α-pinene (23.17%) predominated in *C. viridis*.

Conclusion: Although *C. viridis* seems more potent than *R. officinalis*, the volatile oils of the plants exhibited oviposition and adult emergence inhibitory activities on the weevils.

Key words: *Callosobruchus maculatus*, volatile oils, *Callistemon viridis, Rosmarinus officinalis*, insecticidal activities, 1,8-cineole, α-pinene, camphor

INTRODUCTION

Cowpea weevil, *Callosobruchus maculatus* (Coleoptera, Bruchidae) is one of the major storage pests of different species of cowpea, *Vigna unguiculata* (L) Walp (Leguminosae). Activities of the pests reduce the nutritional and economic values of beans and in cases of extreme uncontrolled infestation, the materials are rendered economically invaluable causing huge losses to farmers. The destruction occasioned by activities of these insects can threaten the food security of the nation and compromise the quantity and quality of proteins they contain. Control of these insects is carried out by application of synthetic chemicals like methyl bromide and aluminum phosphide, some of which have been reported to possess certain carcinogenic properties against handlers [1,2]. Shortcomings include pest resistance, side effects on non-target organisms in addition to many hazards to the environment which informed the ban or discouragement of application of dichloro-diphenyl trichloro ethane (DDT) [3].

Natural products particularly volatiles oils of plant origin have been discovered to be environmentally friendly, and can serve as potent and viable pesticide alternatives due to their availability and safety with multiple effects from contact, deterrent and fumigant properties [4]. Research reports have also depicted some medicinal plants whose activities have been found to reduce the fecundity of the insects and retard the development of the eggs from growing to adults [5]. Volatile oils of *Rosmarinus officinalis* L (Lamiaceae) and *Callistemon viridis* (Curtis) (Myrtaceae) have been established to possess insecticidal and other biological activities [6-8]. This work evaluated chemical composition and probable effects of the volatile oils on the oviposition and adult emergence of *Callosobruchus maculatus* using seed-dressing method.

METHODS

Plant collection and extraction of volatile oils

Fresh green leaves of *Callistemon viridis* were collected from the University of Benin, Ugbowo Campus, while those of *Rosmarinus officinalis* were collected within the University of Benin Teaching Hospital, Benin-city in February, 2017. Leaves of *C. viridis* (6112.4 g) and *R. officinalis* (300 g) were extracted separately using steam distillation method in Clevenger-type apparatus to yield 0.53 and 0.39% respectively, of the volatile oils.

Source and maintenance of insect culture

The strain of bean weevils used, *Callosobruchus maculatus* was collected from infested stored bean seeds purchased from Uselu market, Benin City. Insects were cultured in the laboratory at ambient conditions (25 ± 2 ^{o}C) on clean, insect-free bean *Vigna unguiculata* seeds in a closed container for several consecutive generations to obtain stable Fi generations.

Evaluation of effects of volatile oils on insect fecundity

The method of Gbolade *et al* [5] was used with little modification. Experiment was carried out in Petri dishes (9cm diameter). Effects of each of the volatile oils were evaluated at concentrations of 2.5, 5 and 20 µL. Each concentration (in the Petri dish) was made up to 300 µL with pure acetone, and fifty (50) clean un-infested beans (about 23 g) were quickly added, covered and shaken gently to ensure uniform covering of all seeds. Control seeds were treated with 300 µL acetone. The experiments were carried out in triplicates. Sets of treated seeds were later transferred into different 250 mL translucent plastic containers with tightfitting covers. Based on preliminary observations made on the sensitivities of the insects to the volatile oils, seeds were treated with volatile oil of *R. officinalis* 48 h. before introduction of insects, while other seeds were treated with *C. viridis* volatile oil seven days before the introduction of insects. Thereafter, five (5) pairs of *Callosobruchus maculatus* (both sexes) were introduced into each container, covered and incubated for 14 days. On day 14 after treatment (DAT), the number of eggs laid on each set of beans was counted manually and recorded. From 20 to 40 DAT, the number of adults emerging from each container was counted manually and recorded. Other parameters like number of holes on each seed and weight loss were also recorded.

