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Insecticidal Activity of Powder and Essential Oils of *Vepris heterophylla* (Rutaceae) and *Syzygium aromaticum* (Myrtaceae) Towards *Callosobruchus maculatus* F. walp (Coleoptera: Bruchidae) on Post-Harvest *Vigna unguiculata* (Fabaceae) in the Far-North Region of Cameroon

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Authors' contributions

This work was carried out in collaboration among all authors. Authors MSGN and KH designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors NNE and NI managed the analyses of the study and managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The bioinsecticidal effect of powders and essential oils of *S. aromaticum* (L.) flower buds and *V. heterophylla* (Engl.) leaves against adult cowpea weevil *C. maculatus* was studied. Powders were tested by direct contact only while essential oils were tested by direct contact and indirect contact (inhalation and repellency). In 500 mL glass jars, the individual and combined powders were applied to 100 g of cowpea seeds at 0.5 g, 1 g, 1.5 g and 2 g doses for both leaves and flower

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buds of *V. heterophylla* and *S. aromaticum* respectively. The essential oils were also applied individually and after equilibrium combination on 50 g of cowpea at 4 µL/mL, 8 µL/mL, 12 µL/mL, 16 µL/mL. All the infestations were then achieved by adding 20 bruchids of 48 h old. The essential oil obtained was purified and analyzed with Gas Chromatography and Mass Spectrometry (GC-MS). 72 h after the individual treatment, 100% mortality was obtained at 2 g dose for *V. heterophylla* and 0.5 g dose for *S. aromaticum* compared to the control jars (1%). The combined powders were found to be more effective with 100% of mortality at a dose 1 g after 48 h of exposure. For essential oils, 100% and 90% of mortality were obtained respectively at 8 µL/mL dose for *S. aromaticum* (160 µL/kg) and 16 µL/mL dose for *V. heterophylla* (320 µL/kg) 72 h after treatment. In addition, the essential oil of *S. aromaticum* showed relatively higher repellant properties with an average repellency percentage of PR = 89.37% than that of *V. heterophylla* (PR = 70.62%). 100% of mortality was induced after inhalation of essential oils of *S. aromaticum* and *V. heterophylla* at doses 10 µL and 40 µL respectively after 72 h of exposure time. Results obtained from the GC-MS showed that the major components in the essential oil of *S. aromaticum* were eugenol (83.40%) while that of *V. heterophylla* were Spathulenol (23.66%), Beta-Caryophyllene oxide (16.46%) and 6-Isopropenyl-4 (16.30%). The powders and essential oils of the tested plants applied individually or after equilibrium combination showed interesting prospects for controlling *Callosobruchus maculatus* and effectively preserving cowpea seeds in storage warehouses.

Keywords: Insecticidal; combination; infestation; inhalation and repellency.

1. INTRODUCTION

Worldwide, climatic change, economic instability, the current COVID-19 pandemic and various conflicts threaten food security [1]. In 2020, 155 million people were living in acute food insecurity since they were exposed to hunger and malnutrition, which increased the risk of developing diseases [2]. This represents an increase of about 20% compared to the last five years, despite the laudable efforts of organizations like World Food Programme (WFP) and World Health Organization (WHO). Most of these people are found in Africa and under developed countries like Cameroon, where agriculture is the main activity in rural areas [3,4,5]. In Cameroon, several regions are affected by food insecurity. The Far-North region has the highest prevalence (1.8%) of acute malnutrition which is very close to the emergency threshold of 2% [6,1]. However, this region is an agro-ecological zone for most cereals and legumes nationwide [7,8,9]. Among these legumes, cowpea also called *Vigna unguiculata* is a cash crop and constitutes the basis of the population's diet [10,9,11]. Its leaves and stems constitute hay for livestock and also help to fertilize soils by symbiotic fixation of atmospheric nitrogen. Its seeds are very rich in calories and proteins (24-39%) and contain most amino acids essential for human nutrition [12]. Given this richness in proteins and by comparison with the quantity of protein recommended by the WHO which is 0.75 g/kg/day, cowpea seeds can constitute an important component for food security and be employed in the alleviation of recurrent acute malnutrition problems [13,14,3]. Since 2003, cowpea seeds have taken over all other crops; it has gone from a subsistence crop to a cash crop and a lean crop. Congolese and Nigerians are always frequent in the Far-North market to fetch for these precious grains [15]. Its production has increased from 82.286 tons to 116.207 tons on an area of 111.286 hectares from 2014 to 2018 [15]. Cowpea cultivation is achieved once a year during the short rainy season which lasts three months and only storage makes it available throughout the year [16]. However, during storage, cowpea constitutes a favorite substrate for molds and insect pests, favored by unstable climatic change [10,17]. Cowpea's high protein content also makes it attractive to insect pests and more particularly to *Callosobruchus maculatus*, whose larvae first consume cotyledons by making galleries [18,19]. Within two months of storage if no protective measures are taken, this beetle is able to generate losses up to 100%, which is a big loss of income for the farmers [10]. These severe post-harvest losses most often oblige some authors to say that, in Africa farmers work for insect pests [20,10,21]. Consequently, smallholders' farmers are forced to sell their cowpea at a low price from the harvest in November and buy them very expensive in June during sowing [22,10,23]. To prevent or limit these post-harvest losses, farmers make more use of synthetic chemical insecticides which are certainly effective. But, the misuse of these chemical insecticides leads to the appearance of resistant insect pests and pollutes especially the environment and also cause serious health problems to humans [24,25]. Based on this observation, alternative approaches are directed towards the use of plants with an insecticidal effect since they are very rich source of bioactive chemicals [26,27]. Several investigations have been carried out on the individual insecticidal activities of botanical plants such as *Hyptis spicigera*, *Azadirachta indica*, *Xylopia aethiopica*, *Balanites aegyptiaca* and *Vepris heterophylla* in the Far-North [10,25,27,5]. But, very few studies have been conducted on the mixture of powders or essential oils of leaves and almonds of two different plants to control insect pests in storage warehouse, thereby not only protecting the cowpea but also limiting the over exploitation of insecticidal plants. The present work aimed at evaluating the insecticidal effects of the powders and essential oils of the leaves of a local plant *Vepris heterophylla* (Engl.) Letouzey and the flower bud of *Syzygium aromaticum* (L.) Merr & L.M.Perry taken individually or in equilibrium combination against *Callosobruchus maculatus* adults.

