

BEHAVIOURAL AND OLFACTORY RESPONSES OF *SITOPHILUS ZEAMAI* (COLEOPTERA: CURCULIONIDAE) TO *AFRAMOMUM MELEGUETA* AND *ZINGIBER OFFICINALE* OLEORESINS.

D. A. UKEH; SYLVIA B.A. UMOETOK; I. A. UDO AND KHALID ABDULLAH

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ABSTRACT

The oleoresins extracted from the seeds of alligator pepper, *Aframomum melegueta* and ginger, *Zingiber officinale* rhizome using methanol, was evaluated for bioactivity against the maize weevil, *Sitophilus zeamais* in the laboratory. Using a 4-arm olfactometer, solutions of the oleoresins at a concentration of 1 mg/ml exhibited significant olfactory repellent activity against male and female *S. zeamais* when tested alone, and in combination with maize seeds. These findings provide a scientific basis for the observed repellent properties of the oleoresins and demonstrate the need for their development in stored product pest protection in Africa where these plants are readily available.

KEYWORDS: Oleoresin, *Aframomum melegueta*, *Zingiber officinale*, *Sitophilus zeamais*, Olfactometer.

INTRODUCTION

The maize weevil, *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) is an important field to store pest of cereals in the tropics causing considerable losses estimated at about 96 million metric tonnes the world over (FAO, 1985; Throne, 1994). Post-harvest crop losses due to storage pests such as *S. zeamais* have continued to persist and pose major problems to food security in Africa (Markham *et al.*, 1994). Weight losses of over 30% have been reported in West Africa after a few months of storage (Kossou and Bosque-Perez, 1998). Ukeh and Udo (2008) reported the prevalence of *S. zeamais*, *Rhyzopertha dominica*, *Callosobruchus maculatus*, *Tribolium castaneum*, *Sitotroga cerealella* and *Plodia interpunctella* as the dominant pests of stored crops in Cross River State, Nigeria. In Ghana, out of an estimated total annual harvest of 250, 000 - 300, 000 tonnes of maize, about 20% was lost to storage pests like *S. zeamais* (Obeng-Ofori and Amiteye, 2005). Post-harvest losses caused by insect infestation and spoilage reduce the availability of maize in Cameroon throughout the year, and for the western highlands of Cameroon, losses in stored maize of 12-44% due to *S. zeamais* during the first 6 months of storage has been reported (Bouda *et al.*, 2001). Average dry weight losses of farm-stored maize for a storage period of 6 months caused by *S. zeamais* and *Prostephanus truncatus* (Horn.) (Coleoptera: Bostrichidae) in Togo has been estimated to range between 7% and 30% (Pantenius, 1988; Richter *et al.*, 1997). In Ethiopia in general, post-harvest losses caused by *S. zeamais* ranging from 20-30% are common, and studies in the Bako areas have shown grain damage levels up to 100% in some samples from

farm stores after 6-8 months (Emana, 1999; Demissie *et al.*, 2008). Apart from weight losses, the feeding damages caused by the larvae and adults *S. zeamais* were responsible for the reduction in aesthetic and market values, germination and nutritive value of maize from this region (Ukeh, 2008). The sources of infestation are the field prior to harvest, storage granaries containing infested commodities, alternative hosts or old and abandoned granaries. Alternatively, weevils may gain access passively when other commodities are brought in from infested stores or by transportation on vehicles, farm equipment, sacks, and baskets or even on clothing (Cox and Collins, 2002).

Although several methods have been developed as a part of an Integrated Pest Management (IPM) strategy, at present effective control of *S. zeamais* relies on the fumigation of stores using methyl bromide and phosphine (hydrogen phosphide) (Liu *et al.*, 2006). Methyl bromide, a broad-spectrum fumigant, has been identified as a chemical substance that contributes to degradation of the stratospheric ozone layer in the earth's atmosphere and is therefore being phased out (Fields and White, 2002; Rajendran and Sriranjini, 2008). Other notable drawbacks of phosphine include its corrosiveness to metals, insect resistance and control failures in some countries (Fields and White, 2002). It has therefore become absolutely necessary to look for cheap, environmentally sound and effective methods for reducing *S. zeamais* damage on stored grains.

