Effectieveness of plant oils and essential oil of *Ocimum* plant species for protection of stored grains against damage by stored product beetles

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Abstract

Biological activity of 1,8 cineole, eugenol and camphor, major components of essential oils of *Ocimum kenyense*, *O suave* and *O kilimandscharicum*, respectively, against *Sitophilus zeamais* and *Prostephanus truncatus* was investigated in the laboratory using contact toxicity, grain treatment and repellency assays Each compound applied topically or impregnated on whole maize grains was highly toxic to both beetle species. Beetle mortality was dose-dependent. Grain treated with each compound was highly toxic with the lowest dosage of 0 5 μl or mg/kg grain achieving complete control of all insects exposed within 24 h. Development of eggs and immature stages within grain kernels as well as progeny emergence was completely inhibited in treated grain. Camphor and eugenol were also highly repellent to the beetles, with overall repellency in the range of 80 – 100%. There was, however, a highly significant loss of toxicity in grain after only 24 h following treatment. Mixing each compound with plant oils (coconut, sunflower, sesame or mustard) enhanced toxicity and maintained efficacy for 90 days after treatment.

Introduction

Sitophilus zeamais (Motschulsky) (Coleoptera: Curculionidae) cause serious damage to stored grain and grain products in Africa (Dick 1988) The recent accidental introduction of the larger grain borer, *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae) into Africa has added a new dimension to insect pest infestation in storage through its remarkable ability to damage well-dried maize, even when stored on the cob (Dick 1988) This beetle is currently a more serious pest in parts of sub-Saharan Africa than in its native Central America (Boxall et al. 1997). Treatment of stored grain with synthetic insectation in storage. However, increasing cost of application and erratic supply in developing countries due to foreign exchange constraints have stimulated interest in the re-evaluation of traditional botanical pest-control agents to establish the scientific basis for their continued use regarding their efficacy, active constituents and appropriate application technology (Hassanali et al 1990; Weaver et al 1991; 1994; Poswal & Akpa 1991; Regnault-Roger et al 1993; Bekele 1994; Talukder & Howse 1995; Jembere et al 1995)

The use of locally available plant materials to reduce insect damage to stored foodstuffs is a common practice in traditional farm storage in developing countries (Poswal & Akpa 1991) Protection of stored products generally involves mixing grain with protectants derived from local plants (Hassanali et al. 1990)

Ocimum kenyense Ayobangira, *O. suave* Willd and *O kilimandscharicum* Guerke (Labiatae) are medicinal plants widespread in India and Africa (Kokwaro 1976; Paton 1991) The leaves have traditionally been used in eastern and southern Africa for the treatment of various ailments and as insect repellents, particularly against mosquitoes (Kokwaro 1976) Some farmers also mix stored foodstuffs with dry leaves of *Ocimum* plants for protection against pest damage (Kokwaro 1976; Hassanali et al 1990) Ground leaves and essential oil extract of these plants were recently shown in the laboratory to be effective protectants of maize and sorghum against attack by *S. zeamais* Motschulsky (Coleoptera: Curculionidae), *R dominica* (Fabricius) (Coleoptera: Bostrichidae) and *Sitotroga cerealella* (Olivier) (Coleoptera: Gelechiidae) in storage (Bekele 1994; Jembere et al. 1995) This paper reports the results of further bioassays on the biological activity of 1,8 cineole, eugenol and camphor, the major components identified from the leaves of *O kenyense*, *O. suave* and *O. kilimandscharicum*, respectively (Bekele 1994), alone or in combination with plant oils (coconut, sunflower, sesame and mustard) for protection of stored maize againts damage by *S zeamais* and *P truncatus*.

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Material and Methods

Insects

Siitophilus zeamais and *P. trucatus* were cultured on whole maize grains in controlled environment room at 27±2 ℃ and 65 – 70% rh in the dark. Parent adults were obtained from laboratory stock culture maintained at the institute for Stored Product Protection in Berlin, Germany Hundred adults of each species were put into glass jars containing 500 g maize grain for three weeks after which the adults were sieved out