2. MATERIALS AND METHODS

2.1 Experimental Site

The research was carried out in the Far-North Region of Cameroon. Fresh mature leaves of *V. heterophylla* were collected in September 2018 from Mokolo (10°44'54"N, 13°47'53"E, at 901 m above sea level) in the Mayo Tsanaga Division. Flower buds of *S. aromaticum* were purchased at main market in Maroua (10°36'23"N, 14°19'53"E, at 400 m above sea level) in the Diamare Division. All the plant materials collected were taken to the laboratory of the Institute of Agricultural Research for Development (IRAD) of Maroua, Cowpea section.

2.2 Preparation of Plant Materials and Compounds Analysis

Leaves were shade-dried at 30.9 ± 2.13°C temperature and 35 ± 5.02% relative humidity for 10 days. Flower buds of *S. aromaticum* were washed thoroughly with water and shade-dried for 21 days [28]. The dried leaves and flower buds were weighed with a balance (Startorius 10^{-4}) and pulverized in an electrical blender (Binatone Model 373) separately. The powders obtained were further sieved to pass through 1 mm^2 perforation to obtain homogenous powders. The fine powders were kept in airtight biodegradable plastic papers to avoid absorption of moisture and stored at room temperature through out the experiments [28].

Extraction of the two essential oils was made by hydrodistillation. 1000 g of pulverized plant material was placed in a muslin cloth of dimension 20 cm by 4 cm and then transferred into the thimble and extracted with ethanol in a soxhlet apparatus model 77-520 (Hospital Equipment Manufacturing Co, Limited India) in the laboratory of ENSAI of the University of Ngaoundéré Cameroon. The extraction was carried out for 3-4 h and was terminated when the solvent in the thimble became clear. The thimble was removed from the unit and the solvent recovered by distillation in the soxhlet extractor. Rotary evaporator was used to separate the solvent from the oil after collection of the resulting extracts from the soxhlet, then the oil was exposed to air so that traces of the volatile solvent evaporates, leaving the oil extract which were stored in the refrigerator at 4°C [28,29]. The essential oil yield was calculated according to the method described by Mohamed et al. [30]. It was expressed as a percentage: Yield = (P1 / P2) x 100; where P1: Weight of oil in g; P2: Weight of plant material used in g.

Chemical analysis of the essential oils: Gas chromatography coupled with Mass Spectrometry (GC-MS) analysis was used to reveal profiles of compounds contained in the essential oils. 1 µl of each oil was extracted with acetonitrile and analyzed using Agilent Technologies. The models of machine are as follows: Mass spectrum (5975C VLMSD), Injector (7683B Series) and GC (7890A). The capillary column was HP-5MS. The column has dimensions of: 30 cm in length, 0.320 mm internal diameter, and film thickness was 0.25 µm. Helium was used as the carrier gas. The GC oven temperature was set at 80°C for 2 min. The temperature increased steadily at 6°C per minutes to 240°C and was held for 6 min. The running time of each sample was 36 min. The peak of each chemical compound is expressed based on its retention time and balance [28].

2.3 Collection and Desinfection of Cowpea Seeds

Brown eyed variety of cowpea seed was collected in November 2018 in the local market of Moulvoudaye, 70 km away from Maroua in the Mayo Kani Division which is the predilection zone. The cowpea seeds were selected to exclude dirty and broken seeds [11]. To make sure that all developed stages have been killed, the un-infested seeds were kept in a deep freezer at -5°C temperature for 48 h. Sterilized seeds were removed and sundried for 2 h before sealing in the biodegradable plastic paper.

2.4 Rearing of *Callosobruchus maculatus*

Cowpea seeds infested by *C. maculatus* adults were gotten from permanent rearing at the cowpea section IRAD Maroua, from infested cowpea seeds. These seeds were sieved, adult *C. maculatus* were collected and introduced in a plastic bucket containing 5 kg of sterilized cowpea seeds. The bucket was covered with muslin cloth and fastened with rubber bands till the emergence of adult *C. maculatus* under the same laboratory condition (30.9 ± 2.13°C and 35 ± 5.02% RH) [11]. The fresh healthy adults obtained were used for further experiments from 24 to 48 h of aged.