Some traditional indigenous measures have been taken by small-scale farmers to protect stored maize from pest infestation (Hassanali *et al.*, 1997; Poswall and Akpa, 1991; Oparaeke and Kuhiep, 2006). But the precise processing and application of plant protectants varies from place to place, and appears to

D. A. Ukeh, Department of Crop Science, University of Calabar, PMB115, Calabar Nigeria
Sylvia B.A. Umoetok, Department of Crop Science, University of Calabar, PMB115, Calabar Nigeria
I. A. Udo, Department of Crop Science, University of Calabar, PMB115, Calabar Nigeria
Khalid Abdullah, Agricultural Research Institute, Dera Ismail Khan, Pakistan

depend on the availability, type and efficacy of suitable plants in different geographical locations. Important sources of repellents against stored-product pests are the essential oils extracted from aromatic plant species commonly used in food flavouring and in perfumery (Coppen, 1995; Isman, 2000; 2006). Chemotaxis along an odour gradient is probably the most important way for stored product insect orientation. Thus, it could be useful to reduce the concentration of a gradient by masking the attractive odours of host plants such as maize by repellent smells from oleoresins extracted from spices (Adler *et al.*, 2000; Ukeh, 2008). In Nigeria, the seeds, roots and leaves of ginger, *Zingiber officinale* Roscoe (Zingiberaceae), (Abubakar *et al.*, 2007) and alligator pepper, *Aframomum melegueta* K. Schum (Zingiberaceae) (Ajaiyeoba and Ekundayo, 1999) are used in spicing meat, sauces and soups and mixed with other herbs in traditional medicine for the treatment of body pains, catarrh, congestion, diarrhoea, sore throat, bronchitis, diabetes mellitus, cancer and rheumatism. Many farmers in the West African sub-region have limited economic means to store and preserve their harvested grains for longer periods due to *S. zeamais* infestation. The aim of this study was to evaluate the bioactivity of oleoresins extracted from the seeds of *A. melegueta* and rhizome of *Z. officinale* as potential candidates for *S. zeamais* control in Nigeria.

MATERIALS AND METHODS

Insect culture

Maize weevil was obtained from stock culture maintained by Central Science Laboratory, Sand Hutton, York, England, and reared on untreated Nigerian "Ikom white" maize, *Zea mays* (L.) and "local yellow" maize seeds in Kilner jars, at constant temperature and humidity (CTH) room running at 25 °C, 65% relative humidity on a 12:12 DL (darkness and light) photoperiod.

Plant materials collection and preservation

The seeds of *A. melegueta* and rhizomes of *Z. officinale* were purchased from Watt market in Calabar, Nigeria. The plant materials were dried in the shed to approximately 15% moisture content before transportation, and in Aberdeen, UK preserved in the refrigerator at -20 °C until required for the bioassays.

Preparation of Oleoresins

Seeds (100 g) from dried fruits of *A. melegueta* were ground into fine powder using a laboratory pestle and mortar (Maldenwanger, Berlin). The plant powder was then extracted with methanol (200 ml) for 24 hours at room temperature with additional stirring using a magnetic stir bar (IKA Labortechnik Staufen, Germany). The extract was filtered through filter paper and the residue re-extracted for another 24 hours before filtration. Magnesium sulphate was added to the combined filtrate to remove traces of moisture and then filtered again. Methanol was then removed by evaporation under vacuum using a rotary evaporator (Rotavapor Buchi 461, Switzerland) at room temperature to obtain the condensing pungent pale yellow oleoresin. *Z. officinale* oleoresin was obtained using the same procedure as described above. Solutions of the oleoresins in redistilled diethyl ether (10 mg in 10 ml)

were prepared, sealed under nitrogen and packed for laboratory bioassays against the maize weevil, *S. zeamais*.

