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Research Article

Phytotoxicity of Some Essential Oil Components to Cowpea (*Vigna unguiculata* (L.) Walp.) Seeds

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Abstract

Plant essential oils and their components are used to protect stored grains against insect pests. Cereals and grain legumes are stored for future use as food and seed for further propagation. Phytotoxicity of four essential oil components, carvacrol, 1-8-cineole, eugenol, and (-)-menthone, to cowpea *Vigna unguiculata* (L.) Walp was tested at 10 and 20 µl/L doses. Effects of these oil components on seed germination and seedling growth parameters (i.e., shoot length, number of leaves, moisture content, root-shoot ratio, root length, root volume, root surface area and length of root hairs) were determined at 1, 2, 3, and 6 months after fumigation. Differences in germination and seedling growth between fumigated seeds and unfumigated seeds (control) were used as indices of phytotoxicity of the oil components. Generally, phytotoxicity of the oil components to cowpea seeds was dependent on dose, oil components, and storage period. Seed germination and seedling growth were negatively affected by carvacrol, 1-8-cineole, and eugenol at 2 and 3 months after treatment. 1-8-cineole was the most toxic while (-) - menthone was the least toxic component to cowpea seeds, irrespective of dose and storage period. Higher germination and seedlings growth of 1-8-cineole-fumigated seeds were obtained at 6 months compared to 2 and 3 months, suggesting possible biodegradation or degassing processes. This study showed that carvacrol, 1-8-cineole, and eugenol reduced cowpea germination and seedling growth. In addition, germination of seeds fumigated with essential oil components and presumably natural essential oils, does not imply that the germinated seeds are viable.

INTRODUCTION

Cow peas, *Vigna unguiculata* (L.) Walp are major human food legume crop grown in the semi-arid tropics regions of Africa, Asia, Europe, United States, and Central and South America [1]. The high protein content of cowpea seeds makes them an important source of protein in many developing regions [1]. Insect pests are a serious constraint to farmers in developing countries during the storage of their crops after harvest. Cowpea suffers the highest levels of postharvest losses of any food grain crop in the tropics [2]. A 70% loss could occur in stored cowpeas within three months if no prophylactic measures are applied [3-5].

Aside from toxicity to humans, non-target animals, and the environment, the majority of farmers in developing countries cannot afford the cost of using synthetic chemical pesticides [6]. These problems have shifted attention of researchers to exploring botanicals as a part of ecologically tolerable measures to solve global problems of environmental pollution and provide good quality foods for consumers [6-8].

Many studies have demonstrated contact and fumigant toxicities of essential oils of aromatic plants and their components to several species of stored-products insects at different life stages [9-15]. Aromatic plants are rich in active compounds and are considered a primary source of potential allele chemicals [16]. Many aromatic plants are toxic; a small quantity of which may cause serious reductions in plant viability [17-20]. Cowpea seeds are stored either for consumption or planting. Seed quality sets limits on production, hence is a major factor in the success of farmers. Therefore, it is important to study effects of plant oils

and their components on viability of oil-protected seeds.

Seed viability entails germination (emergence of the radicle through the seed coat) and development of seedling structures that indicate ability of the seedling to develop further into a satisfactory plant under favorable conditions [21,22]. Most previous studies that tested phytotoxicity of botanical oils to seeds used whole essential oils. They also limited their investigations to germination tests within a few days after treatments. While some researchers reported no significant negative effect on germination [9,23,24], others have demonstrated strong allelopathic effects of plant oils on seed germination [25-27]. The disparities in allelopathic activities of plant oils were attributed to qualitative and quantitative variations in the components of the oils tested [27]. Most of these studies were conducted with whole essential oils, topically admixed with the seeds, or added to water or soil to plant the seeds. Many of the oil-treated seeds were not stored for appreciable periods after treatment to assess effect of storage periods on phytotoxicity of the oils tested. Presently, we are not aware of any detailed studies on effects of fumigation with botanical oil components on viability of cowpea seeds.

In this study, the effects of fumigation with four essential oil components of aromatic plants: carvacrol, 1-8-cineole, eugenol, and (-)-menthone on viability of cowpea seeds stored for up to six months after fumigation were tested. These essential oil components have been reported to be toxic to many stored-products insects [8,28-30]. The results of this study will provide information on suitability of these essential oil components for protecting cowpea seeds in storage.

MATERIALS AND METHODS

Cowpea seeds

Cowpea (cultivar California black-eye #0094) seeds were purchased from a local grocery store in Auburn, AL, USA. The seeds were kept in polythene bags, and sealed until they were used for the bioassays.

Fumigation with essential oil components

The four essential oil components, 1-8-cineole (99%), carvacrol (98%), eugenol (99%) and (-)-menthone (90%), tested in the study were obtained from Sigma-Aldrich (St. Louis, MO). These four monoterpenes were selected because their pure form, and many essential oil blends or extracts that have them as major components have been reported to be toxic to some invertebrate pests of stored products [8,13]. In addition, the selected monoterpenes were the most toxic to the cowpea weevil, *Callosobruchus maculatus* (F.) in our earlier study [15]. The oil components were tested at 10 and 20 µl/L air. These were doses at which 90-100% of beetles died within 24 h in previous studies on fumigation toxicity of essential oil components against *C. maculatus* [15].