2.5 Experimental Set Up for Insecticidal Activity

2.5.1 Contact toxicity of individual and mixed powder of *V. heterophylla* and *S. aromaticum* towards *C. maculatus* adults

In 500 mL capacity of glass jars (32 jars for treatment and 8 jars for control), 100 g of cowpea seeds were introduced followed by 0.5 g, 1 g, 1.5 g and 2 g of *V. heterophylla* and *S. aromaticum* powders taken individually. After a balanced combination (proportions of 50% LVh + 50% Sa; LVh: Leaves *V. heterophylla*, Sa: *S. aromaticum*), the same quantity of powder was weighed and introduced into another 16 jars containing 100 g of cowpea each. The various proportions correspond to 5 g /kg, 10 g /kg, 15 g /kg and 20 g /kg of cowpea. All the glass jars were stirred gently for 2 min to coat the seeds with the powders and 20 unsexed bruchids aged of 48 h at most, were introduced in the glass jars [31]. Then, these glass jars were covered with muslin cloths (diameter 0.5 mm) to prevent the exit of the bruchids and fastened with rubber stings. Control glass jars were free from treatment and contain 100 g of cowpea and 20 bruchids. For the positive control, a chemical insecticide powder called Malagrain was used. Daily observation was made up and dead weevils were counted. The glass jars were refrigerated for 2 min to incapacitate the live weevils before counting [32].

For all the experiments, repetitions have been done four times. For all the treatment, treated jars (Mo) were expressed according to Abbott's formula [33] in corrected mortality (Mc) taking into account the natural mortality observed in the control jars (Mt), Mc = [(Mo - Mt) / (100 - Mt) x 100].

2.5.2 Contact toxicity of individual and mixed oils of *V. heterophylla* and *S. aromaticum* towards *C. maculatus* adults

For each test, 1 mL of acetone solution was taken and added separately to the known doses of essential oils: 4 µL, 8 µL, 12 µL and 16 µL/mL for *V. heterophylla* and *S. aromaticum* (corresponding respectively to 80 µL/kg, 160 µL/kg, 240 µL/kg and 320 µL/kg). After dilution, 1 mL of each solution was measured using a micropipette and added to 50 g of seeds contained in glass jars of 500 mL capacity. The whole content of glass jar was mixed with the cowpea using glass rod in order to ensure uniform coating of the cowpea with oil. The treated cowpea was exposed to air for 30 min for complete evaporation of the acetone [28]. All glass jars were infested with 20 unsexed adult insects aged 2 days, and the glass jars were covered with muslin cloth and sealed with the rubber stings. For the control, 50 g of cowpea seeds were introduced in the glass jars and the seeds were free from treatment with the essential oil. After an equilibrium combination of the two essential oils, the same doses were taken and applied. The rest of the experiment was carried out as when these essential oils were taken individually. Four repetitions were made for all treatments with the essential oil. DD force, a chemical insecticide was used for positive control.

2.5.3 Repellent effect of essential oils on filter paper

This test was carried out according to the preferential zone method on filter paper described by McDonald et al. [34]. Thus, the filter paper disc of 10 cm in diameter was separated into two equal parts. An acetone solution was prepared with essential oils at different doses (4 µL, 8 µL, 12 µL and 16 µL/mL of acetone for *S. aromaticum* and *V. heterophylla*). Using a micropipette, 0.5 mL of each solution was measured and distributed evenly over one half of the filter paper and the other half received only acetone (0.5 mL). After 30 min, the solvent was evaporated. The two halves of the disc were glued with a cell tape and introduced into petri dishes of the same size [29,35]. All petri dishes were infested with 20 unsexed adult insects up to 48 h aged and then covered. Four repetitions were performed for each dose. The control petri dishes received only the solvent (0.5 mL). After two hours of treatment under laboratory conditions, the counting of the number of insects present on the half-disc treated with essential oils was carried out. The percentage repellency (PR) was calculated according to the formula described by Nerio et al. [35] as follows:

PR (%) = [(Nc - Nt) / (Nc + Nt)] x100

Nc: number of bruchids present on the half-disc treated only with acetone;
Nt: number of bruchids present on the half-disc treated with the different doses.

The average repellency percentage for each essential oil was calculated and assigned to one of the different repellant classes according to McDonald et al. [34], varying from 0 to V: class 0 (PR <0.1%), class I (PR = 0.1-20%), class II (PR = 20.1-40%), class III (PR = 40.1-60%), class IV (PR = 60.1-80%) and class V (PR = 80.1-100%).

LD_{50} values were calculated for the toxicity of powders and essential oils in cowpea seeds. Thus, the percentages of mortalities were transformed into probit units and the values obtained were correlated with the logarithm of the doses in order to obtain the lethal dose for 50% of the population of *C. maculatus* for each plant material tested [36].

2.5.4 Effect of inhalation of *V. heterophylla* and *S. aromaticum* oils on *C. maculatus* adults

The biocidal effect of essential oils after their inhalation by *C. maculatus* adults was evaluated according to the method described by Papachritos and Stamopoulos [37]. In glass jars with a capacity of 500 mL, 2 g of cotton was fixed by a thread in the center of the lids. The following doses of the essential oils: 0 µL, 10 µL, 20 µL, 30 µL and 40 µL corresponding to calculated concentrations of 0, 10, 20, 30 and 40 µL/liter of air volume were injected into the mass of cotton using a micropipette. 20 bruchids aged from 0 to 48 h at most were placed in the glass jars and sealed with an adhesive tape. At the same time, the control jar was made essentially with bruchids. Four repetitions were performed for each treatment. Dead bruchids were counted from the first day to the third day for each treatment.