Bioassay method

Behavioural bioassays were performed in a 4-arm olfactometer modified after Pettersson (1970). The olfactometer consisted of three layers of 6 mm thick transparent Perspex screwed together. A four-pointed star-shaped exposure chamber was milled into a circular plate measuring 12 x 12 x 1.2 cm, with a hole (3 mm diameter) drilled into the walls at each of the four cardinal points. Another plate (10.2 x 10.2 x 0.6 cm) served as the floor and the third plate of the same size but with a hole (4 mm diameter) in its centre, served as a cover. Since *S. zeamais* cannot walk on smooth surfaces a sheet of Fisherbrand QL 100 filter paper (Springfield Mill, Maidstone, Kent, England) was used as a floor covering. The olfactometer side arms made of socket glass were inserted through the holes of the chamber walls. Air was supplied by the Air entrainment system (KNF Neuberger, Germany) through Teflon tubing (Camlab Ltd., UK). Immediately after the pump, the air was divided through 2 charcoal filters to purify it. From each charcoal filter, the air stream was then further divided and pushed through two flow meters (GPE Ltd., Leighton Buzzard, UK) to give a total of four air flows going into the behaviour chambers. Each air stream then passed through a glass side arm with a net-covered inlet to prevent insect entry, which contained the stimulus source impregnated into clean filter paper discs. Solutions of oleoresins of *A. melegueta* and *Z. officinale* at a concentration of 1 mg/ml in 10 µl diethyl ether were the stimuli tested singly and in combination with 2 g yellow maize grains for bioactivity against *S. zeamais*. The test arm contained the stimulus while the 3 control arms contained 10 µl of freshly prepared redistilled diethyl ether loaded on filter paper discs. The test insects were 3 days old virgin adults which have been starved for 24 hours and kept singly in Petri dishes prior to the commencement of the bioassays. Each weevil was observed for 10 minutes using a stopwatch, and each trial was replicated 12 times in a complete randomized design. Test individuals and olfactometers were changed between replicates, while odour samples or stimuli were replaced after every 2 replications. From each glass side arm, air was delivered into the bioassay exposure chamber by the four air-delivery tubes. The rate of airflow through each side arm was set at 200 ml min⁻¹. The air streams formed four distinct zones in the chamber as shown by the smoke tests. The air was pulled from the chamber at the rate of 800 ml min⁻¹ through the central hole in the cover plastic plate. A computer programme (OLFA programme, 33100 Udine, Italy) was used to obtain data. The data recorded included the time spent by the weevil in the different areas of the olfactometer and the number of entries or visits into each area or odour zone.

Data analysis

The time spent in each olfactometer arm was tested using a one-way analysis of variance (ANOVA) followed by comparison of means by Tukey's 95% simultaneous confidence intervals (MINITAB 15 Statistical Software). Data on the number of entries or visits to the odour-treated arm was compared with the

number of visits in control arms using a “global” Chi-square contingency table (Zar, 1999).

RESULTS AND DISCUSSION

When solutions of *A. melegueta* and *Z. officinale* oleoresins were tested for bioactivity against *S.*

zeamais, no significant activity was observed in the control experiments involving the solvent, 10 μ l diethyl ether and clean filter paper control arms in the mean time spent (Figure 1) and mean number of visits (Table 1).

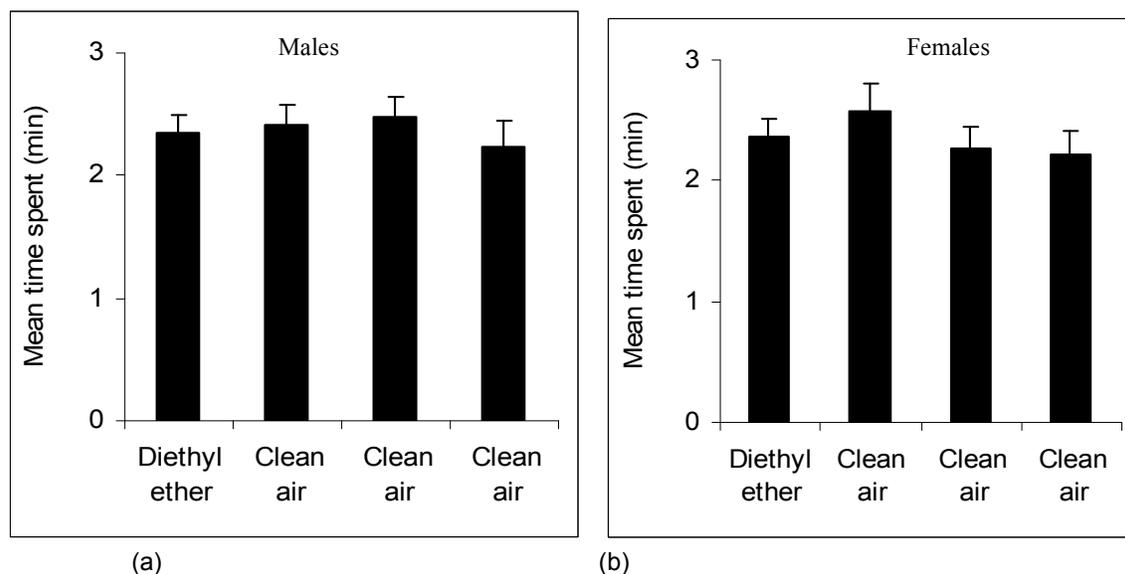


Figure 1: Mean time spent in the arm out of 10 min by *S. zeamais* males (a) and females (b) in response to 10 μ l diethyl ether and three control arms in a four way olfactometer. Control arms contained clean filter paper discs. Bars = standard errors (SE) of the means, n = 12.