Fumigation of cowpea seeds was accomplished in 1 L wide-mouthed Ball® Glass Mason jars containing 50 g (~170-180) of cowpea seeds. Two doses of essential oil, 10 µl/L and 20 µl/L were tested. Each dose was replicated four times for each of the storage period (1, 2, 3, and 6 months). The oil component was spread evenly on a 9cm diameter **Whatman**™ no.1 filter paper (Fisher Scientific, 1004-090 Grade 4) glued to the underside of the lid of the jar as described previously [15]. Jars with no filter papers served as the controls. The fumigation lasted for 5 days, after which the lid and filter paper that served as carrier of essential oil components in each jar was removed and replaced with a screen lid (16 x 16 mm) held in place with the band of the lid. The fumigated seeds were stored in the laboratory under normal storage conditions of 12:12 light and dark regime at 26 ± 2°C, and 65 ± 5% RH. Viability tests were conducted on the fumigated grains at 1, 2, 3 and 6 months after fumigation.

Seed planting

Fumigated seeds were planted in Sunshine potting mixture #8, consisting of 70-80% Canadian sphagnum grower grade peat moss, coarse grade perlite, coarse grade vermiculite, dolomitic limestone for pH adjustment, gypsum and a wetting agent (Sun Gro Horticulture, WA, USA) using Anderson pots AB 410, (4 in. "W, 10 in. "H and 142.8 cm^3 "V; Stewe and Son, Inc. Tangent, OR, USA). The dimension of the pot was selected to allow for roots to grow freely. Ten seeds (~ 0.3-0.35g) were selected randomly from each treatment for planting. Selected seeds were pretreated with 70% ethanol for 1 min and rinsed with sterile water twice, followed by 1% sodium hypochlorite for 30 seconds and then rinsed with sterile water 5 times to disinfect the seeds before planting [32]. Each dose was replicated 4 times. A replicate consisted of five pots; a total of twenty pots of plants per dose for each storage period. Naturally, physiology of each seed, like other living thing varies hence, the number of pots (5) per replicates was to minimize error and prevent wrong conclusions in the experiments. The viability test was conducted in a Percival Scientific incubator at optimum conditions for growing cowpea, 28 ± 2°C 12 hours (light) and 22 ± 2°C 12 hours (daily) at 65 ± 5% RH [22,33]. A 12:12 light and dark regime was maintained using 40 Watt fluorescent tubes (122 cm) positioned at each of the four corners of the incubator to provide white light. Seeds were planted 2.5 cm deep in soil [33] and supplied 50 ml of water every other day or as required. The treated seeds were planted and observed ten days for germination and seedling growth [22]. The procedure was used for seeds of all treatments and storage periods.

Seed germination and primary growth assessment

Percentage of seed germination was recorded 5-7 days after planting. Seeds were considered germinated with the emergence of radicles through the seed coat [34]. Effect of treatments on primary growth was determined using basic growth parameters: length of aerial part of seedling, number of seed leaves, moisture content, root-shoot ratio, root volume, root surface area, total root length and length of root hairs (root ≤ 0.5 mm Ø). Destructive analysis was done on the 11^{th} day after each set of plantings.

Length of the aerial part of the shoot was measured (cm) from the soil line to the tip of topmost leaf on each seedling. Number of leaves was counted. Roots were held under slow-running tap water to remove soil. Paper towel was used to blot wash water on each seedling before recording wet weight. Roots were severed from the shoot at the soil line and analyzed using a Win RHIZO 2009 *a, b, c*, scanner (Regent Instruments Canada Inc.) The roots were scanned at x6 to x8 to obtain total root length, length of

root hairs (≤ 0.5 mm Ø), root volume (cm^3), and root surface area (cm^2). Roots and shoots were then oven-dried at 68°C for 48 h [35]. The dry weight was used to calculate root-shoot ratio.

Data analysis

Data were angular-transformed for normality, and analyzed using One-way Analysis of Variance (ANOVA). Tukey's HSD test was used to separate the means. Analysis was done in SPSS version 16.0. A significance of α=0.05 was used to estimate differences.

RESULTS

Germination

Seed germination was oil component, dose and storage period dependent (Table 1). Percentage germination ranged between 80 and 100% at 10 µl/L dose and 60 to 95% at 20 µl/L dose. Generally, seed germination declined with increasing dose and storage period. Seed germination declined at 2 and 3 month and increased a little at 6 month except for eugenol-treated seeds at 10 µl/L dose (Table 1). The reduction in germination was apparently greater in 1-8-cineole and eugenol-treated seeds at 10 and 20 µl/L doses (Table 1). All planted seeds (100%) in the untreated germinated. There were no significant differences in germination percentages between seeds fumigated with 10 µl/L of the oil monoterpenes and the control at 1 month ($F_{4, 15}$ = 2.400, P = 0.096) and 6 months ($F_{4, 15}$ = 1.636, P = 0.217) after treatment. Seeds fumigated with (-) - menthone had the highest germination percentages at both doses: 10 µl/L(90 – 100%) and 20 µl/L (80 – 95%) (Table 1).

