Bioactivity of essential oils from five spices against *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae)

Samantha Ramlal[1], Ayub Khan[1*], Russel Ramsewak[2] and Faisal Mohammed[2]

[1]Department of Life Sciences, and [2]Department of Chemistry
The University of the West Indies, St. Augustine, Trinidad, West Indies

**Corresponding author email*: ayub.khan@sta.uwi.edu

Sitophilus zeamais **is a serious pest of stored products worldwide. We report for the first time on the repellent and contact toxicity of the essential oils of *Myristica fragrans, Cinnamomum verum, Pimenta racemosa, Syzygium aromaticum* and *Cuminum cyminum* against an important stored product pest, *Sitophilus zeamais* feeding on *Pisum sativum*. Eugenol was the dominant constituent of both *P. racemosa* (76.9%) and *S. aromaticum* (55%), while cinnamaldehyde (62%), cuminaldehyde (89.8%) and elemicin (59%) were the most prevalent components of *C. verum, C. cyminum* and *M. fragrans* respectively. Essential oils of *M. fragrans, P. racemosa, C. verum* and *S. aromaticum* were repellents at concentrations of 4 and 8µL/mL. *M. fragrans* essential oil was the most toxic against *S. zeamais* with 100% mortality occurring within 2 days at 100µL/mL. Significant mortality was also observed with *C. verum* essential oil causing 78% and 97% of deaths at concentrations of 75 and 100µL/mL respectively. The LC50 values for *M. fragrans* and *C. verum* oils were 10.16 and 56.47µL/mL with the corresponding LT50 values of 1.44 and 2.48 days respectively. These results indicate that the essential oils from *M. fragrans* and *C. verum* may be explored as potential natural repellent and contact insecticides against *S. zeamais* in stored products.**

Keywords: Essential oils, bioactivity, maize weevil, GC/MS analysis, probit analysis

The maize weevil, *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) is a pest that causes severe damage to stored cereal crops in tropical and sub-tropical regions (Throne 1994; Danho et al. 2002). The primary cereal affected is corn (*Zea mays* L.), while its secondary hosts may include non-cereal commodities such as dried yam (*Dioscorea* spp.) and dried cassava (*Manihot esculenta* Crantz) Gnonlonfin et al. 2008). Most farmers in developing countries have open storage systems for maize and post-harvest losses in untreated, open stored maize due to *S. zeamais* as high as 90% have been documented (Throne 1994; Markham et al. 1994). *S. zeamais* is a small weevil measuring 2.5 - 4.5mm in length. It is predominantly found in warm, tropical regions of the world especially where maize is cultivated. Infestation by *S. zeamais* usually commences in the field just before the maize crop reaches maturity while the destructive activities occur in storage (Zakka et al. 2013). Adult females deposit their eggs in individual maize kernels and upon hatching the larva begins tunneling through the interior of the grain. *S. zeamais* has four larval instars and a pupal stage all of which occur inside the kernel (Zakka et al. 2013; Longstaff 1981). Grain damage by both larvae and adults cause reduction in grain quality and weight which decrease commercial value considerably thus affecting both farmers and traders (Mwololo et al. 2012). Feeding by *S. zeamais* also decreases the viability and the nutritional value of the grains (Caneppele et al. 2003). The infestation also enhances mould development including *Aspergillus* spp. which creates aflatoxins making the grain unfit for consumption (Caneppele et al. 2003; Sinha and Sinha 1991).

Synthetic insecticides including phosphine, pirimiphos methyl and malathion are primarily used to protect stored cereal from post-harvest losses caused by insect pests as these are considered the most effective and efficient control strategy (Conti et al. 2010; Ileke and Oni 2011). Despite their widespread usage in stored product insect management there are undesirable aspects of their use including presence of chemical residues in food products, pest resistance and resurgence, high cost (due to frequent applications as a result of insect resistance), harmful effects on

beneficial and non-target organisms and environmental pollution (Cherry et al. 2005). These problems have increased public consciousness of the hazards of synthetic insecticide usage and have led to the search for alternative pest management strategies. The use of controlled or modified atmosphere storage, physical measures (e.g. use of inert materials and sanitation) and the use of essential oils have been studied to develop safe and effective alternative pest control methodologies for post-harvest storage environments (Conti et al. 2010; Ileke and Oni 2011; Cherry et al. 2005). Some of these controls have already been integrated with others and implemented under practical conditions, for example modified atmosphere in rice mills in Portugal for control of both *S. zeamais* and *Sitophilus oryzae* L. (Coleoptera:Curculionidae (Carvalho et al. 2012).