Extraction and chemical composition of essential olis

The essential oil extract was isolated separately from the leaves, inflorescences and succulent stems of *O. kenyense*, *O. Suave* and *O kilimandscharicum* by steam distillation using Clavenger apparatus and the condensing oils were collected in n-hexane solvent (Aldrich HPLC grade) (Jembere et al. 1995). A VG analytical mass spectometery (VG12-250), GC-MS equipped with data system and Hewlett Packard 5790 gas chromatograph with aplitless injector and flame ionization detector was used to analyse the essential oils. The constituent compounds were identified by spectral comparison with synthetic standards. A total of 11,7 and 10 compounds from different functional groups (alcohol, cyclic hydrocarbon, ketone, ether) were identified with 1, 8 cineole, eugenol and camphor forming the major essential oil components comprising 40, 60 and 70% of the total collection from *O kenyense*, *O. suave* and *O. kilmandscharicum*, respectively (Table 1) 1,8 cineole, eugenol and camphor (99% purity and with similar enantiomer as those naturally present in the essential oil of the three plants) were purchased from Aldrich Limited, Germany for the different bioassays.

Contact toxicity by topical application

Tests for contact toxicity by topical treatment were carried out using the standard method described by McDonald et al. (1970). Different dosages of test compound 1, 5, 10, 20, 40, 60 or 100 μg (camphor) or 0.5, 1, 3, 5 and 10 μl (1,8 cineole, eugenol) in 1 ml acetone were prepared. Three to seven-day old insects were first transferred into Petri dishes lined with moist filter paper and chilled at 5℃ for three minutes to reduce their activity to enable topical treatment to be done. The immobilized insects were picked individually for treatment Two μl of test solution was applied to the dorsal surface of the thorax of each insect with a micro-applicator. Fifty beetles in five replicates of 10 insects each were treated with each dose. Similar numbers were treated with solvent only as control. After treatment, insects were transferred into 11.0 cm diameter glass Petri dishes (10 insects/Petri dish) containing 20 g maize kernels Insects were examined daily for two days and those that did not move of respond to three probings with a blunt probe were considered dead. Insect mortalities were recorded at 48 h after treatment.

Table 1. Essential oil components of *Ocimum. kenyensis*, *O. suave* and *O. kilimandscharicum* in Kenya.

Common name	Formula	% Composition
O. kenyense		
1,8 cineole	$C_{10}H_{18}O$	38.93
β-selinene	$C_{15}H_{21}$	23.57
methyl chavicol	$C_{10}H_{18}O$	12.86
isoeugenol	$C_{10}H_{12}O$	8.46
ethyl isovalerate	$C_7H_{11}O_2$	2.99
β-pinene	$C_{10}H_{18}$	2.86
α-humulene	$C_{15}H_{24}$	2.78
4-terpineol	$C_{10}H_{18}O$	0.79
Y-cardinene	$C_{15}H_{24}O$	0.78
trans-caryophyllene	$C_{15}H_{24}$	0.6
α-terpineol	$C_{10}H_{16}$	0.55
O. suave		
eugenol	$C_{10}H_{12}O_2$	59.65
tarans-β-ocimene	$C_{10}H_{16}$	14.52
β-cubebene	$C_{15}H_{24}$	5.26
trans-caryophllene	$C_{15}H_{24}$	3.27
trans-α-ocimene	$C_{10}H_{16}$	2.14
β-pinene	$C_{10}H_{16}$	1.66
linalool	$C_{10}H_{16}$	1.41
O. kilimandscharicum		
camphor	$C_{10}H_{16}O$	70.43
1,8 cineole	$C_{10}H_{18}O$	7.20
limonene	$C_{10}H_{16}O$	6.23
camphene	$C_{10}H_{16}O$	5 07
trans-caryophyllene	$C_{15}H_{24}$	2.80
4-terpineol	$C_{10}H_{18}O$	1.44
myrtenol	$C_{10}H_{16}O$	1.30
α-terpineol	$C_{19}H_{18}O$	0.60
endo-borneol	$C_{10}H_{18}O$	0.60
linalool	$C_{10}H_{18}$	0.47

Toxicity and persistence in grain

The effect of essential oil-treated maize grain on adult mortality of *S. zeamais* and *P. truncatus* was studied in the laboratory. The grain was separtely treated with different dosages of 1,8 cineole, eugenol (0, 0.5, 1, 5 and 10 μl/kg) and camphor(0, 1, 5, 20, 40, 80 and 100 mg/

kg) dissolved in acetone. Test solutions were mixed with 250 g samples of grain in 1l glass jars and stirred continuously for 10 minutes to ensure and even spread of the material over the surface of the grains, and kept for three hours to allow the solvent to evaporate completely The grain was then infested with three to seven days-old adult beetles (20 beetles per jar) and each jar was covered with a nylon mesh secured with a rubber band Each treatment was replicated five times and mortality counts were made after 24 h. To assess the persistence of the treatments, beetles in a similar experiment were exposed to treated grain glass 1, 10, 20 or 40 days after application Mortality counts were made after a 24h exposure