Table 1 Mean number of visits made by adult *Sitophilus zeamais* in response to volatiles from 10 μ l stimuli of diethyl ether, or 10 μ l diethyl ether containing 1 mg/ml *Aframomum melegueta* or *Zingiber officinale* oleoresins tested alone or in combination with maize grains in olfactometry bioassays

Test Stimuli	Mean no. visits in olfactometer arm		n	χ^2 *	P
	T	C			
1. 10 μ l Diethyl ether against blanks					
Males	2.25	2.08	12	0.2	0.978
Females	2.33	2.56	12	0.33	0.954
2a. 1 mg/ml <i>Aframomum melegueta</i> oleoresin					
Males	1.33	2.72	12	8.04	0.045*
Females	1.17	2.56	12	10.15	0.017*
2b. 1 mg/ml <i>A. melegueta</i> oleoresin + 2 g maize					
Males	2.08	2.89	12	2.32	0.509
Females	1.5	2.28	12	2.8	0.424
3a. 1 mg/ml <i>Zingiber officinale</i> oleoresin					
Males	2.25	4.08	12	8.39	0.039*
Females	1.42	3.75	12	7.52	0.042*
3b. 1 mg/ml <i>Z. officinale</i> oleoresin + 2 g maize					
Males	2.83	3.81	12	2.5	0.475
Females	1.67	2.81	12	4.92	0.178

T is the mean value of test arm

C is the mean value of the mean of three control arms

* χ^2 analysis was performed on the total number of visits (n = 12) into the test, control 1, control 2 and control 3 arms in a 4 way contingency table.

However, 10 μ l diethyl ether containing 1 mg/ml *A. melegueta* oleoresin showed significant ($P < 0.001$) repellent activity against male and female *S. zeamais*

individually, in the mean time spent when compared with the control arms. *A. melegueta* oleoresin was also repellent against males ($P = 0.026$) and females

($P=0.029$) in combination with 2 g yellow maize seeds in the mean time spent when compared with the control arms containing 10 μ l diethyl ether (Figure 2a, b, c, d). In the number of visits, the males ($\chi^2 = 8.04$, $df = 3$, $P=0.045$) and females ($\chi^2 = 10.15$, $df = 3$, $P=0.017$) significantly preferred control arms to the test arm when tested alone. But both sexes failed to make any significant choice between the test and control arms when equal amounts of *A. melegueta* oleoresin was tested in combination with 2 g yellow maize kernels (Table 1). Olfactometry bioassays also showed that *Z. officinale* oleoresin was repellent to the male ($P<0.001$) and female ($P<0.001$) weevils alone, and to the male ($P<0.001$) and female ($P<0.001$) in combination with 2 g

yellow maize seeds in the mean time spent in the arms (Figure 3a, b, c, d). For the number of visits, the males ($\chi^2 = 8.39$, $df = 3$, $P=0.039$) and females ($\chi^2 = 7.52$, $df = 3$, $P=0.042$) significantly preferred the solvent control arms to the test, in response to *Z. officinale* oleoresin alone. However, both sexes failed to show significant choice of test or control arms when *Z. officinale* oleoresin was presented in combination with 2 g yellow maize seeds (Table 1). The weevils spent less time in the olfactometer arm with yellow maize plus *Z. officinale* oleoresin but the frequency of visits was not significantly ($P=0.05$) different between the test and control arms.