3. RESULTS

3.1 Assessment of the Toxicity of Powder of *S. aromaticum* and *V. heterophylla* Applied Individually or in Combination

Table 1 shows the percentages of *C. maculatus* mortality with respect to individual powders. Those percentages vary with the doses applied and the time of exposure. 72 h after treatment, *S. aromaticum* powder induced maximum mortality (100%) at 0.5 g dose while *V. heterophylla* powder induced the same mortality rate at 2 g dose. The LD_{50} of *V. heterophylla* powders was higher 1.02 g/100 g while that of *S. aromaticum* was lower 0.40 g/100 g 24 h after treatment (Table 3). These LD_{50} show that *S. aromaticum* powder is more effective than that of *V. heterophylla* against *C. maculatus*. The effect of the combined powders is also shown in Table 1. 100% mortality induced at 1 g dose is observed during the same exposure period (72 h), a lower dose compared to the individual dose of *V. heterophylla* powder applied under the same conditions. The LD_{50} of the combined powder is 0.63 g/100 g (Table 3).

3.2 Toxicity of Individual and Combined Essential Oil of *S. aromaticum* and *V. heterophylla* Towards *C. maculatus* Adults

The individual effect of essential oils (EO) of *S. aromaticum* and *V. heterophylla* is shown in Table 2. Both EO were effective against *C. maculatus* on cowpea seeds and the death rate varied with the dose of oil used and exposure time after treatment. Maximum mortality (100%) was obtained with EO of *S. aromaticum* at the 8 µL/50 g doses. The EO of *V. heterophylla*, resulted in 90% at the 16 µL/50 g doses three days after treatment.

The LD_{50} of the EO of *V. heterophylla* was higher (8.65 µL/50 g) than that of *S. aromaticum* (4.08 µL/50 g), 24 h after treatment (Table 3). From the two essential oils tested, that of *S. aromaticum* was found to be more effective than that of *V.*

heterophylla at lower doses. The maximum mortalities obtained confirms the efficacy of the EO of *S. aromaticum* over that of *V. heterophylla* for the same targets such as adults of *C. maculatus*. After equilibrium mixture, 100% mortality was observed at 8 µL/50 g dose on the third day of exposure. Also, the quantity of essential oil used after combination is lower than the quantity used individually and the LD_{50} of the combined essential oil is 4.67 µL/50 g (Table 3).

3.3 Repellent Effect of the Two Essential Oils on Filter Paper

The result of the repellent activity of EO is displayed in Table 4. The two EO tested are repellent against *C. maculatus* adults even at the lowest dose (4 µL). After an exposure period of 2 h, the repellency percentage of the EO of *S. aromaticum* and *V. heterophylla* tested increased as the dose increased. This percentage varied from 75% at 4 µL dose to 100% at 12 µL dose for the EO of *S. aromaticum* while it varied from 42.5% at 4 µL dose to 87.5% at 16 µL dose for the EO of *V. heterophylla*. However, the EO of *S. aromaticum* had relatively higher repellant properties with an average repellency percentage PR = 89.37% compared to that of *V. heterophylla* (PR = 70.62%), although both EO were strongly repellent. By applying the classification method of McDonalds et al. [34], the average repulsion rates obtained allows us to observe and classify the EO of *S. aromaticum* in class V (very repellent) and the EO of *V. heterophylla* in class IV (repellent).

3.4 Effect of Inhalation of the Two Essential Oils

The death rate of *C. maculatus* adult increased with the EO doses applied and the exposure time (Table 5). The maximum (100%) mortality was recorded at 20 µL dose, after 48 h of exposure of *C. maculatus* indirectly towards the EO of *S. aromaticum* while the same rate was recorded at 40 µL dose after an exposure period of 72 h to the EO of *V. heterophylla*. Thus, the EO of *S. aromaticum* once again displayed a stronger biocidal effect by inhalation on adults of *C. maculatus* during a short period of exposure than the EO of *V. heterophylla*.

Table 1. Insecticidal activity of the individual and combined powders of *V. heterophylla* and *S. aromaticum* towards *C. maculates*

Tests	Plants	Quantity of powder used (g /100 g)	Exposure time/% of mortality		
			24 h	**48 h**	**72 h**
Individual	*S. aromaticum*	0.5	65 ± 2.3b	90 ± 0.81b	100 ± 0.0a
		1	97.5 ± 0.5a	100 ± 0.0a	100 ± 0.0a
		1.5	100 ± 0.0a	100 ± 0.0a	100 ± 0.0a
		2	100 ± 0.0a	100 ± 0.0a	100 ± 0.0a
		value F	35.18***	15.6**	7.9***
	V. heterophylla	0.5	22.5 ± 0.95d	42.5 ± 0.5b	65 ± 1.29c
		1	30 ± 0.81cd	47.5 ± 1.5ab	82.5 ± 1.7bc
		1.5	50 ± 0.81bc	55 ± 1.29ab	95 ± 0.57bc
		2	52.5 ± 0.95b	70 ± 1.4a	100 ± 0.0a
		value F	11.05***	21.8***	30.17***
Equilibrium combination	*S. aromaticum+ V. heterophylla*	0.5	32.5 ± 0.95d	45 ± 0.57ba	55 ± 0.57ba
		1	75 ± 1.3cd	100 ± 0.0a	100 ± 0.0a
		1.5	100 ± 0.0a	100 ± 0.0a	100 ± 0.0a
		2	100 ± 0.0a	100 ± 0.0a	100 ± 0.0a
		value F	34.81**	10.4**	8.49***
	Control	0	0 ± 0.0	0 ± 0.0	1 ± 0.2
	Positive control	0.25	100 ± 0.0	100 ± 0.0	100 ± 0.0