Effect on hidden eggs and immature stages

The effect of the compounds on the development of eggs and immature stages of *S zeamais* and *P truncatus* within maize kernels was bioassayed. Batches of 200 g of maize in 300 ml glass jars were infested with 50 three-to seven-day-old unsexed adults to allow ovipostion The parent adults were sieved out after seven days One day after adult removal, four batches of the grain were treated different dosages of 1,8 cineole, eugenol (0, 0.5, 1, 5 and 10 μl/kg) and camphor (0,1,5,20,40,80 and 100 mg/kg) dissolved 10 ml acetone to test the effect of the treatments on the eggs Thereafter, these treatments were repeated at one, two and three weeks after adult removal to determine their effect of the treatments on the early larval, late larval and pupal stages of the beetles, respectively Adults subsequently emerging were counted for a period of seven weeks following the removal adults

Progeny production

The effect of each compound on the mumber of progeny produced by *S zeamais* and *P truncatus* was investigated in maize treated with different different dosages of 1,8 cineole, eugenol (0,0.5,1,5 and 10 μl/kg) and camphor (0,1,5,20,40,80 and 100 mg/kg) dissolved in acetone Batches of 250 g maize in 100 ml glass jars were mixed with test solutions dissolved in 10 ml acetone Control treatments consisted of maize mixed with acetone only These were stored in the laboratory for 1,10,30 or 60 days prior to the introduction of 20 three-to seven-days old adults of mixed sex into separate glass jars. After a three-week oviposition period, the parent adults were sieved out insects subsequently emerging were counted daily for eight weeks after the introduction of the insects into the jars There were four replicates of each treatment

Repellency bioassay

The repellent action of 1,8 cineole, eugenol and camphor against *S. zeamais* and *P truncatus* was evaluated using the area preference method described by McDonald et al (1970) Test arenas consisted of 11 cm Whatman No. 1 filter papers cut in half Different test solutions were prepared by dissolving 5,10,30,50 or 100 mg camphor or 1, 3,5 and 10 μl (1,8 cineole, eugenol) in 1 ml acetone Each solution was applied to a half filter paper disc as uniformly as possible with a pipette. The other filter papaer halves were treated with acetone only Chemically-treated and control half discs were air-dried to evaporate the solvent Full discs were then remade by attaching treated halves to untreated halves of the same dimensions with cellotape Each filter paper was placed in a Petri dish and 10 adult beetles of mixed sex for each species were released separately at the centre of each filter paper disc and then covered. Each treatment was replicated 10 times. The number of insects present on control (N_C) and treated (N_T) strip were recorded after 30 minutes exposure Percent repellency (PR) values were computed as $PR = [(N_C - N_T)/(N_C + N_T)] \times 100$ PR data were analysed using ANOVA after transforming them into arcsin values

Plant oil/essentialoil mixture

Locally extracted coconut oil and refined oils (sunflower, sesame, mustard) were purchased from Makola market, Accra, Ghana and WELP products, Berlin Germany, respectively. Different doses, 0.5,1,5 μl(1,8 cineole and eugenol) and 0.5, 1, 5 mg (camphor)/kg of grain were mixed with the plant oils at the rate of 5 ml/kg og grain. Samples of 500 g of maize were mixed with test solutions by tumbling in 1l glass jars. The effect of the oils alone and in combination with the chemicals on adult mortality and progeny production was bioassayed Twenty one-week-old adults of mixed sex were exposed to treated grain which had been stored for three hours, 10, 30, 60 and 90 days. Mortality counts were made after a 24 h exposure time. Thereafter, the treatments were infested with 30 *S zeamais* and *P truncatus* adults of mixed sex for seven days to allow oviposition and then removed F_1 progeny subsequently emerging were counted for eight weeks after the introduction of the insects. In a similar experiment, beetles were exposed to oil treated grain at rate of 10 ml/kg without chemicals. Each treatment in the two experiments was replicated five times.

Data analysis

Percent adult mortality obtained for the various experiments were analysed using ANOVA after transforming them into arcsin values Where ANOVA revealed significant differences between the means, LSD was used to separate them Re-transformed data are presented in the tables.