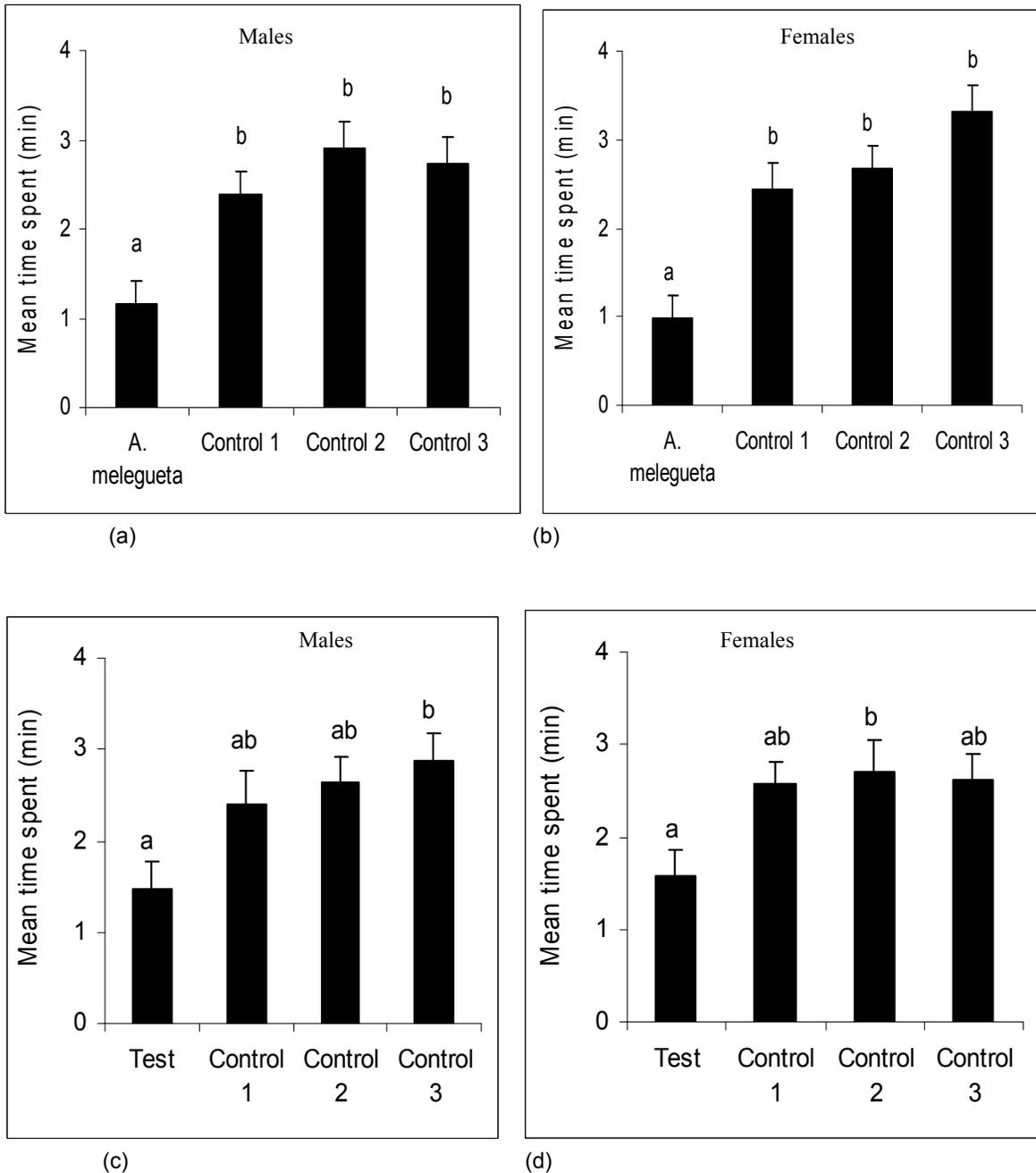


Figure 2 Mean time spent out of 10 min by males (a) and females (b) *S. zeamais* in response to 10 μ l solvent containing 1 mg/ml *Aframomum melegueta* oleoresin. Mean time spent by males (c) and females (d) *S. zeamais* in response to 10 μ l solvent containing 1 mg/ml *A. melegueta* oleoresin tested in combination with 2 g maize grains in a four way olfactometer. Control arms contained 10 μ l diethyl ether loaded on filter paper discs. Bars = standard errors

of the means, $n = 12$. Bars followed by the same letter are not significantly different from each other ($P > 0.05$). a-d: a (males), $P < 0.001$; b (females), $P < 0.001$; c (males), $P = 0.026$; d (females), $P = 0.029$.