****p= .0001, **p= .001. For the same product, mean ± standard error followed by the same letter in the column did not differ significantly according to the Newman and Keuls test (p= .05). Each datum represents the mean of four replicate values*

Table 2. Insecticidal activity of the individual and combined essential oil of *V. heterophylla* and *S. aromaticum* towards *C. maculatus*

			Exposure time /% of mortality		
Tests	**Plants**	**Quantity of essential oil(µL/50g)**	**24 h**	**48 h**	**72 h**
Individual	*S. aromaticum*	4	45 ± 1.3cd	60 ± 1.4cd	78 ± 1.8bc
		8	60 ± 0.81b	82.5 ± 0.95ab	100 ± 0.0a
		12	100 ± 0.0a	100 ± 0.0a	100 ± 0.0a
		16	100 ± 0.0a	100 ± 0.0a	100 ± 0.0a
		value F	23.13***	21.36**	11.59***
	V. heterophylla	4	30 ± 0.81d	42.5 ± 0.84d	60 ± 1.4c
		8	47.5 ± 0.7cd	60 ± 0.81cd	72.5 ± 1.2bc
		12	55 ± 1.7bc	72.5 ± 0.95bc	82.5 ± 0.91ab
		16	70 ± 1.4b	80 ± 1.0abc	90 ± 0.86ab
		value F	24.14***	51.38***	15.8***
Equilibrium combination	*S. aromaticum* + *V. heterophylla*	4	52.5 ± 0.93cde	67.5 ± 0.95fg	85 ± 2.15cde
		8	65 ± 1.5bcd	85 ± 1.2de	100 ± 0.0a
		12	92.5 ± 0.5bc	100 ± 0.0cde	100 ± 0.0ab
		16	100 ± 0.0ab	100 ± 0.0bc	100 ± 0.0abc
		value F	16.72**	14.4**	28.49***
	Control	0	0 ± 0.0	0 ± 0.0	0 ± 0.0
	Positive control	5	100 ± 0.0	100 ± 0.0	100 ± 0.0

****p= .0001, **p= .001. For the same product, mean ± standard error followed by the same letter in the column did not differ significantly according to the Newman and Keuls test (p= .05). Each datum represents the mean of four replicates values*

Table 3. Action speed of powders and essential oils of *V. heterophylla* and *S. aromaticum* towards *C. maculatus* after 24 h

Powders (g /100 g) /Essential oil (µL /50 g)	**n**	**R^2**	**Regression equation y**	**LD_{50}**
Powder of *V. heterophylla*	4	0.9185	1.9757x + 4.9756	1.02
Powder of *S. aromaticum*	4	0.9614	4.8451x + 6.8843	0.40
ECP of Sa+Vh	4	0.9083	6.5328x +6.3101	0.63
EO of *V. heterophylla*	4	0.9717	1.6073x + 3.4854	8.75
EO of *S. aromaticum*	4	0.7816	5.2542x +1.7846	4.08
ECEO of Sa+Vh	4	0.7868	4.6546x +1.8605	4.67

ECP: Equilibruim Combination of Powder; ECEO: Equilibruim Combination of Essential Oil; EO: Essential Oil; Sa: Syzygium aromaticum; Vh: Vepris heterophylla

Table 4. Repellence of essential oils of *V. heterophylla* and *S. aromaticum* towards *C. maculatus* adults

Plants	**Doses of essential oil (µL)**	**Index of repulsion (%)**	**Probability value**	**Repulsive classes**	**Degree of repulsion**
S. aromaticum	4	75 ± 1.73	0.0001***	IV	repulsive
	8	85 ± 2.21		V	very repulsive
	12	100 ± 0.0		V	very repulsive
	16	100 ± 0.0		V	very repulsive
	Mean	89.37 ± 0.26		V	very repulsive
V. heterophylla	4	42.5 ± 0.95	0.0001***	III	moderable repulsive
	8	67.5 ± 0.95		IV	repulsive
	12	85 ± 1.0		V	very repulsive
	16	87.5 ± 0.72		V	very repulsive
	Mean	70.62 ± 0.90		IV	repulsive

Table 5. Mortalities of *C. maculatus* adults induced after inhalation of essential oils at different doses

			Exposure time /% of mortality		
Tests	**Plants**	**Quantity of essential oil (µL)**	**24 h**	**48 h**	**72 h**
Individual	*S. aromaticum*	0	0	0	0
		10	25 ± 1.29ab	37.05 ± 1.025b	57.5 ± 0.5ab
		20	65 ± 1.73b	100 ± 0.0a	100 ± 0.0a
		30	100 ± 0.0a	100 ± 0.0a	100 ± 0.0a
		40	100 ± 0.0a	100 ± 0.0a	100 ± 0.0a
		value F	36**	15***	13***
	V. heterophylla	0	0	0	0
		10	15 ± 1.0cd	27.5 ± 0.95ab	35 ± 1.29ba
		20	26.25 ± 0.5d	40 ± 0.81ab	52.5 ± 0.95a
		30	35 ± 1.29d	55 ± 0.57b	70 ± 1.82ab
		40	47.5 ± 0.95d	72.5 ± 1.71bc	100 ± 0.0a
		value F	25***	41***	35***
Equilibrium combination	*S. aromaticum* + *V. heterophylla*	0	0	0	0
		10	25 ± 1.29bcd	30 ± 2.16ba	47.5 ± 1.89ac
		20	40 ± 1.15bc	70 ± 1.15b	95 ± 1.0ac
		30	80 ± 0.81cd	100 ± 0.0a	100 ± 0.0a
		40	100 ± 0.0a	100 ± 0.0a	100 ± 0.0a
		Value F	54***	30***	42**