The present study investigates the use of essential oils as an alternative control strategy for *S. zeamais.* Essential oils are natural volatile compounds produced by aromatic plants and are characterized by a unique fragrance (Bakkali et al. 2008). They have been found to have toxic, repellent, insecticidal and antifeedant effects to various insect species (Conti et al. 2010; Ileke and Oni 2011; Bakkali et al. 2008). Essential oils possessing low mammalian toxicity, fast degradation and with local availability are a safe alternative to conventional synthetic insecticides against stored grain pests (Liu et al. 2010). A wide range of investigations to validate essential oils' monumental contribution to stored grain protection have been documented (Regnault-Roger 1997; Cosimi et al. 2009; Chu et al. 2011; Brahmi et al. 2016) and is on-going as more research is being conducted.

The objectives of the present study were to extract, determine the percentage composition of the essential oils from clove (*Syzygium aromaticum*), cumin (*Cuminum cyminum*), nutmeg (*Myristica fragrans*), cinnamon (*Cinnamomum verum*), West Indian bay leaf (*Pimenta racemosa*) and elucidate their toxicity and repellent activity against *S. zeamais*. These essential oils were used as they are readily available, non-toxic to consumers at concentrations tested and may form part of a sustainable management programme for *S. zeamais*.

Materials and methods

Rearing of Sitophilus zeamais

The initial *S. zeamais* stock was cultured from parent insects obtained from a store bought pack of yellow split pea (*Pisum sativum* L.). Fifteen mixed sexed adults were placed into each of three polythene containers (18cm x 14cm x 9cm) with tightly fitted mesh covered lids each containing 700g of *P. sativum* as the food source. Containers were kept at room temperature (27^0C) in darkness for 45 days to allow for population growth.

Extraction and analysis of essential oils

Essential oils were extracted from dried flower buds of clove, seeds of cumin and nutmeg, the bark of cinnamon and leaves of West Indian bay leaf. *S. aromaticum*, *C. cyminum*, *M. fragrans* and *C. verum* were ground to a powder using a grinding mill while *P. racemosa* leaves were chipped into smaller pieces and milled separately to a paste using a kitchen blender prior to steam distillation. Essential oil was extracted from 500g of each sample in 300mL of distilled water in a Clevenger-type steam distillation apparatus for 6 hours per sample. Each distillate was placed into a separation funnel and oils extracted using dichloromethane (at 2x200mL dichloromethane). The oils collected were dried over anhydrous sodium sulphate, filtered and stored in glass vials. The vials were labeled, weighed and the mass of each of the oils determined.

Essential oils were analyzed using a Perkin Elmer Clarus 500 Gas Chromatography-Mass Spectrometer (GC-MS) with a split-splitless injector and Elite 5MS fused-silica capillary

column (30m × 0.25mm i.d., 0.5µm film thickness). The carrier gas (helium) was used at a flow rate of 0.5mL/min and the sample was introduced *via* splitless injection with the injector port maintained at 250ºC. The oven temperature-programmed run was as follows: initial temperature at 80ºC then increased at a rate of 5ºC/min to a final temperature of 300ºC. The interface temperature was kept at 220ºC and the MS scan involved the ionization technique of electron impact with a scanning range from 50 to 450 amu at 0.1 second intervals. Identification and percentage composition of the chemical constituents were inferred based on comparison of the obtained mass spectra with authenticated NIST and WILEY libraries supplied with the GC-MS.

Repellent activity

The repellent effect of essential oils of clove, cumin, nutmeg, cinnamon and West Indian bay leaf was evaluated against *S. zeamais* adults. Twenty-five Whatman® No. 4 filter discs (9 cm diameter) were cut in halves. Four concentrations (1, 2, 4 and 8 µL/mL acetone) of each essential oil were prepared. One milliliter of each concentration was uniformly applied to one half of the filter paper using a micropipette. The other half was treated with 1mL of acetone and served as the control. Both halves were allowed to air dry for 10 minutes and then re-joined and secured with clear sellotape. The re-made disc was placed tape side down in a 9cm Petri dish together with 10 unsexed adult *S. zeamais.* Insects were released at the centre of the filter disc and covered with mesh covered petri dish covers. There were five replicates per treatment. The number of insects on the treated and control halves of the disc was recorded after 2 and 4 hours. The procedure was repeated for each concentration of each essential oil. The repellent activity of the essential oils was categorized based on the Repellent Index of Kogan and Goeden (1970) as either attractant (RI > 1 + SD), indifferent (RI between 1 – SD and 1 + SD) or repellent (RI < 1 – SD). Percentage repellency of the essential oils was also classified as Class 0 – 0-0.1% repellency, Class I – 0.1 – 20%, Class II – 20.1- 40%, Class III – 40.1-60%, Class IV – 60.1-80%, Class V – 80.1-100% (Juliana and Su 1983).