Results

Contact toxicity by topical application

Toxicity of 1, 8 cineole, eugenol and camphor applied topically to the beetles is shown in Table 2 Toxicity increased with increasing dosage. Every single dosage produced significantly higher mortality than the control treatment For 1,8 cineole and eugenol, dosages of ⩾70 μl and for camphor 60 μg/insect produced 100% mortality of the two beetle species within 48 h of application.

Table 2. Toxicity of 1,8 cineole, eugenol and camphor applied topically.

Dosage (μl/beetle)	Percent adult mortality after 48 h	
	S zeamais	*P truncatus*
1,8 cineole		
0	0^{d}	0^{d}
1	42^{c}	45^{c}
3	72^{b}	78^{b}
5	90^{a}	91^{a}
7	100^{a}	100^{a}
10	100^{a}	100^{a}
Eugenol		
0	0^{d}	0^{d}
1	42^{c}	50^{c}
3	84^{b}	87^{b}
5	92^{ab}	93^{ab}
7	100^{a}	100^{a}
10	100^{a}	100^{a}
Camphor		
0	0^{f}	0^{f}
1	15^{e}	23^{e}
5	33^{d}	44^{d}
10	58^{c}	63^{c}
20	81^{b}	85^{b}
40	88^{b}	91^{ab}
60	100^{a}	100^{a}
100	100^{a}	100^{a}

Mean of 10 replicates of 10 insects each Column means for each compound followed by different letter(s) are signifiantly different at the 0.05 level, LSD.

Toxicity and persistence in grain

Table 3 shows the precent mortality of *S zeamais* and *P. truncatus* in maize grains treated with each compound after different intervals of storage The compounds were highly toxic to the beetles with the lowest dose of 1 mg (camphor) and 0.5 μl/kg (1,8 cineole or eugenol) inducing 100% mortalities in the two beetle species within 24 h. Their effectiveness was, howere, significantly reduced by the length of storage after treatment. For grain bioassayed after 10 days of storage following application, mortality decreased to less than 30% even at the highest dosage of 100 mg (camphor) or 10 μl (1,8 cineole or eugenol)

Effect on eggs and immature stages

1,8 cineole, eugenol or camphor completely inhibited the development of eggs, larvae and pupae of *S zeamais* and *P truncatus* concealed within maize kernels. When maize kernels containig eggs, first and second larval instars or pupae were treated with each compound, no progeny emerged after eight weeks compared with the untreated grain in which an average of 427 *S. zeamais* and 75 *P. truncatus* adults were recorded

Progeny production

Each of the three compounds completely inhibited oviposition and subsequent progeny by *S. zeamais* and *P truncatus*. No progeny was produced in essential oil treated maize stored for 1, 10, 30 or 60 days before infestation with *S. zeamais* and *P. truncatus* compared to the untreated controls which recorded an average of 625 *S. zeamais* and 102 *P truncatus* adults over the experimental period

Repellency

Eugenol and camphor were highly repellent to *S. zeamais* and *P. truncatus* with over all repellency in the range of 80 – 100% (Table 4) 1,8 cineole evoked strong repellent action against only *S. zeamais*. Repellency was dose-dependent with the highest dosage of 100 mg of camphor and 10 μl of eugenol evoking over 90% repellency against the two beetle species.

Plant oil/essential oil mixture

All treatments with plant oils only or in combination with each essential oil caused significant ($P < 0.05$) mortality compared to untreated grain. Plant oils when used alone were less effective against the beetles than oils combined with either 1,8 cineole, eugenol or camphor (Tables 5 and 6). Mortality was significantly reduced by the storage period following application of the protectants except treatments combining plant oils with essential oil which achieved complete control of all beetles exposed after 90 days storage following application Of the plant oils tested, coconut, sesame and mustard oil were more effective than sunlower. Similarly, all treatments with plant oils and essential oil compounds only or in combination completely inhibited progeny production by both *S zeamais* and *P truncatus*

Discussion

1,8 cineole, eugenol or camphor applied topically to insect bodies or impregnated on maize grains was highly toxic *S. zeamais* and *P. truncatus* The 24 h adult mortality on grain treated with the chemicals was uniformly high for both beetle species at 1.0 mgk/kg or 0.5 μl/kg. Products prepared from these essential oils could thus be used in severely infested grain stocks for immediate control of pest species present, although they have a rather low residual action.