****p= .0001, **p= .001. For the same product, mean ± standard error followed by the same letter in the column did not differ significantly according to the Newman and Keuls test (p= .05). Each datum represents the mean of four replicates values*

Results show that the powder and EO of *S. aromaticum* are more effective (LD_{50}= 0.40 g/100 g for the powder and 4.08 µL/50 g for the EO) than those of *V. heterophylla* (LD_{50} = 1.02 g/100 g for powder and 8.75 µL/50 g for EO).

3.5 Chemical Analysis of the Essential Oil and Yield

The yield of EO obtained by hydrodistillation was not the same for the leaves of *V. heterophylla* and the spice *S. aromaticum*. The production of essential oil of *S. aromaticum* is more important than that of the leaves of *V. heterophylla* (Table 6).

Figs. 1 and 2 represent the chromatograms of *S. aromaticum* and *V. heterophylla* respectively. From these chromatograms, the mass spectrometry was done and its analysis showed that, both EO merely contain monoterpenes and sesquiterpenes components. The essential oil of *V. heterophylla* contains mainly: Spathulenol (23.66%), Caryophyllene oxide (16.46%), 6-Isopropenyl-4 (16.30%) and β - Caryophyllene (12.21%) (Table 7). For the essential oil of *S. aromaticum* the most active compound is Eugenol (83.40%) (Table 8).

4. DISCUSSION

The treatment carried out with the powders of *V. heterophylla* and *S. aromaticum* have adulcidal effects against *C. maculatus* at different doses. Mortalities observed could probably be due to the action of certain major compounds such as

Table 6. Production of essential oil in percentages

Plants	Quantities of fresh leaves/flower buds (g)	Quantities of oil (g)	Yield (%)
V. heterophylla	1000	09.47	0.95
S. aromaticum	1000	15.4	1.54