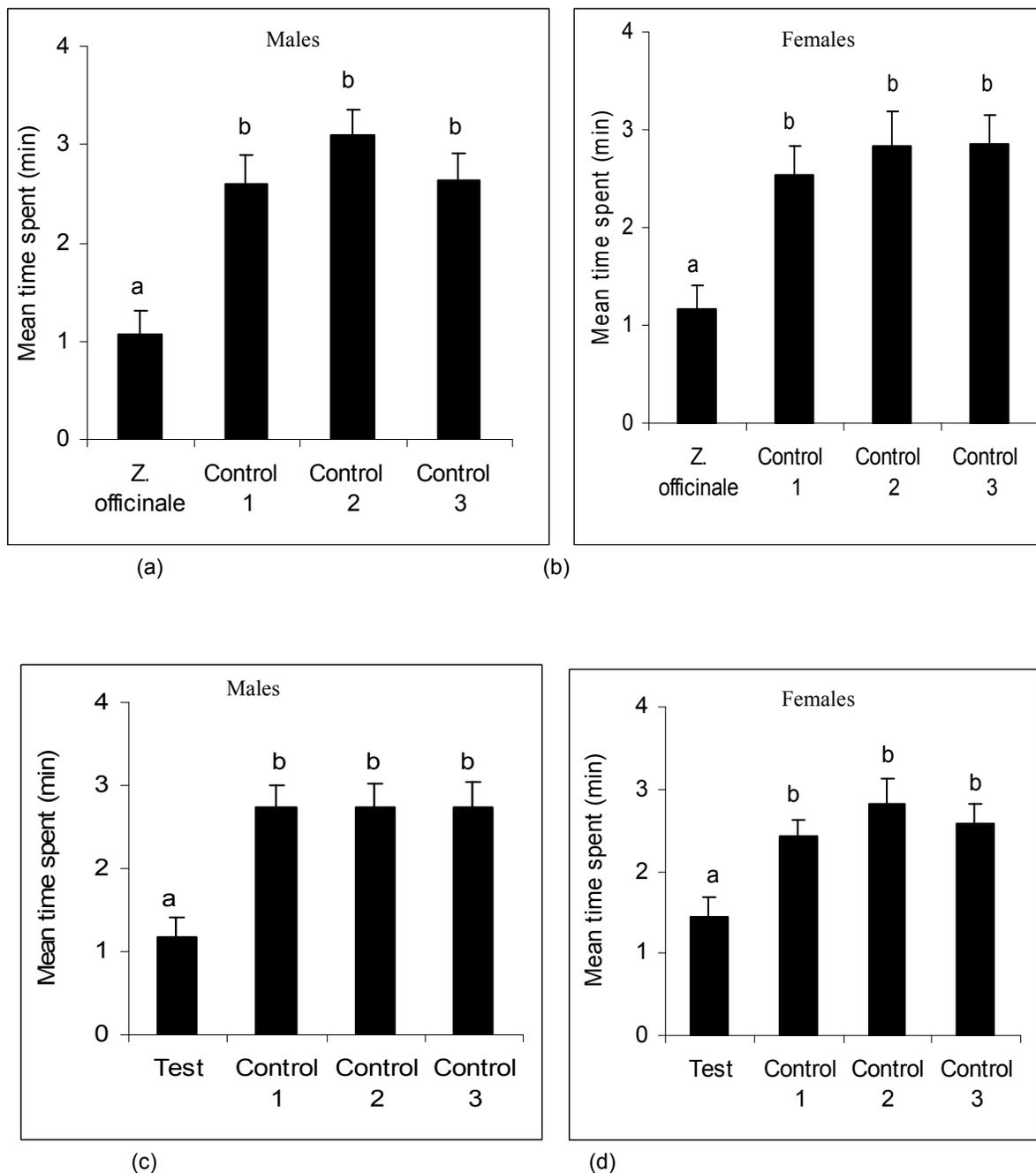


Figure 3 Mean time spent in the arm out of 10 min by males (a) and females (b) *S. zeamais* in response to 10 μ l diethyl ether containing 1 mg/ml *Zingiber officinale* oleoresin. Mean time spent by males (c) and females (d) *S. zeamais* in response to 10 μ l diethyl ether containing 1 mg/ml *Z. officinale* oleoresin tested in combination with 2 g maize grains in a four way olfactometer. Control arms contained 10 μ l diethyl ether loaded on filter paper discs. Bars = standard errors of the means, $n = 12$. Bars followed by the same letter are not significantly different from each other ($P > 0.05$). a-d: a (males), $P < 0.001$; b (females), $P < 0.001$; c (males), $P < 0.001$; d (females), $P < 0.001$.