Talbe 3. Percent mortalıty of *Sıtophilus zeamaıs* and *Prostephanus truncatus* ın (a) 1,8 cıneole, (b) eugenol and (c) camphor treated maize graıns after dıfferent ıntervals of storage

Dosage (μl/kg)	Tıme after treatment				
	3 hours	1 day	10 days	20 days	40 days
(a) *S zeamaıs*					
0	0±0.0[d]	0±0.0[d]	0±0.0[d]	0±0.0[d]	0±0.0[d]
0.5	100±0.0[a]	2±0.6[d]	0±0.0[d]	0±0.0[d]	0±0.0[d]
1	100±0 0[a]	13±1 6[c]	3±0.7[d]	0±0.0[d]	0±0.0[d]
5	100±0.0[a]	19±2.4[bc]	3±0.6[d]	0±0.0[d]	0±0.0[d]
10	100±0.0[a]	28±2.7[b]	4±0.8[d]	0±0.0[d]	0±0.0[d]
P. truncatus					
0	0±0.0[d]	0±0.0[d]	0±0.0[d]	0±0.0[d]	0±0.0[d]
0.5	100±0.0[a]	5±1.0[d]	0±0.0[d]	0±0.0[d]	0±0.0[d]
1	100±0.0[a]	20±1.8[c]	5±1.2[d]	1±0 0[d]	0±0.0[d]
5	100±0.0[a]	32±2 7[b]	5±1.4[d]	0±0.0[d]	0±0.0[d]
10	100±0.0[a]	39±2.9[b]	5±1.0[d]	1±0.0[d]	0±0.0[d]
(b) *S. zeamaıs*					
0	0±0.0[d]	0±0 0[d]	0±0.0[d]	0±0.0[d]	0±0.0[d]
0.5	100±0 0[a]	3±0.6[d]	4±0.0[d]	0±0.0[d]	0±0.0[d]
1	100±0 0[a]	14±1.6[c]	7±0.7[d]	2±0.0[d]	0±0.0[d]
5	100±0.0[a]	39±2.4[b]	19±0.6[c]	4±0.0[d]	0±0.0[d]
10	100±0.0[a]	48±2.7[b]	23±0.8[c]	5±0.0[d]	0±0.0[d]
P truncatus					
0	0±0.0[d]	0±0.0[d]	0±0.0[d]	0±0.0[d]	0±0.0[d]
0.5	100±0.0[a]	10±1.0[d]	3±0.0[d]	0±0.0[d]	0±0.0[d]
1	100±0.0[a]	27±1.8[c]	8±1.2[d]	2±0.0[d]	0±0.0[d]
5	100±0.0[a]	42±2.7[b]	25±1.4[c]	5±0.0[d]	1±0.0[d]
10	100±0.0[a]	57±2.9[b]	27±1 0[c]	7±0.0[d]	2±0.0[d]
(c) *S zeamaıs*					
0	0±0.0[d]	0±0.0[d]	0±0.0[d]	0±0.0[d]	0±0.0[d]
1	100±0.0[a]	2±0.6[d]	0±0.0[d]	0±0 0[d]	0±0.0[d]
5	100±0 0[a]	4±0.9[d]	2±0.6[d]	0±0 0[d]	0±0.0[d]
20	100±0.0[a]	16±2.1[c]	12±1.4[c]	11±1.5[c]	0±0.0[d]
40	100±0.0[a]	20±2.4[c]	14±1.6[c]	11±1.7[c]	0±0.0[d]
80	100±0.0[a]	33±3.1[b]	14±1.9[c]	12±1.6[c]	0±0.0[d]
100	100±0.0[a]	44±2.9[b]	18±2.0[c]	12±1 5[c]	0±0.0[d]
P truncatus					
0	0±0.0[d]	0±0.0[d]	0±0 0[d]	0±0.0[d]	0±0.0[d]
1	100±0.0[a]	4±1.2[d]	2±0.7[d]	0±0.0[d]	0±0.0[d]
5	100±0.0[a]	8±1.5[d]	4±0.8[d]	0±0.0[d]	0±0.0[d]
20	100±0.0[a]	18±2.6[c]	7±1.8[d]	5±1.2[d]	0±0.0[d]
40	100±0.0[a]	20±2.5[c]	17±1.8[c]	6±1.6[c]	0±0.0[d]
80	100±0.0[a]	35±2.4[b]	20±2.4[c]	16±1.4[c]	0±0.0[d]
100	100±0.0[a]	45±3.1[b]	20±2 6[c]	16±2.3[c]	3±1.1[d]

Mean of fıve replıcates of 20 ınsects each Mortalıt recorded after 24 h expousre to treated graıns stored for 3 hours, 1,10,20 and 40 days Means for each specıes followed by dıfferent letters are sıgnıfıcantly dıfferent at the 0 05 level, LSD test

Table 4. Percent repellency of the essential oils against *Sitophilus zeamais* and *Prostephanus truncatus* in the choice arena.