Chemical composition of volatile oils

In order to determine the respective chemical composition of the two volatile oils, microlitre quantities were separately subjected to GC-MS analyses on GCMS-QP2010 PLUS (Shimadzu, Japan) with column oven and injection temperatures of 60 and 250 ^{o} C respectively, split injection mode; column flow rate of 0.99 mL/min; flow pressure of 56.2 kPa and FTD detector. Components of each oil were identified by comparison with NIST05s. Library.

Statistical analysis

All data were expressed as mean ± SEM and one-way Analysis of Variance (ANOVA) statistical test using Graph Pad Instant R version 2.05 (UK) was used to test for significance. P< 0.05 was considered significant.

RESULTS

Insecticidal effects of *Rosmarinus officinalis* volatile oil

Volatile oil of *R. officinalis* was observed to repel insects. Weevils used to infest the bean seeds pretreated with 2.5-20 µL oil were all dead within 24 h. Fresh insects introduced after the 24 h were also discovered dead after further 24 h without ovipositing. In both cases, control insects remained alive and continued to oviposit. However, weevils introduced after 48 h. of seed treatment with oil were discovered to lay eggs which were less and significantly (p<0.01) different from controls. After 14 days of incubation, control weevils had laid an average of 282 eggs, whereas seeds treated with the least concentration, 2.5 µL per 50 seeds had laid 51 eggs on them (Table 1). Increasing the concentration of volatile oil to 5.0 and 20 µL produced 26 and 24 eggs respectively, while further increase did not reduce oviposition appreciably. Anti-oviposition effects of oil (81.70, 90.79 and 91.26 % reductions in fecundity) were evident at 2.5 to 20 µL (Table 1). It also produced marked reduction in adult emergence of insects. While 233 represented adult insects' emergence (F_1 generation) in controls, oil-treated seeds produced 41, 17 and 17.00 adults respectively at 2.5, 5, 20 µL. These results implied marked reduction in percentage adult emergence from 82 observed in control to 68 in seeds treated with highest concentration (20 µL) of volatile oil. Other parameters like average number of holes on the seeds and weight loss were also significantly reduced in treated seeds (Table 1).

Table 1: Inhibitory effects of *Rosmarinus officinalis* volatile oil on oviposition and adult emergence of *Callosobruchus maculatus*

Concentration (µL)	Number of eggs laid	Inhibition (%) of oviposition	Adult emergence	Percentage adult emergence	Number of holes	Weight loss (mg)
Control (Acetone 300 µL)	282.33± 77.99	-	233.33± 72.99	82.64 ± 2.92	186.0 ±70.03	2.64 ± 0.85
2.5	51.67±22.84*	81.70	41.67±20.34*	80.65±22.19*	28.00±12.58*	0.73±0.27*
5.0	**26.00**±10.26*	90.79	17.33±10.87*	66.65±17.69*	11.33±6.84*	0.00*
20.0	24.67±8.41*	91.26	17.00±11.15*	68.91±27.59*	11.00±7.77*	0.01±0.01*

Values are significantly ($p<0.05$) different from controls. Oil exhibited inhibitory effects on insect oviposition and adult insect emergence.

Insecticidal effects of *Callistemon viridis* volatile oil

Volatile oil was observed to reduce significantly the average number of eggs laid by the weevils in a concentration-dependent manner (Table 2). Control seeds produced average of 62 eggs which was reduced to 18.67, 5.33, and 5.33 in seeds treated with 2.5, 5, and 20 µL, respectively of volatile oil. Also, anti-oviposition effects of 2.5 to 20 µL oil represented 69.99 to 91.40 % reductions in fecundity. Average number of adult weevils that emerged were observed to reduce significantly ($p< 0.05$) compared to control seeds which produced 46 while seeds treated with 2.5, 5.0 and 20 µL of oil gave 5, 0 and 0 eggs respectively. Average number of holes on seeds and weight loss were also remarkably reduced below the controls (Table 2).