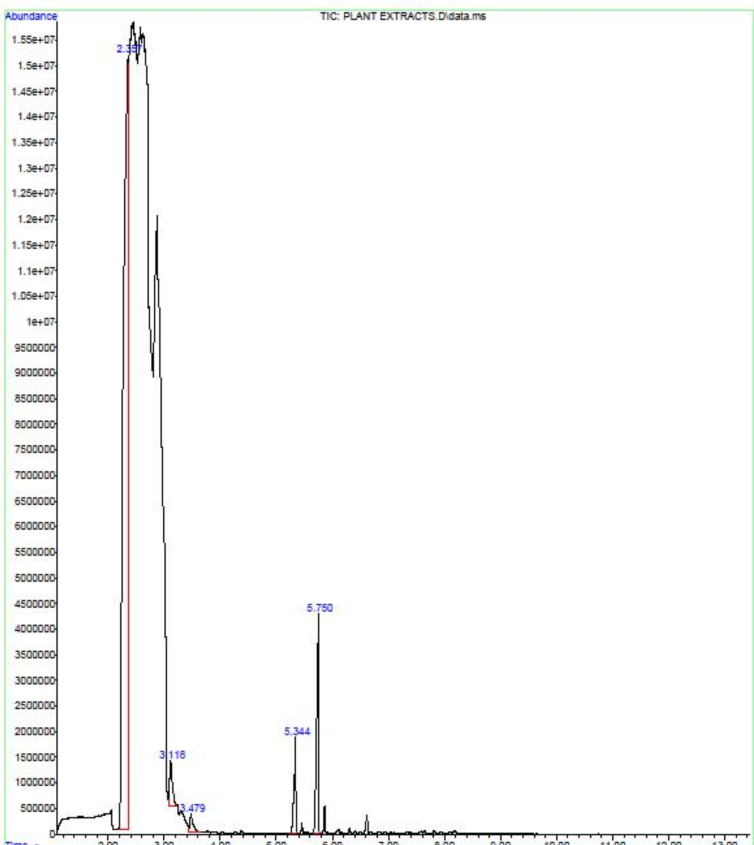

Fig. 1. Chromatogram of *S. aromaticum*

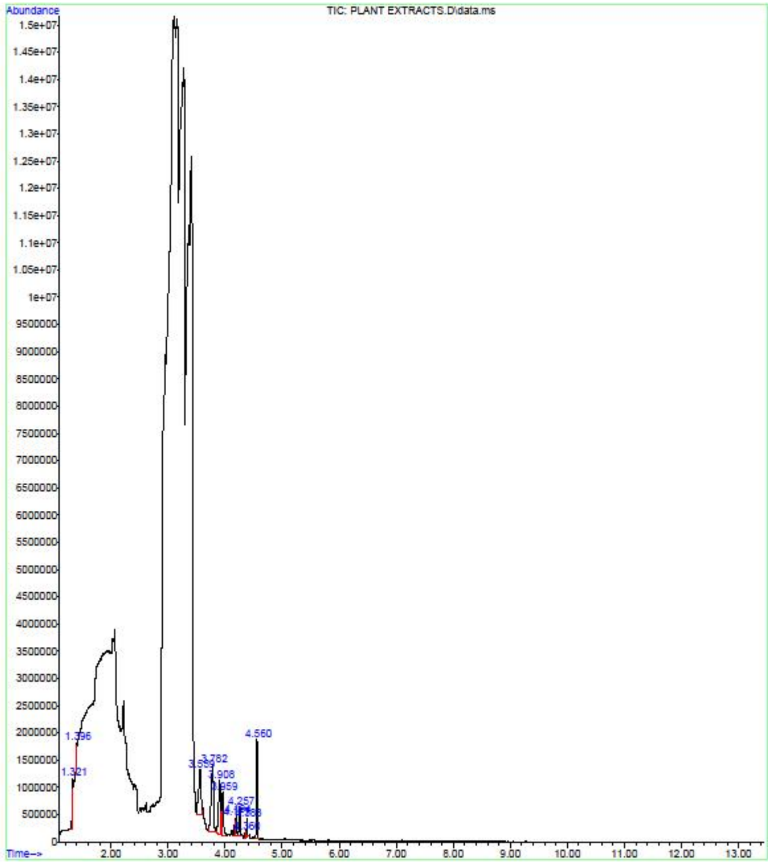

Fig. 2. Chromatogram of *V. heterophylla*

Table 7. Major compounds elucidated from GC-MS analysis of fractioned acetonitrile essential oil of *V. heterophylla*

Compounds	% Area	RT(min)	molecular Formula	CAS
Sabinene	4.90	1.321	$C_{10}H_{16}$	003387-41-5
α-Pinene	3.60	1.396	$C_{10}H_{18}$	000498-15-7
Caryophyllene oxide	16.46	3.559	$C_{15}H_{24}O$	001139-30-6
Spathulenol	23.66	3.782	$C_{15}H_{24}O$	006750-60-3
6-Isopropenyl-4	16.30	3.908	$C_{15}H_{24}O$	1000189-10-2
Alpha elemol	8.96	3.959	$C_{15}H_{26}O$	000639-99-6
7-Hydroxyfarnesen	3.04	4.177	$C_{15}H_{24}O$	1000374-20-4
Nerolidol 2	2.54	4.194	$C_{15}H_{26}O$	1000285-43-6
Nerolidol	5.41	4.257	$C_{15}H_{26}O$	026560-14-5
cis-Z-.alpha.-Bisabolene	1.01	4.360	$C_{15}H_{24}O$	1000131-71-2
hydroxycitronellal	1.91	4.383	$C_{10}H_{20}O_2$	000107-75-5
β - Caryophyllene	12.21	4.560	$C_{15}H_{24}$	000118-65-0

CAS: Chemical Abstract Service; RT: Retention Time

saponins which are found in these powders and which could diffuse easily through the cell membrane at the origin of the Knock Down effects on *C. maculatus*. The powder of *S. aromaticum* reveals a very interesting insecticidal activity with respect to *C. maculatus* whose females are most active during the first three days of their imaginal state [38]. Katunku and Ukwela [39] reported that saponins affect the respiratory system of insects and causes expiratory effect due to their deterrent action on them. The action of powders on this insect could be the same as the action of inert particle or dust in *C. maculatus*. In this respect, Roghaiyeh et al.

Table 8. Major compounds elucidated from GC-MS analysis of fractioned acetonitrile essential oils of *S. aromaticum*

Compounds	% Area	RT(min)	Molecular Formula	CAS
Eugenol	83.40	2.357	$C_{10}H_{12}O_2$	000097-53-0
Caryophyllene oxide	2.79	3.118	$C_{15}H_{24}O$	001139-30-6
Acrolein	1.18	3.479	C_3H_4O	000458-36-6
Estragole	3.55	5.344	$C_{10}H_{12}O$	000140-67-0
Anethole	9.07	5.750	$C_{10}H_{12}O$	000104-46-1

CAS: Chemical Abstract Service; RT: Retention Time

[40] reported that, mixing ash or kaolin on cowpea cause more rapid water loss and increase mortality due to their abrasive and desiccant properties. Results are similar to those of Tapondjou et al. [29] who, by evaluating the insecticidal effect of the powders of *C. ambrosioides* and *E. saligna* leaves against *C. maculatus*, showed that after four days, the highest doses of powder (0.4 g in the case of *C. ambrosioides* and 10 g in the case of *E. saligna*) induced 92% and 57% mortality respectively. Also, the results showed that, maximum mortality (100%) was obtained at low doses (1 g) after a balanced combination of the two powders. This suggests that, *V. heterophylla* powder's effects being low at the 0.5 g dose becomes important after combination with the powders of another plant family. From these results, it can be suggested that the combination of the powders would increase the insecticidal compounds and the consequence would be the occurrence of high mortalities due to the additive effects of these compounds in a limited time [41]. Singh and Allen [42] showed that not only the combination of powders increases the majority compounds, but that the minor compounds in contact with the more active others, were able to significantly increase their activity. This suggests the need to make combinations between different species of plants or between plants and spices. Mortalities obtained are higher than those of Musa et al. [43] who reported that the combination of powders from the leaves of *V. amygdalina* and *O. gratissimum* in equal proportions (50:50) induced 60% mortality with respect to *C. maculatus* after 72 h of exposure. Therefore, the powders tested in this work were more effective than the later and therefore, the combination of plant powders and species or their essential oils could be another alternative method to reduce the overexploitation of most insecticidal plant.