Dosage (μl/disc)	Mean percent repellency(PR)	
	S. zeamais	*P. truncatus*
1,8 cineole		
0.5	19d	14d
1	40c	20d
3	55b	23d
5	62ab	41c
10	70a	53b
Eugenol		
0.5	52d	49d
1	70c	64c
3	82b	80b
5	89ab	86ab
10	92a	94a
Camphor		
5	65c	63c
10	78b	76b
30	85ab	86ab
50	93a	93a
100	96a	94a

Mean of five replicates of 20 insects each Column means for each compound followed by different letter(s) are significantly different at the 0.05 level, LSD test

Eugenol and camphor were also highly repellent to the beetles relative to the controls Jembere et al. (1995) showed the repellency of crude essential oil extract of *O. suave* and *O. kilimandscharicum* against *S. zeamais*, *R. dominica* and *S. cerealella* in laboratory bioassays Thus, the repellent effect of *O. suave* and *O kilimandscharicum* may be attributed to its major components, eugenol and camphor, respectively. This repellent action, coupled with the complete inhibition of the development of eggs and immature stages hidden inside grain kernels as well as progeny emergence in maize grains treated with 1,8 cineole, eugenol or camphor increases the protectant potential of these essential oils against pest infestation during storage.

The mode of action of the compounds was not determined in the present study A common structural feature of terpenoids is their hydrocarbon skeleton which confers upon them a common property of hydrophobicity. Many hydrophobic compounds are associated with protein deactivation and enzyme inhibition (Ryan & Byrne, 1988). These workers attributed the toxicity of several terpenoids, representing a range of functional groups including pulegone (ketone), linalool (alcohol) and 1,8 cineole (ether) against *T castaneum* (Herbst) (Coleptera: Tenebrionidae) to their reversible competitive inhibition of acetylcholinesterase by apparently occupying the hydrophobic site of the enzyme's active centre. Eugenol and camphor, being ketones may have a similar mode of action

Small-scale farmers in East Africa mix foodstuffs with dried leaves of *O kenyense*, *O suave* and *O kilimandscharicum* to protect foodstuffs against postharvest damage by pests. Bekele(1994) and Jembere et al. (1995) showed the effectiveness of ground leaves and essential oil extract *O. kenyense*, *O. suave* and *O. kilimandscharicum* in protecting maize and sorghum against attack by *s zeamais*, *R. dominica* and *S cerealella* The efficacy of the three plant species in providing protection against insect damage in storage may consequently be attributed to the high concentration of 1,8 cineole, eugenol and camphor in the leaves, although other minor toxic components (Table 1) could be important.

The plant oils (coconut, sunflower, sesame and mustard) applied at 5 or 10 ml/kg of maize caused significant mortality of *S. zeamais* and *P. truncatus* compared to untreated controls. Dead beetles from grain treated with plant oils showed signs of rapid immobilization with their legs flexed and clinging to either the grain or the container surface. Beetles killed in grain treated with essential oils appeared paralysed with their metathoracic wings unfolded and stretched outside the elytra (Obeng-Ofori et al 1996) Several previus studies have demonstrated the effectiveness of different vegetable oils in protecting grain against major stored product insects pests (Yun-Tai & Burkholder 1981; Pereira 1983; Kumar & Okonronkwo 1991; Obeng-Ofori 1995) Although the mode of action of such vegetable oils is yet to be confirmed, based on similar observations, it has been suggested that insect death is due to anoxia or interference in normal respiration resulting in suffocation (Hewlett 1975; Schoonhoven 1978; Don-Pedro 1989). The decrease in beetle mortality with storage following treatment with vegetable oils obtained in the present study supports the above mechanism. Presumably, with storage the oils become absorbed by the grain, thereby reducing its availability for pick-up by the beetles (Tembo & Murfitt 1995). Insect mortality in grain treated with oils has also been attributed to direct toxicity by penetrated fractions (Hill & Schoonhoven 1981).