GC-MS chemical composition of *Rosmarinus officinalis* volatile oil

Volatile oil *R. officinalis* mainly yielded 1,8-cineole (31.72 %) and camphor (30.11 %) as major constituents, as well as α-terpineol (14.34 %) and borneol (13.69 %) in moderate amounts (Table 3). Oxygenated monoterpenes constituted 93.64% of the oil, while the non-oxygenated compounds gave 6.56%. Total monoterpene composition of oil was 95.23 %. Sesquiterpenes (4.77 %) were dominated by azulene (4.05 %). Linalool (2.99%) and azulene (4.05%) represented minor constituents while others including α-pinene (0.66%) existed in trace quantities.

Table 2: Inhibitory effects of *Callistemon viridis* oil on oviposition and adult emergence of *Callosobruchus maculatus*

Concentration (µL)	Number of eggs laid	Inhibition (%) of oviposition	Adult emergence	Percentage adult emergence	Number of holes	Weight loss (mg)
Control (Acetone 300 µL)	62.00±9.45	-	46.67±8.10	75.27±3.14	32.00±4.36	0.53±0.39
2.5	18.67±8.51*	69.88	5.33±2.91*	28.55± 10.02	3.33±2.40*	0
5.0	5.33±1.33*	91.40	0	0	0*	0
20.0	5.33±1.86*	91.40	0*	0	0*	0

*Values are significantly ($p<0.05$) different from controls. Oil exhibited inhibitory effects on insect oviposition and adult insect emergence

Table 3: GC-MS analysis of leaf volatile oil of *Rosmarinus officinalis*

S/No	Compounds	Retention time (min.)	% Composition	Type	Nature
1.	α-pinene	5.373	0.66	Non-oxygenated	Monoterpene hydrocarbon
2.	1,8-Cineole (Eucalyptol)	6.823	**31.72**	Oxygenated	Monoterpene
3.	Linalool	7.861	2.99	Oxygenated	Monoterpene
4.	Camphor	8.659	**30.11**	Oxygenated	Monoterpene
5.	Borneol	9.054	13.69	Oxygenated	Monoterpene
6.	α -terpineol	9.384	14.34	Oxygenated	Monoterpene
7.	Bornyl acetate	10.571	0.79	Oxygenated	Monoterpene
8.	Unidentified	11.839	0.18		Monoterpene
9.	Azulene	12.492	4.05	Non oxygenated	Sesquiterpene hydrocarbon
10.	Cis-beta-ocimene	12.952	0.46	Non oxygenated	Monoterpene
11.	Unidentified	13.185	0.28	Non oxygenated	Monoterpene
12.	Unidentified	13.497	0.29	Non oxygenated	Monoterpene

13.	Germacrene D	13.798	0.44	Non oxygenated	Sesquiterpene
	Total		100		

Table 4: GC-MS chemical compositions of the volatile oil of *Callistemon viridis*

Compounds	Retention time	% Composition	Type	Nature
α-pinene	5.378	**23.17**	Non oxygenated	Monoterpene
1,8-cineole (Eucalyptol)	6.823	**72.98**	Oxygenated	Monoterpene
Myrcenol	9.391	3.85	Oxygenated	Monoterpene
Total		100		

Volatile of *C. viridis* was found to be simple in composition, comprising α-pinene (23.17%) and 1,8-cineole (72.98 %) as predominant constituents, and myrcenol (3.85%) in minor amount. All constituents are monoterpenoid in nature, with α-pinene being the only hydrocarbon while others are oxygenated compounds (Table 4).

DISCUSSION

The safety, deterioration, and retention of nutritional and commercial values of stored grains and legumes particularly cowpeas are constantly threatened by perpetual infestation by insect pests. Majority of them are controlled using synthetic chemicals with the attendant undesirable effects. Results of this work have further established the use of essential/volatile oils as viable and potent alternative to control coleopteran insects in stored grains.

Oils used in this study could be applicable as contact poisons, fumigants, repellants, antifeedants, oviposition inhibitors/ deterrents for stored grains [9,10]. This study has revealed potentials of the volatile oils of *R. officinalis* and *C. viridis* in reducing oviposition of cowpea weevils on seeds. The fact that the first set of insects introduced into the treated seeds died within 24-48 h implied the remarkable efficacy of oils when used as fumigants. Treatment of seeds with volatile oils solubilized in acetone ensured uniform surface coating of seeds. Remarkable reductions observed in number of eggs laid compared with controls were indicative of oviposition deterrence of the volatile oils which could be due to interference in the pheromone production by insects which may impair attraction of opposite sex. It is also possible that insects experienced some level of morbidity due to inhalation of constituents of volatile oils leading to serious impairment of oxygen intake and consequent paralysis and death. Furthermore, the observed reduction in oviposition could be due to repellent effects of volatile oils under study. In relation to controls, *R. officinalis* and *C. officinalis* oils used can be described as effective anti-oviposition agents.