Seedling assessment

Shoot assessment: The greatest values of the parameters assessed in cowpea seedlings were obtained in the controls (Tables 2-5 and Figures 1-4). Seedlings of (-)-menthone-treated seeds had the greatest shoot and root qualities among the seedlings of oil-treated seeds at 10 and 20 µl/L doses and at 1 to 3 and 6 month observation periods (Tables 2-5). Carvacrol and eugenol-treated seeds consistently had the lowest values in most of the shoot features assessed (Tables 2-4). Phytotoxic effect of the monoterpenes on seedling shoot increased with increasing dose. The greatest seedling length (Table 2), number of leaves (Table 3) and moisture contents (Table 4) in seedlings treated with 10 µl/L oil components were 20.8 cm, 7.7 and 3.2 g, respectively while at 20 µl/L the values were 18.3 cm, 7.6 and 2.8 g, respectively. Carvacrol, 1-8-cineole and eugenol reduced root:shoot ratio of seedlings (Table 5). The greatest root: shoot ratios in seedlings of all oil-treated seeds at 10 (0.204) and 20 µl/L (0.201) were recorded in seedlings of (-)-menthone-treated seeds. The least root: shoot ratio (0.1) was recorded in seedlings of 1-8-cineole-treated seeds except at 6 month in 10 µl/L treatment (Table 5). The root: shoot ratio of seedlings in treated seeds differed significantly from the controls (P < 0.05) (Table 5). Tukey' HSD test revealed no significant difference between reduction in root:shoot ratio due to carvacrol, 1-8-cineole and eugenol treatments at 2 (P = 0.329) and 3 (P = 0.204) month in 20 µl/L dose levels (Table 5). There were significant differences between seedlings of monoterpene-treated and untreated cowpeas in most of the above-mentioned parameters at the two doses tested and exposure periods (P = 0.001) (Tables 2-5).

Root assessment: In general, the monoterpenes reduced root length (Figure 1), surface area (Figure 2), volume (Figure 3) and length of root hairs (Figure 4) of seedlings from 1 to 6 month at 10 and 20 µl/L. Carvacrol, 1-8-cineole and eugenol greatly reduced root qualities. (-)-menthone was least toxic among the oil components while 1-8-cineole was most toxic to cowpea seeds as revealed in most of the root features assessed in this study (Figures 1-4). Root length in all treatments declined with increasing storage period (Figure 1). Seedlings of (-)-menthone-treated seeds had the greatest root length which declined from 288cm at 1 month to 264cm at 6 month in 10 µl/L dose and from 271cm at 1 month to 226cm at 6 month in 20 µl/L dose (Figure 1). The least root length was recorded in 1-8-cineole in all treatments at 1-3 month storage periods but it increased a little at 6 month in 10 µl/L dose (Figure 1). Root surface area in 1-8-cineole-treated seedlings was significantly (P < 0.001) lower than the surface area obtained in seedlings of carvacrol and eugenol-fumigated seeds at 10 and 20 µl/L during 1-6 month storage periods (Figure 2). Root surface area reduction in fumigated seeds was greater in fumigated seeds that were stored for 2 and 3 month (Figure 2).

Table 1: Germination percentage of fumigated cowpea seeds (Mean ± SE).

		Storage period (Month)			
Dose (µl)	**Oil component**	**1**	**2**	**3**	**6**
	Carvacrol	95.0 ± 2.5^{a}	90.0 ± 3.5^{ab}	80.0 ± 5.0^{a}	90.0 ± 5.0^{a}
	1-8-Cineole	90.0 ± 5.0^{a}	85.0 ± 2.5^{a}	80.0 ± 3.5^{a}	90.0 ± 3.5^{a}
10	Eugenol	100.0 ± 0.0^{a}	85.0 ± 2.5^{a}	85.0 ± 2.5^{a}	85.0 ± 4.3^{a}
	(-)Menthone	100.0 ± 0.0^{a}	100.0 ± 0.0^{b}	95.0 ± 4.3^{ab}	90.0 ± 3.5^{a}
	*Control	100.0 ± 0.0^{a}	100.0 ± 0.0^{b}	100.0 ± 0.0^{b}	100.0 ± 0.0^{a}
	Carvacrol	85.0 ± 4.3^{ab}	65.0 ± 2.5^{a}	60.0 ± .0^{a}	85.0 ± 4.3^{ab}
	1-8-Cineole	80.0 ± 3.5^{a}	60.0 ± 3.5^{a}	60.0 ± 0.0^{a}	80.0 ± 3.5^{a}
20	Eugenol	80.0 ± 3.5^{a}	65. ± 2.5^{a}	60.0 ± 0.0^{a}	80.0 ± 3.5^{a}
	(-)Menthone	95.0 ± 4.3^{ab}	85.0 ± 2.5^{b}	80.0 ± 3.5^{b}	85.0 ± 2.5^{ab}
	*Control	100.0 ± 0.0^{b}	100.0 ± 0.0^{b}	100.0 ± 0.0^{c}	100.0 ± 0.0^{b}

Each value is a mean of four replicates. For each dose, means within a column (month) followed by different letter (s) are significantly different (P < 0.05; ANOVA, Tukey's HSD test).
*All seeds planted in controls germinated.