The results of this study showed that *A. melegueta* and *Z. officinale* oleoresins appear to repel *S. zeamais* even in the presence of the host plant, *Z. mays*. The oleoresin (when resins are associated with volatile oils) of *A. melegueta* and *Z. officinale* are combination of both the important characteristics such as aroma and

pungency compounds in the same extract. Olfactory cues provided by plants have been shown to act as key factors in the process of host selection by many phytophagous insects (Visser, 1986), and this could be effectively utilized for the protection of stored grains especially in the developing world. Masking or coating of

sacks containing stored grains with oleoresin could repel stored product pests such as *S. zeamais* from the product by interfering with the odours from host grains thereby protecting them from infestation. Some plant oils and oleoresins have been reported to show a broad spectrum of activity such as insecticidal, antifeedant, repellent, oviposition deterrent and growth regulatory activities against insect pests and plant pathogenic fungi (Koul *et al.*, 2008). The use of tropical plant oils and oleoresins to control insect and tick pests through repellency, immobilization or antifeedance has been reported elsewhere. Dormont *et al.* (1997) reported that field sprays of mountain pine (*Pinus uncinata* Ram.) cones with oleoresin extracts of Swiss stone pine (*Pinus cembra* L.) cones significantly reduced the overall damage of specialized cone insects in the French Alps. None of the cones sprayed with oleoresin were attacked, whereas 11% and 31% of the unsprayed control cones were damaged by insects. Specific cone damage due to a cone weevil, *Pissodes validirostris* Gyll. (Coleoptera: Curculionidae), and the cone Pyralid, *Dioryctria mutata* Fuchs (Lepidoptera: Pyralidae), were also significantly decreased in one year. Crude *Z. officinale* extracts have been reported to exhibit antifeedant and insect growth disruption activity against the armyworm, *Spodoptera litura* (Fabricius) larvae (Sahayaraj, 1998), and to control the cowpea bruchid, *Callosobruchus maculatus* (Fabricius) (Echendu, 1991), and the cowpea aphid, *Aphis craccivora* (Koch) (Ofuya and Okuku, 1994). Similarly, Agarwal *et al.* (2001) reported the insect growth regulatory and antifeedant activity of *Z. officinale* oleoresin against *Spilosoma obliqua* (Walker), and antifungal activity against *Rhizoctonia solani* (Kuhn). *S. obliqua* is an insect pest of vegetables and oilseeds, and *R. solani* is a soil fungus causing damping off of seedlings, root/stem rot and stem canker in many vegetables and field crops. Singh *et al.* (2008) also reported 100% mycelial zone inhibitory activity of *Z. officinale* oleoresin against food-borne pathogenic fungi *Fusarium moniliforme* at 6 µl dose using inverted Petri plate technique. Antiectoparasitic activity of the gum resin, gum haggard, from the East African plant, *Commiphora holtziana* (Burseraceae) against the cattle tick, *Boophilus microplus* and the red poultry mite, *Dermanyssus gallinae* have also been established (Birkett *et al.*, 2008).

The characteristic pungent and aromatic odour of these Zingiberaceae plants, *A. melegueta* and *Z. officinale* are reported to be the products of their natural organic constituents such as monoterpene and sesquiterpene hydrocarbons and alcohols (Bartley and Foley, 1994). While the marked repellent activity of *A. melegueta* and *Z. officinale* oleoresins against *S. zeamais* is believed to be due to the presence of various phenolic compounds in them. *Z. officinale* oleoresin has been reported to encompass several closely related phenolic alkenones such as gingerols, shogaols, zingiberones, paradols, gingerdiols and other pungent principles like diarylheptanoids, gingerenones, dehydroshogaol and cyclic diarylheptanoids that are biogenetically derived from phenylalkanes (Kikuzaki *et al.*, 1992; Kikuzaki and Nakatani, 1993; Wu *et al.*, 1998). [6]-Gingerol and [6]-shogaol are the main components accounting for 50% of the gingerol and shogaol groups, which differ from each other in the lengths of their respective aliphatic chains (Yoshikawa *et al.*, 1993). The

chemical composition of oleoresins or oils from the same plant or plant part could vary due to the environment, genetic or other factors such as production conditions, or the nature of the solvent used for the extraction. Data from this study have shown that *A. melegueta* and *Z. officinale* oleoresins have the potentials in stored product protection under tropical small scale granary against known storage pests.

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