Adult emergence can be used as a measure of probable ovicidal or larvicidal effects of the volatile oils. Volatile oils of both plants significantly ($p<0.05$) reduced adult emergence on day 41 far below the controls. From this study, *C. viridis* oil (28.55 to 0%) appeared more effective than *R. officinalis* (80.65 to 68.91%) when tested at 2.5 to 20 µL in inhibiting adult insect emergence. With relatively high post-experiment percentage adult emergence, these volatile oils can be said to have mild effect on egg viability or subsequent metamorphosis. These oils may have rendered the eggs (or any of the other stages of development) incapable of further metamorphosis through alteration or disruption of important enzymatic biochemical. With complete inhibition of adult emergence at minimal concentration, 5µL by *C. viridis* volatile oil, it therefore seems to be more potent than *R. officinalis* oil as cowpea protectant during storage. Other parameters like average number of holes on seeds are direct reflections of the extent of insect infestation and effects. Adult emergence was proportional to average number of holes on the seeds. For both volatile oils, average number of holes decreased markedly with decrease in adult emergence, but was more pronounced in volatile oil of *C. viridis* particularly at concentrations of 5 and 20 µL.

Both average number of holes and average weight loss can be used to estimate the extent to which the economic and nutritional values of cowpea seeds have been compromised by developmental activities of weevils' larvae and pupae. These go along with consumption of seeds' nutrients which ultimately lead to reduced weight, unattractive appearance and low economic and nutritional values. Average values of these parameters are proportional to average adult emergence. Activities of volatile oils are reflections of the chemical compositions which are naturally monoterpenes or sesquiterpenes of different forms and could be oxygenated or non-oxygenated in varying proportions. This work revealed *R. officinalis* oil to be dominated by oxygenated monoterpenes, while *C. viridis* comprised both oxygenated and non-oxygenated monoterpenes.

Constituents of volatile oils are aromatic in nature and have been associated with an array of biological effects. Volatile oil of *R. officinalis* has been previously reported to contain varying proportions of similar major constituents like 1,8-cineole, camphor and borneol [6, 11] as in this present study. *C. viridis* volatile oil investigated in this study, shared similar composition as that of related species, *Callistemon citrinus* [12] and *C. viminalis* [13]. *C. citrinus* oil was also found to be toxic to adult insects when applied by fumigation. These major components, 1,8 cineole and α-pinene appear to be characteristic of *Callistemon* genus. Variations in composition of major and trace compounds in these volatile oils, and others could be due to environmental factors [14].

Although, *R. officinalis* (Lamiaceae) and *C. viridis* (Myrtaceae) investigated herein belong to different families, two of their constituents (α-pinene and 1,8-cineole) are similar but in varying proportion, and have been differently associated with many insecticidal effects [15,16]. Consequently, it is reasonable to assume they have contributed to the insecticidal effects in this study. In addition, 1,8-cineole has been reported to reduce oviposition of *C. maculatus* and found highly toxic to adult and the egg stage even at low concentrations [17]. On the other hand, α-pinene has been reported to cause inhibition of development and production of F_1 generation in *Sitophilus zeamais* up to 94% through contact and fumigation methods [18]. These activities have been linked to inhibition of acetylcholinesterase and exertion of neurotoxicity [19]. The presence of high amounts of 1,8 cineole and α- pinene in *C. viridis* oil may account for its higher insecticidal potency over *R. officinalis*.

CONCLUSION

The remarkable inhibition of oviposition and adult emergence demonstrated by volatile oils of *R. officinalis* and *C. viridis* against cowpea weevil in this study suggest possible potential for their future application as suitable alternatives in protecting stored products against weevils. This study also reported greater insecticidal potency for *C. viridis* oil.

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