Table 2: Above ground length (cm) of seedlings of fumigated cowpea seeds (Mean ± SE).

		Storage period (Month)			
Dose (µl)	**Oil component**	**1**	**2**	**3**	**6**
	Carvacrol	19.09 ± 0.6^{a}	16.95 ± 0.2^{a}	16.86 ± 0.1^{a}	16.25 ± 0.8^{a}
	1-8-Cineole	19.80 ± 1.5^{a}	17.30 ± 0.3^{a}	16.68 ± 0.4^{a}	18.89 ± 0.2^{ab}
10	Eugenol	19.82 ± 0.2^{a}	18.23 ± 0.5^{a}	17.79 ± 0.7^{a}	16.60 ± 1.1^{a}
	(-)Menthone	20.55 ± 0.4^{a}	20.75 ± 0.2^{b}	18.82 ± 0.9^{a}	18.47 ± 0.8^{ab}
	Control	24.55 ± 0.4^{b}	21.58 ± 0.4^{b}	21.73 ± 0.2^{b}	21.30 ± 0.3^{b}
	Carvacrol	17.15 ± 1.1^{a}	16.40 ± 1.3^{a}	17.19 ± 0.5^{a}	17.48 ± 0.5^{a}
	1-8-Cineole	18.20 ± 0.2^{a}	15.45 ± 0.3^{a}	17.95 ± 0.6^{a}	18.30 ± 0.6^{ab}
20	Eugenol	16.01 ± 0.6^{a}	16.60 ± 0.5^{a}	16.88 ± 0.3^{a}	18.10 ± 0.2^{a}
	(-)Menthone	17.95 ± 0.6^{a}	17.25 ± 0.3^{a}	17.13 ± 0.6^{a}	18.03 ± 0.2^{a}
	Control	21.83 ± 0.4^{b}	21.20 ± 0.3^{b}	22.84 ± 0.3^{b}	21.33±0.2^{b}

Each value is a mean of four replicates. For each dose, means within a column (month) followed by different letter (s) are significantly different (P<0.05; ANOVA, Tukey's HSD test).

Table 3: Number of leaves on seedlings of fumigated cowpea seeds (Mean ± SE).

		Storage period (Month)			
Dose (µl)	**Oil component**	**1**	**2**	**3**	**6**
	Carvacrol	6.50 ± 0.3^{a}	6.05 ± 0.9^{a}	6.60 ± 0.3^{ab}	6.00 ± 0.3^{a}
	1-8-Cineole	6.75 ± 0.6^{a}	6.75 ± 0.6^{a}	7.55 ± 0.7^{b}	6.45 ± 0.2^{a}
10	Eugenol	6.91± 0.4^{a}	5.70 ± 0.2^{a}	5.50 ± 0.2^{a}	5.25 ± 0.4^{a}
	(-)-Menthone	7.20 ± 0.2^{a}	7.65 ± 0.3^{ab}	7.50 ± 0.2^{ab}	6.30 ± 0.2^{a}
	Control	10.35 ± 0.1^{b}	10.05 ± 0.3^{b}	10.25 ± 0.4^{c}	9.25 ± 0.1^{b}
	Carvacrol	5.70 ± 0.2^{a}	6.20 ± 0.3^{ab}	6.25 ± 0.4^{a}	5.90 ± 0.1^{ab}
	1-8-Cineole	6.40 ± 0.1^{ab}	5.70 ± 0.2^{ab}	6.40 ± 0.4^{a}	6.55 ± 0.2^{b}
20	Eugenol	5.15 ± 0.1^{a}	5.55 ± 0.2^{a}	5.20 ± 0.3^{a}	5.05 ± 0.4^{a}
	(-)-Menthone	7.55 ± 0.5^{b}	7.20 ± 0.6^{b}	6.50 ± 0.1^{a}	6.13 ± 0.1^{ab}
	Control	10.30 ± 0.2^{c}	10.20 ± 0.2^{c}	9.85 ± 0.3^{b}	9.30 ± 0.1^{c}

Each value is a mean of four replicates. For each dose, means within a column (month) followed by different letter (s) are significantly different (P<0.05; ANOVA, Tukey's HSD test).

Table 4: Moisture content (g) in seedlings of fumigated cowpea seeds (Mean ± SE).