Mortality bioassay

The mortality of *S. zeamais* adults when treated with the essential oils of clove, cumin, nutmeg, cinnamon and bay leaf was studied at room temperature in darkness. Concentrations of 0, 50, 75 and 100µL of each essential oil per mL of acetone were prepared and 5mL mixed with 40g *P. sativum* in 375mL glass jars. The jars were tumbled for 5 minutes to allow the test solutions to be evenly distributed throughout the surface of the food media. Samples were allowed to air dry for 10 minutes for the solvent to evaporate. Ten unsexed *S. zeamais* adults were introduced to each jar which was covered with nylon mesh and firmly held in place with rubber bands. There were five replicates for each concentration of each essential oil. Dead insects were counted and removed daily for 7 days.

Statistical analysis

Percentage repellency was determined using the formula and classification of Juliana and Su (1983). The Repellent Index (Kogan and Goeden 1970) was also determined at four concentrations and two time periods for each essential oil. The mortality data were corrected for control mortality (Abbott 1925) and then subjected to probit analysis to determine LC_{50} and LT_{50} values for each essential oil (Finney 1971).

Results

Yield and composition of essential oils

The yields of the essential oils extracted from 500g raw material of each spice were: nutmeg 3.34g (0.67% yield, bay leaf 7.65g (1.53%), cinnamon 5.06g (1.01%), cumin 7.34g (1.45%) and Clove 84.12g (16.82%). The composition of the different essential oils analysed using GC-MS is shown in Table 1. Eugenol ($C_{10}H_{12}O_2$ MW = 164.204) (76.9%) (Figure 1) and Chavicol ($C_9H_{10}O$ MW = 134.175) (Figure 2) were the main constituents in *P. racemosa*. In *S. aromaticum* eugenol and caryophyllene ($C_{15}H_{24}$ MW = 204.357) (Figure 3) were the major components comprising 55.1% and 26.6% respectively. (E)-Cinnamaldehyde (C_9H_8O MW = 132.162) (Figure 4) and trans-cinnamic acid (Figure 5) accounted for 62.0% and 23.3% respectively of the major constituents of *C. verum.* In *C. cyminum*, cuminaldehyde ($C_{10}H_{12}O$ MW = 148.205) (Figure 6) was the main component (89.8%), while elemicin ($C_{12}H_{16}O_3$ MW = 208.257) (59.1%) (Figure 7) and myristicin ($C_{11}H_{12}O_3$ MW = 192.214) (23.5%) (Figure 8) were the two key constituents in the essential oil of *M. fragrans.*

Table 1: Chemical composition of the five essential oils tested

Compound	Percentage composition				
	Pimenta racemosa (West Indian bay leaf)	*Cinnamomum verum* (Cinnamon)	*Syzygium aromaticum* (Clove)	*Cuminum cyminum* (Cumin)	*Myristica fragrans* (Nutmeg)
.Alpha.-isopropylbenzyl alcohol	---	---	---	8.46	---
1-Acetyl-4,6,8-trimethylazulene	0.073	---	---	---	---
1-decene, 2,4-dimethyl-	---	---	0.047	---	---
2-(1-hydroxyethyl) norbornadiene	---	---	0.265	---	---
2-(2-hydroxyphenoxy)-1-phenylethanol	---	---	0.318	---	---
4-hydroxymethylbenzaldehyde	---	---	0.201	---	---
4-N-Propylacetophenone	0.131	---	---	---	---
α-Cadinol	0.443	---	---	---	---
Allo-Aromadendrene	0.227	---	---	---	---
Anethole	0.248	---	0.758	0.386	---
α-Pinene	––	---	---	0.075	---
Aromadendrene	0.284	---	---	---	---
α-Terpinene	––	---	---	0.471	---
Benzaldehyde, 3-(phenylmethoxy)-	---	0.240	---	---	---
Benzeneacetic acid, .alpha.-methylmethyl ester	---	0.373	---	---	---
Benzeneacetic acid, .alpha.-oxo-ethyl ester	---	0.804	---	---	---
Benzeneacetic acid, .alpha.-oxo-methyl ester	---	0.222	---	---	---
Bicyclo[2.1.1]hex-2-ene, 2-ethenyl	---	---	0.103	---	---
Bicyclo[4.1.0]heptane,-3-cyclopropyl,-7-hydroxymethyl, trans	---	---	0.666	---	---
Caryophyllene	0.312	---	**26.63**	---	---
Caryophyllene Oxide	0.198	---	---	---	---
Chavicol 4-(2-propenyl)phenol	**19.644**	---	---	---	