Table 5. Percent adult mortality of *Sitophilus zeamais* in plant oil/1,8 cineole, eugenol or camphor treatd maize after different intervals of storage.

Treatments	Days of storage after treatment				
	3 h	10	30	60	90
Control	0[f]	0[f]	0[f]	0[f]	0[f]
1,8 cineole (0.5 μl/kg)	100[a]	0[f]	0[f]	0[f]	0[f]
1,8 cineole (1.0 μl/kg)	100[a]	2[f]	0[f]	0[f]	0[f]
1,8 cineole (5.0 μl/kg)	100[a]	3[f]	0[f]	0[f]	0[f]
Eugenol (0.5 μl/kg)	100[a]	0[f]	0[f]	0[f]	0[f]
Eugenol (1.0 μl/kg)	100[a]	3[f]	0[f]	0[f]	0[f]
Eugenol (5.0 μl/kg)	100[a]	5[f]	3[f]	0[f]	0[f]
Camphor (0.5 mg/kg)	100[a]	0[f]	0[f]	0[f]	0[f]
Camphor (1.0 mg/kg)	100[a]	1[f]	0[f]	0[f]	0[f]
Camphor (5.0 mg/kg)	100[a]	3[f]	0[f]	0[f]	0[f]
Coconut oil at 10 ml/kg	100[a]	100[a]	100[a]	80[bc]	72[c]
Coconut oil at 5 ml/kg	100[a]	42[d]	6[f]	0[f]	0[f]
+ 1,8 cineole	100[a]	100[a]	100[a]	100[a]	100[a]
+ eugenol	100[a]	100[a]	100[a]	100[a]	100[a]
+ Camphor	100[a]	100[a]	100[a]	100[a]	100[a]
Sunflower oil at 10 ml/kg	100[a]	83[bc]	43[d]	35[d]	17[e]
Sunflower oil at 5 ml/kg	100[a]	26[e]	5[f]	0[f]	0[f]
+ 1,8 cineole	100[a]	100[a]	100[a]	100[a]	100[a]
+ eugenol	100[a]	100[a]	100[a]	100[a]	100[a]
+ Camphor	100[a]	100[a]	100[a]	100[a]	100[a]
Sesame oil at 10 ml/kg	100[a]	42[a]	4[f]	0[f]	0[f]
+ 1,8 cineole	100[a]	100[a]	100[a]	100[a]	100[a]
+ eugenol	100[a]	100[a]	100[a]	100[a]	100[a]
+ Camphor	100[a]	100[a]	100[a]	100[a]	100[a]
Mustard oil at 10 ml/kg	100[a]	100[a]	90[ab]	80[bc]	72[c]
Mustard oil at 5 ml/kg	100[a]	45[d]	5[f]	0[f]	0[f]
+ 1,8 cineole	100[a]	100[a]	100[a]	100[a]	100[a]
+ eugenol	100[a]	100[a]	100[a]	100[a]	100[a]
+ Camphor	100[a]	100[a]	100[a]	100[a]	100[a]

Mean of five replicates of 20 beetles each Mortality recorded after 24 h exposure to untreated and treated maize stored for 3 hours, 10,30,60 and 90 days. Means followed by different letter(s) are significantly different at the 0.05 level, LSD test

Table 6. Percent adult mortality of *Prostephanus truncatus* in plant oil/1,8 cineole, eugenol or camphor treatd maize after different intervals of storage