		Storage period (Month)			
Dose (µl)	**Oil component**	**1**	**2**	**3**	**6**
	Carvacrol	2.627 ± 0.2^{bc}	2.097 ± 0.1^{a}	1.771 ± 0.2^{a}	1.370 ± 0.2^{a}
	1-8-Cineole	2.014 ± 0.0^{a}	1.957 ± 0.0^{a}	1.510 ± 0.2^{a}	1.553 ± 0.1^{ab}
10	Eugenol	2.585 ± 0.2^{ab}	1.832 ± 0.2^{a}	1.531 ± 0.1^{a}	1.293 ± 0.1^{a}
	(-)-Menthone	3.199 ± 0.0^{cd}	2.896 ± 0.2^{b}	2.016 ± 0.4^{a}	1.995 ± 0.1^{b}
	Control	3.681 ± 0.1^{d}	3.366 ± 0.1^{b}	3.423 ± 0.1^{b}	2.722 ± 0.1^{c}
	Carvacrol	1.917 ± 0.0^{a}	1.178 ± 0.0^{a}	1.208 ± 0.1^{a}	1.244 ± 0.0^{a}
	1-8-Cineole	1.596 ± 0.1^{a}	1.185 ± 0.1^{a}	1.293 ± 0.2^{a}	1.333 ± 0.1^{a}
20	Eugenol	1.993 ± 0.1^{a}	1.224 ± 0.1^{a}	1.278 ± 0.0^{a}	1.229 ± 0.1^{a}
	(-)-Menthone	2.835 ± 0.2^{b}	2.105 ± 0.1^{b}	1.768 ± 0.2^{a}	1.679 ± 0.0^{a}
	Control	3.559 ± 0.0^{c}	3.559 ± 0.0^{b}	3.560 ± 0.1^{b}	2.675 ± 0.1^{b}

Each value is a mean of four replicates. For each dose, means within a column (month) followed by different letter (s) are significantly different (P<0.05; ANOVA, Tukey's HSD test).

In 10 µl/L treatments, root volumes of seedlings obtained in (-)-menthone-treated seeds ranged from 1.4 to 1.5 cm^3 while those obtained with carvacrol, 1-8-cineole and eugenol ranged between 1.0 and 1.3 cm^3 (Figure 3). There were significant differences between root volumes in seedlings of fumigated seeds and seedlings in controls at 1 month and 3 month ($P < 0.05$). The root volumes obtained in seedlings of carvacrol, 1-8-cineole and eugenol-treated seeds were significantly lower than that of (-)-menthone and controls at 3 month. There were no significant differences between seedlings of treated seeds and controls at 2 months ($F_{4, 15}$ =1.829, $P = 0.176$) and 6 months ($F_{4, 15} = 0.463$, $P = 0.762$) after treatment. In the 20 µl/L treatments, root volumes ranged between 1.3 and 1.4 cm^3 in seedlings of (-)-menthone-treated seeds and between 0.8 and 1.2 with carvacrol, 1-8-cineole and eugenol. There were no significant differences ($F_{4, 15} = 0.348$, $P = 0.842$) between seedlings of fumigated seeds and control seedlings only at 6 month. The oil components had varied effects on root parameters assessed at 1-3 month. Root volumes of seedlings in controls ranged from 1.41 to 1.66 cm^3. Length of root hairs in 10 µl/L treatments ranged between 53.20 and 125.89cm. The range of root hairs was between 51.41 and 78.43cm in 20 µl/L treatments. Eugenol and carvacrol-treated seedlings consistently had the shortest root-hairs in 10 µl/L treatments while eugenol and 1-8-cineole consistently had the shortest root-hairs in 20 µl/L treatments. (-)-menthone had the longest root hairs at the two doses tested at all exposure periods (Figure 4). The length of root hairs ranged from 130 to 135cm in controls.

DISCUSSION

This study demonstrated fumigant phytotoxicity of some essential oil monoterpenes used to store cowpea seeds. The oil components, carvacrol, 1-8-cineole, eugenol and (-)-menthone had varied effects on seed germination and seedling growth at 10 and 20 µl/L compared to the controls. 1-8-cineole, carvacrol and eugenol reduced germination and seedling growth compared to a moderate reduction effect observed in seedlings of (-) -

Table 5: Root-shoot ratio of seedlings of fumigated cowpea seeds (Mean ± SE).

		Storage period (Month)			
Dose (µl)	**Oil component**	**1**	**2**	**3**	**6**
	Carvacrol	0.179 ± 0.0^{b}	0.152 ± 0.0^{ab}	0.150 ± 0.0^{b}	0.132 ± 0.0^{a}
	1-8-Cineole	0.123 ± 0.0^{a}	0.104 ± 0.0^{a}	0.132 ± 0.0^{a}	0.156 ± 0.0^{b}
10	Eugenol	0.177 ± 0.0^{b}	0.145 ± 0.0^{ab}	0.139 ± 0.0^{ab}	0.129 ± 0.0^{a}
	(-)-Menthone	0.201 ± 0.0^{b}	0.202 ± 0.0^{bc}	0.204 ± 0.0^{c}	0.182 ± 0.0^{c}
	Control	0.269 ± 0.0^{c}	0.256 ± 0.0^{c}	0.230 ± 0.0^{d}	0.231± 0.0^{d}
	Carvacrol	0.155 ± 0.0^{b}	0.083 ± 0.0^{a}	0.135 ± 0.0^{a}	0.100 ± 0.0^{a}
	1-8-Cineole	0.109 ± 0.0^{a}	0.100 ± 0.0^{a}	0.143 ± 0.0^{a}	0.150 ± 0.0^{bc}
20	Eugenol	0.148 ± 0.0^{b}	0.115 ± 0.0^{a}	0.131± 0.0^{a}	0.128 ± 0.0^{b}
	(-)-Menthone	0.201± 0.0^{c}	0.179 ± 0.0^{b}	0.171± 0.0^{b}	0.172 ± 0.0^{c}
	Control	0.281± 0.0^{d}	0.258 ± 0.0^{d}	0.220 ± 0.0^{c}	0.223 ± 0.0^{d}

Each value is a mean of four replicates. For each dose, means within a column (month) followed by different letter (s) are significantly different (P<0.05; ANOVA, Tukey's HSD test).