Table 1 continued…

Compound	Percentage composition				
	Pimenta racemosa (West Indian bay leaf)	*Cinnamomum verum* (Cinnamon)	*Syzygium aromaticum* (Clove)	*Cuminum cyminum* (Cumin)	*Myristica fragrans* (Nutmeg)
Cinnamaldehyde, (E)-	---	**62.002**	---	---	---
Cinnamyl alcohol	---	0.479	---	---	---
Cinnamyl alcohol acetate	---	0.31	---	---	---
Cumarin	---	6.883	---	---	---
Cuminaldehyde	---	---	---	**89.772**	---
Cyclohexane, 1-butenylidene-	---	---	0.212	---	---
Cyclohexanol, 1-(2-hexenyl)-	0.476	---	---	---	---
Cyclohexene, 1-(3-ethoxy-1-propenyl)-, (z)-	---	---	---	0.126	---
Dioctyl isophthalate	0.05	---	---	---	---
d-Limonene	---	---	---	---	0.159
Elemicin	---	---	---	---	**59.145**
Ethanone, 1,2-diphenyl-	---	---	0.123	---	---
Eugenol	**76.941**	---	**55.147**	---	0.424
Germacene-D	---	---	---	---	0.116
γ-Gurjunene	0.443	---	---	---	---
Glycolophenone	---	3.675	---	---	---
Harmine	0.106	---	---	---	---
Isoelemicin	---	---	---	---	1.541
Isoeugenol	0.082	---	---	---	---
Methanone, [3-(1-methylethyl)phenyl]phenyl-	---	1.408	---	---	---
Methyl nonanoate	---	---	0.071	---	---
Methyleugenol	---	---	---	---	1.249
Myristicin	---	---	---	---	**23.45**
Nerolidol 2	0.135	---	---	---	---
Ocimene	0.137	---	---	0.089	---
Perillaldehyde	---	---	---	0.229	---
Perillyl acetate	---	---	---	0.275	---
Phenol, 2-methoxy-3-(2-propenyl)-	---	---	12.254	---	---
Sabinene	---	---	---	---	1.449
Seychellene	0.072	---	---	---	---
Sylvestrene	---	---	3..210	---	---
Terpinen-4-ol	---	---	---	0.037	---
Tetradecanoic acid	---	---	---	---	12.466
Trans-cinnamic acid	---	**23.345**	---	---	---
Trans-pinocarveol	---	---	---	0.080	---
Vinyl trans-cinnamate	---	0.261	---	---	---
Total	**100**	**100**	**100**	**100**	**100**

Compounds and values in bold are present in essential oils at ≥ 20% of their total composition

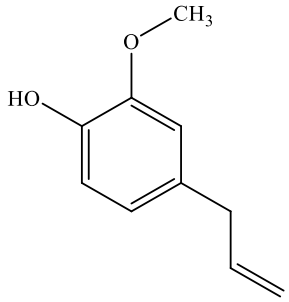

Figure 1: Eugenol (4-Allyl-2-methoxyphenol)

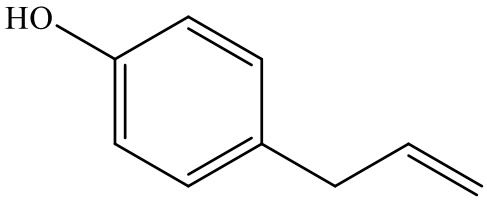

Figure 2: Chavicol (4-(2-propenyl)phenol)

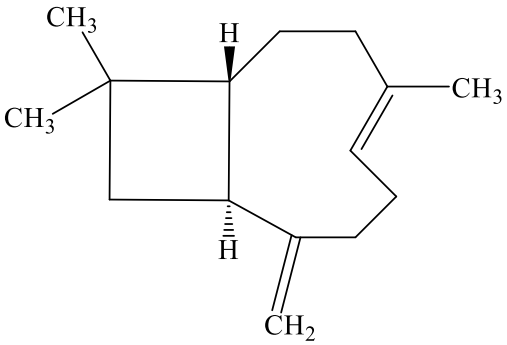

Figure 3: Caryophyllene ((1R,4E,9S)-4,11,11-trimethyl-8-methylidenebicyclo[7.2.0]undec-4-ene)