The essential oils tested are more toxic to *C. maculatus* than the powders of the same plants since they induce maximum mortality within 48 h of exposure. This action of essential oils could be due to the high concentration of active components such as eugenol in *S. aromaticum*, Spathulenol, Beta-Caryophyllene oxide and 6-Isopropenyl-4 in *V. heterophylla* essential oil. Most of these compounds are neurotoxic compounds acting on different targets depending on their chemical nature [44,25,28], although in powders, the major or minor components are dispersed. The essential oil may also have disrupted the normal respiratory activity of the insect and this may lead to asphyxiation and death of the insect. The maximum mortalities obtained were 100% (for *S. aromaticum*) and 90% (for *V. heterophylla*) at the respective doses 8 µL /50 g and 16 µL/50 g. Likewise, Righi-Assia [45] demonstrated that the essential oil of *T. vulgaris* induced a total mortality of *C. chinensis* after 72 h of exposure to the 10 µL dose. Also, Kouninki et al. [46] reported that at a dose of 1% per 100 g of corn seeds, the essential oils of *X. aethiopica* and *H. spicigera* induced 96% and 70% mortality respectively against adults of *S. zeamais* by direct contact and fumigation after 24 h of exposure.

The EO of the two plants tested showed significant variations in their repellant activities compared to the 0% obtained in the control jars. It is important to note that the EO of *S. aromaticum* which has been shown to be the most toxic is also the most repellant. These results are in agreement with those of Papachristos and Stamopoulos [37]. They reported that, among the EO of 13 aromatic plants, five of them (*M. viridis*, *E. globulus*, *M. microphylla*, *R. officinalis* and *L. hybrida*) had effective repellant effects than the EO of three other *T. orientalis*, *C. sinensis* and *P. terebinthus*. However, they had proven that the most toxic EO exhibited at the same time repellent and inhibitory effects on the reproduction of pest insects. The mean percentage of repulsion (89.37%) obtained by the EO of *S. aromaticum*, member of the Myrtaceae family is much higher than that obtained by the EO of *V. heterophylla*

(70.62%) which is member of the Rutaceae family. Similar results were obtained by Hamdani [47]. His work showed that the EO of four Rutaceae (sour orange, lemon, grapefruit and orange) against adults of *A*. *obtectus* have average repellant effects of 70%, 50%, 42.5% and 17.5% respectively compared to the repellant effects induced by plants of the Myrtaceae family. Likewise, Tapondjou et al. [29] demonstrated the high repellent properties of EO from *C*. *ambrosioides* (PR = 89%) and *E*. *saligna* (PR = 71%) for *C*. *maculatus*. These EO could retain their repellency two months after the period of experimentation. The results are also similar to those obtained by Kafle et al. [48]. They reported that *S*. *aromaticum* EO were rich in eugenol, eugenol acetate and beta-caryophyllene; these compounds had very significant repellant activity against *Solenopsis invicta* with the most active being eugenol. The work of Nerio et al. [49] showed that the EO of aromatic plants emit volatile substances such as terpenes which act at a distance and constitute a barrier, preventing insects from carrying out their activity on the surface of the host or seeds.

Inhalation tests showed maximum mortality depending on the volume of air reserved. The EO of *S*. *aromaticum* exhibited maximum inhalation toxicity with respect to *C*. *maculatus* from the dose of 10 µL / liter of air volume followed by that of *V*. *heterophylla* which is 40 µL / liter of air. This may be due to the presence of majority component such as monoterpenic and sesquiterpenes derivatives in the essential oils tested which are volatile components. The result is similar to that of Regnault-Roger [50] who demonstrated that, the odor of EO can act indirectly on the antenna of *C*. *maculatus* and change their behavioral system.

This work reveals and highlights the potential insecticide effect of powders and essential oils of *S*. *aromaticum* and *V*. *heterophylla* in the fight against *C*. *maculatus*. Indeed, the potential of plants for seed preservation against various insects have been the subject of several studies [51,10,5]. It would be important that to effectively control pests of stored foods, farmers should look for plants and spices that can exhibit both biocidal, inhalation and repellent properties such as *S*. *aromaticum*. The yield of EO obtained (0.95% for *V*. *heterophylla* and 1.54% for *S*. *aromaticum*) was very high and can be exploited at the industrial level, compared to the yield (0.84%) obtained by essential oils of *Mentha piperita* which was qualified as very important on an industrial scale by Mohamed et al. [30].

5. CONCLUSION

This study reveals that *S*. *aromaticum* and *V*. *heterophylla* powders and their essential oils have a lethal effect on adults *C*. *maculatus*. However, the powder and essential oil of *S*. *aromaticum* are the most active due to the maximum mortality induced within 72 h of exposure. The GC-MS revealed that in the EO of *S*. *aromaticum* the major component is Eugenol (83.40%) and that of *V*. *heterophylla* is Spathulenol (23.66%). Equilibrium combination of these powders on one hand and essential oils on the other hand induced maximum mortalities but at smaller doses. Thus, to limit the overexploitation of efficient plants, combination methods should be recommended to farmers for the treatment of cowpeas in stock. The use of powders from the leaves of *V*. *heterophylla* and flower buds of *S*. *aromaticum* to protect cowpeas against severe attack of *C*. *maculatus* may represent an additional green alternative solution for smallholders' farmers. Given that *S*. *aromaticum* is an expensive spice, it will be interesting to carry out an economic study of this plant due to its high efficacy at low dose and to focus on a final formulation like a phytopesticide.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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