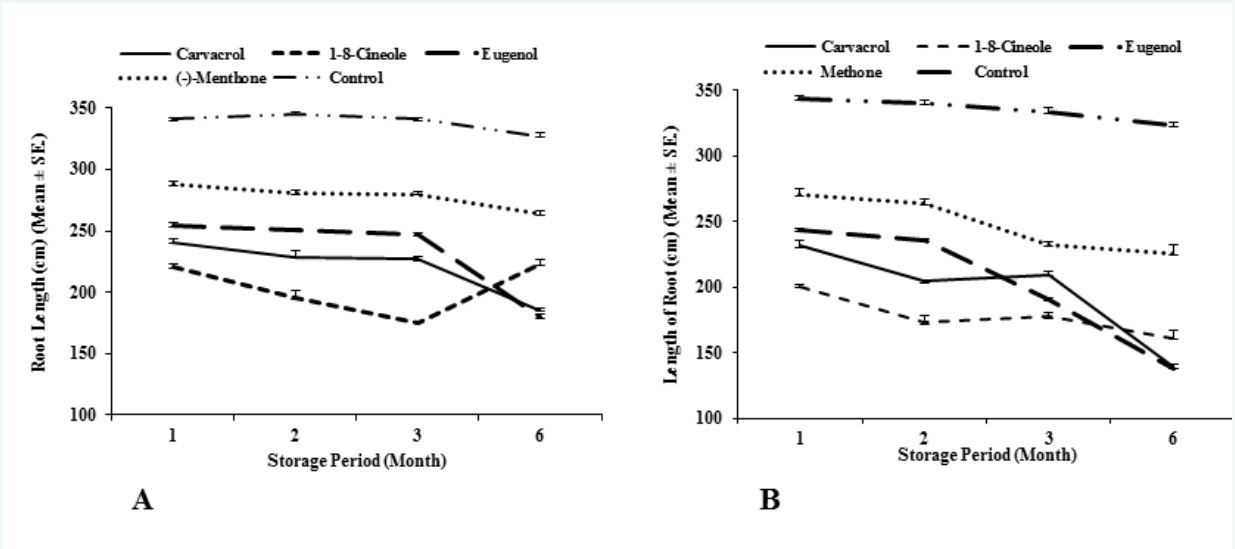

Figure 1 Length of roots of in seedlings of fumigated cowpea seeds 10 µl/L (A) and 20 µl/L (B).

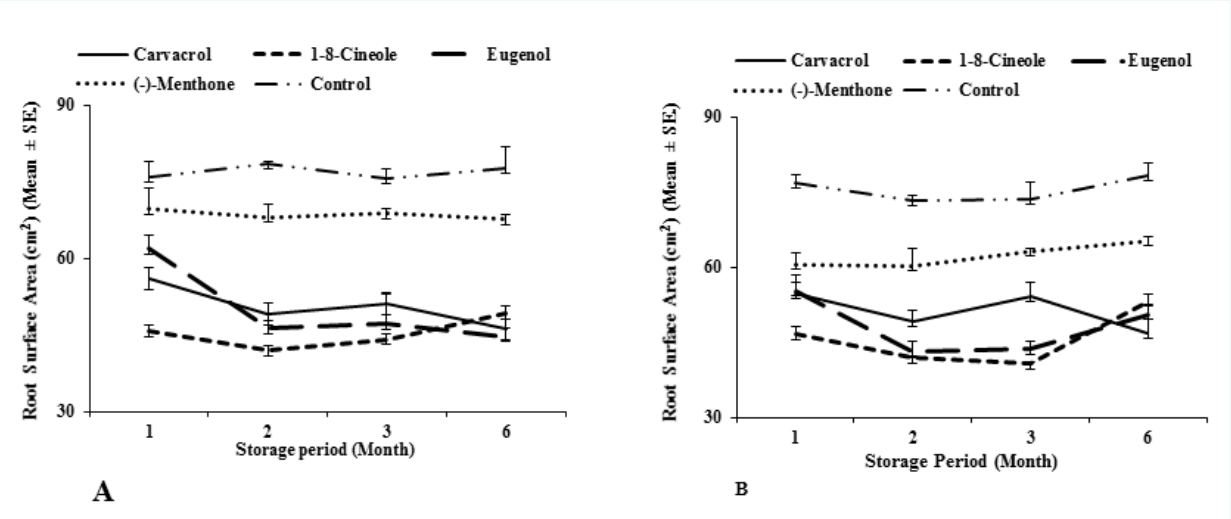

Figure 2 Surface area of roots in seedlings of fumigated cowpea seeds 10 µl/L (A) and 20 µl/L (B).