Treatments	Days of storage after treatment				
	3 h	10	30	60	90 days
Control	0[f]	0[f]	0[f]	0[f]	0[f]
1,8 cineole (0.5 μl/kg)	100[a]	5[f]	1[f]	0[f]	0[f]
1,8 cineole (1.0 μl/kg)	100[a]	2[f]	0[f]	0[f]	0[f]
1,8 cineole (5.0 μl/kg)	100[a]	3[f]	0[f]	0[f]	0[f]
Eugenol (0.5 μl/kg)	100[a]	0[f]	0[f]	0[f]	0[f]
Eugenol (1.0 μl/kg)	100[a]	7[f]	0[f]	0[f]	0[f]
Eugenol (5.0 μl/kg)	100[a]	24[e]	4[f]	0[f]	0[f]
Camphor (0.5 mg/kg)	100[a]	0[f]	0[f]	0[f]	0[f]
Camphor (1.0 mg/kg)	100[a]	2[f]	0[f]	0[f]	0[f]
Camphor (5.0 mg/kg)	100[a]	5[f]	0[f]	0[f]	0[f]
Coconut oil at 10 ml/kg	100[a]	100[a]	100[a]	80[b]	70[b]
Coconut oil at 5 ml/kg	100[a]	49[d]	0[f]	0[f]	0[f]
+ 1,8 cineole	100[a]	100[a]	100[a]	100[b]	100[a]
+ eugenol	100[a]	100[a]	100[a]	100[a]	100[a]
+ Camphor	100[a]	100[a]	100[a]	100[a]	100[a]
Sunflower oil at 10 ml/kg	100[a]	100[a]	70[c]	65[c]	59[c]
Sunflower oil at 5 ml/kg	100[a]	37[a]	4[f]	0[f]	0[f]
+ 1,8 cineole	100[a]	100[a]	100[a]	100[a]	100[a]
+ eugenol	100[a]	100[a]	100[a]	100[a]	100[a]
+ Camphor	100[a]	100[a]	100[a]	100[a]	100[a]
Sesame oil at 10 ml/kg	100[a]	100[a]	80[b]	70[c]	64[c]
Sesame oil at 5 ml/kg	100[a]	46[d]	0[f]	0[f]	0[f]
+ 1,8 cineole	100[a]	100[a]	100[a]	100[a]	100[a]
+ eugenol	100[a]	100[a]	100[a]	100[a]	100[a]
+ Camphor	100[a]	100[a]	100[a]	100[a]	100[a]
Mustard oil at 10 ml/kg	100[a]	100[a]	82[b]	70[f]	64[c]
Mustard oil at 5 ml/kg	100[a]	42[d]	6[f]	0[f]	0[f]
+ 1,8 cineole	100[a]	100[a]	100[a]	100[a]	100[a]
+ eugenol	100[a]	100[a]	100[a]	100[a]	100[a]
+ Camphor	100[a]	100[a]	100[a]	100[a]	100[a]

Mean of five replicates of 20 beetles each Mortality recorded after 24 h exposure to untreated and treated maize stored for 3 hours, 10,30,60 and 90 days Means followed by different letter(s) are significantly different at the 0 05 level, LSD test

The use of oils as synergistic and/or potentiation agents has received less attention compared with their contact toxicity, even though some studies have demonstrated that petroleum and vegetable oils can improve the efficacy and persistence of certain insecticides (Treacy et al 1991; Tembo & Murfitt 1995; Xie & lsman 1995). The reasons for the enhanced toxicity and persistence of 1, 8 cineole, eugenol and camphor when combined with vegetable oils were not investigated in the experiments Since these terpeniods are highly volatile and readily degradable, the most probable effect of oils is to reduce the rate of spray droplet evaporation of the compounds and increase uniformity of distribution over grain surface, thereby increasing insecticide pick-up by the insects The oils can also slow down the rate of penetration of chemicals into plant interiors (e g. grain), which may increase the probability of an insect contacting a lethal dose of the chemicals (Salt & Ford 1984) The oils can increase the rate of insecticide penetration into insect cuticle (Licastro et al 1983; Anderson et al 1986)

The practical use of vegetable oils as grain protectants is limited by the high rates required to disinfest grain (Don-Pedro 1989). For example, plant oils at 10 ml/kg had given the most effective control in previous studies where oil alone was tested In the present experiments, 5 ml oil/kg combined with 0 5 μl (1,8 cineole and eugenol) or 0.5 mg (camphor)/kg of maize achieved complete control of both beetle species exposed to treated maize which has been stored for 90 days following treatment It is even possible to use reduced levels of plant oils when combined with chemicals, thereby making their use more economical Furthermore, unrefined oils produced at village level may also be much cheaper than refined oils and thus could influence the economics of the treatments

Botanical pesticides represent an important potential for integrated pest management strategies in developing countries as they are broad spectrum in action, based on local materials and potentially less expensive Many are also safe to the environment and harmless to mammals, man and beneficial arthropods (Talukder & Howse 1995). Local initiatives aimed at preparation of traditional pesticides at the farm level should be promoted for those resource-poor farmers who have no access to commercial pesticides or cannot afford them Future work on these essential oils would focus on understanding the mode of action of these compounds particularly regarding their penetration through insect cuticle and grain testa as well as their effects on mammals fed on treated food. Detailled toxicological studies are also requred before they could be recommended for use in stored product protection.

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