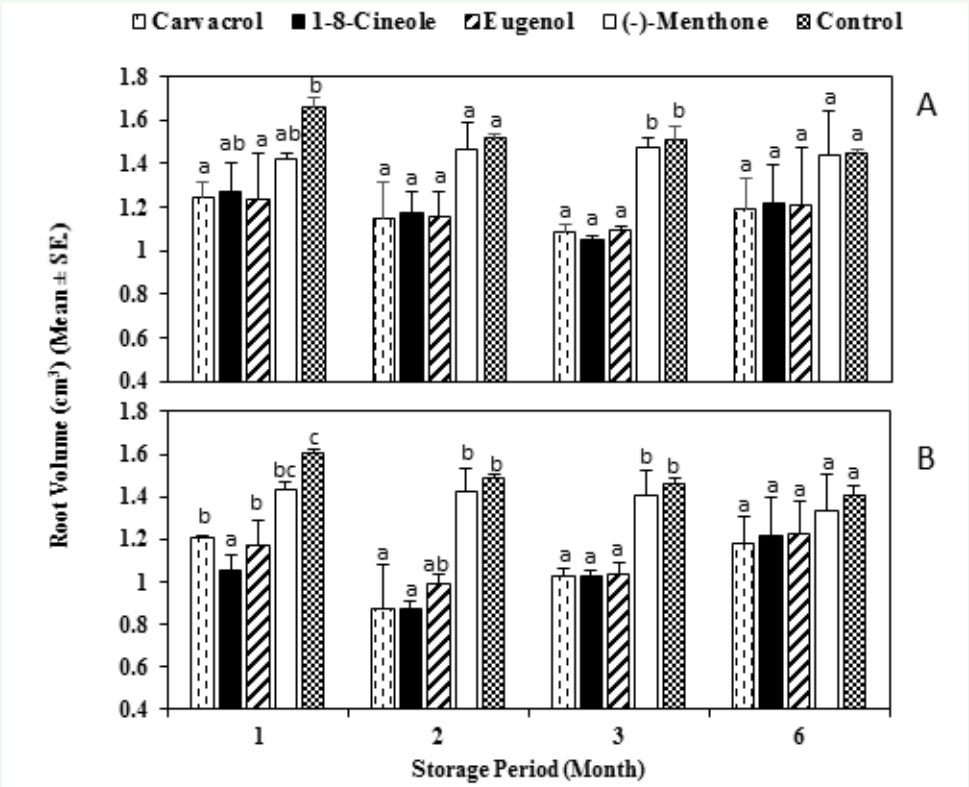

Figure 3 Root volumes of seedlings of fumigated cowpea seeds 10 µl/L (A) and 20 µl/L (B).

menthone-treated seeds. Shoot length, number of leaves and moisture content are good estimates of photosynthetic capacity and transpiration area of seedlings [35]. The four essential oil monoterpenes tested in this study negatively affected shoot length and number of seedling leaves and would be expected to reduce plant growth. Root-shoot ratio is a unit-less ratio that measures the balance between the water absorbing area (root) and the transpiration area (shoot) of seedlings. It is an index of overall health status of plants [35]. The root-shoot ratio of healthy plants under normal conditions ranges from 1:5 (0.2) to1:6 (0.16) [36]. Seedlings with good root volume, root length, root surface area and root hairs can anchor the seedling well into the soil, maximally harness soil nutrients and survive unfavorable conditions [35]. The reduction effect of carvarol, 1-8-cineole, and eugenol on root quality in seedlings of fumigated seeds (Figures (1-3)) may result in slower growth or a lower plant yield. The

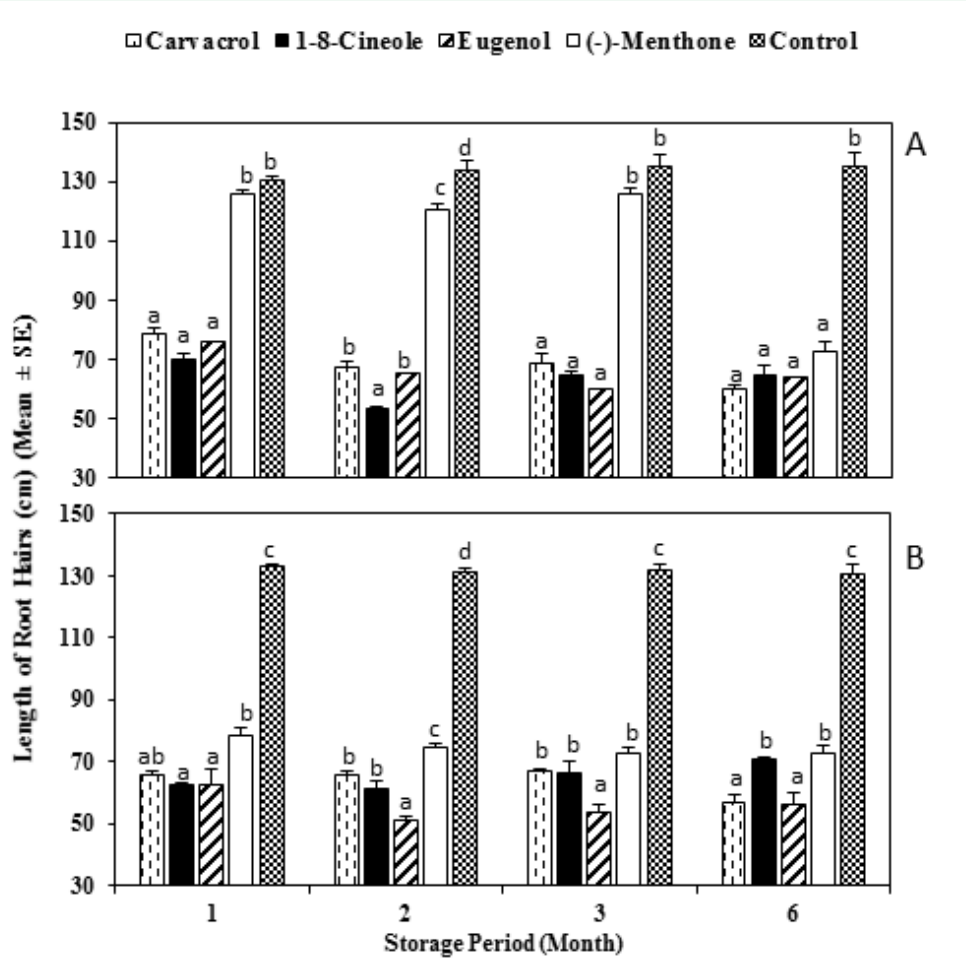

Figure 4 Length of root hairs on seedlings of fumigated cowpea seeds 10 µl/ L (A) and 20 µl/L (B).

length, surface area, and volume in seedlings of (-)-menthone-treated seeds compares favorably with that of seedlings in controls. This indicates that (-)-menthone is relatively safe to be used as protectant on cowpea seeds.

Phytotoxity of these monoterpenes were more pronounced at 2 and 3 month than 1 and 6 months after fumigation. Generally, negative effect of these essential oil components on germination and seedling growth reduced with increasing observation periods notably with 1-8-cineole. The compound 1-8-cineole has the highest vapor pressure (1.648 mmHg at 25°C) and lowest boiling points (176 °C) among the four compounds tested. These physical factors might have caused it to react with cowpea seeds faster, which could lead to its biodegradation. The highest levels of seedlings growth observed in (-)-menthone-fumigated seeds might be due to its relatively low density (0.89 g/ml), which might have reduced its adsorption on seeds, and a relatively greater vapor pressure (0.256 mmHg at 25 °C) than carvacrol (0.030 mmHg at 25 °C) and eugenol (0.010 mmHg at 25 °C) may have enhanced its volatilization. These findings agree with some earlier reports that essential oils and their components are biodegradable [8,7,11,12].

Earlier studies have documented that volatile oils or their constituent monoterpenes inhibit and delay seed germination, and seedling growth of many weeds by obstructing mitochondrial metabolism thereby causing inhibition of cell elongation and reduced photosynthetic activities [37-40]. Yoshimura et al. [41] implicated 1-8-cineole in inhibition of cell elongation and starch concentration in root apical meristem. Koitabashi et al. [42] demonstrated that 1-8-cineole reduced mitotic index of root apical meristem and inhibited the synthesis of both cell nuclear and organellar DNA synthesis. Vaid et al. [43] showed that eugenol decreased seedling length, seedling dry weight and germination of *Cassia occidentalis* (L.) (Stinking weed), and *Bidens pilosa* (L.) (Black-jack). Carvacrol has been reported to inhibit the germination and seedling growth of *Amaranthus retroflexus* (L.) (Tumble weed) and *Chenopodium album* (L.) (Lamb's quarters) at a higher rate than commercial herbicide 2.4.D isooctyl ester [44]. The organic herbicides, EcoSMART (which contains 5% eugenol) and Phydura (12% clove oil) were effective at controlling weeds [45]. It is likely that similar chemical reactions may have occurred between the oil components and cowpea seeds to bring about the reduction effects on seed germination and seedling growth recorded in this study.

CONCLUSION

In summary, the results showed that carvarol, 1-8-cineole, and eugenol are phytotoxic to cowpea seeds while (-)-menthone

is relatively non-toxic. Phytotoxicity of these compounds was more apparent at 2 and 3 months after fumigation. The results showed that fumigation with some essential oil components may affect seed viability. It also showed that phytotoxicity reduced with storage period. Therefore, it is highly imperative to reach a compromise between future use of stored cowpea seeds, choice and doses of essential oils or components to protect the seeds against insect infestation and duration of storage before such seeds could be planted. Based on our previous reports on toxicity of the tested components to adult *C. maculatus* [15] and the results of this study, (-)-menthone could be used to protect cowpeas seeds for consumption and planting.

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