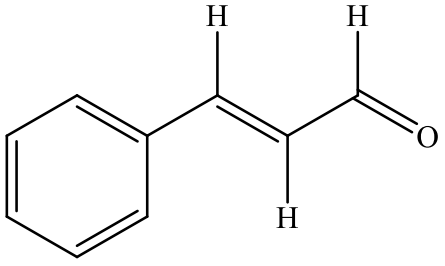

Figure 4: Cinnamaldehyde (E-3-phenylprop-2-enal)

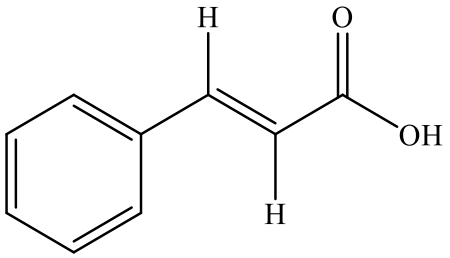

Figure 5: *Trans* – Cinnamic acid ((***E***)-3-phenylprop-2-enoic acid)

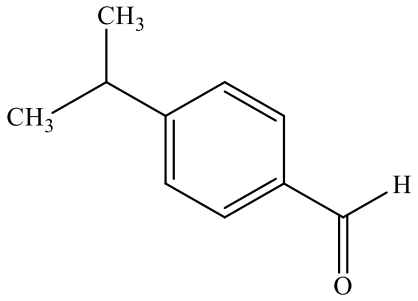

Figure 6: Cuminaldehyde (4-isopropylbenzaldehyde)

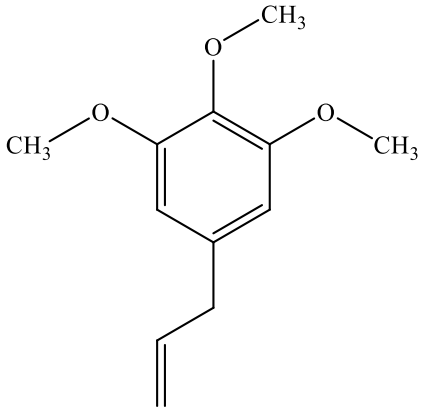

Figure 7: Elemicin (5-Allyl-1,2,3-trimethoxybenzene)

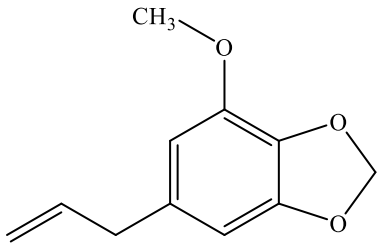

Figure 8: Myristicin (6-Allyl-4-methoxy-1,3-benzodioxole)

Repellent activity

Four of the essential oils –cinnamon, clove, bay leaf and nutmeg showed varying levels of repellent activity against *S. zeamais* whilst, the fifth, cumin oil was an attractant at the lowest concentration (1µL/mL) (Table 2). The repellent effect of the essential oils was greatly influenced by the concentrations administered and the duration of exposure. Clove oil had repellent action at 4 and 8µL/mL (Table 2). Both nutmeg and cinnamon oils were effective against *S. zeamais* only at the maximum concentration of 8µL/mL while bay leaf and cumin oils exhibited no significant (P>0.05) repellency at the end of the 2 hour period. Both cinnamon and bay leaf essential oils demonstrated similar repellent activity after a 4 hour period against *S. zeamais* when applied at concentrations of 4 and 8µL/mL. The clove and nutmeg oils were repellent to *S. zeamais* at the highest concentration of 8µL/mL after 4 hours whilst cumin oil exhibited no significant (P>0.05) effect against the insects. Oils were also classified as repellents based on their percentage repellency Juliana and Su (1983). Among the five oils tested, only cinnamon oil at 8µL/mL after 2 and 4 hours was classified as a Class V repellent while it was a Class IV repellent at 4µL/mL at 2 hours. Nutmeg oil was a Class IV repellent at 8µL/mL at 2h (Table 3).

Table 2: Repellent effect of four concentrations of five essential oils against *Sitophilus zeamais* at 2 hours and 4 hours

Essential oil	Concentration (µL/mL)	Mean ± SD value of Repellent Index (RI)[1]	
		2 hours	4 hours
Cinnamomum verum (Cinnamon)	1	1.28 ± 0.33 (I)	0.96 ± 0.33 (I)
	2	0.88 ± 0.30 (I)	0.80 ± 0.24 (I)
	4	1.08 ± 0.54 (I)	0.36 ± 0.30 (R)
	8	0.20 ± 0.14 (R)	0.16 ± 0.09 (R)
Cuminum cyminum (Cumin)	1	1.00 ± 0.32 (I)	1.32 ± 0.23 (A)
	2	1.00 ± 0.37 (I)	1.32 ± 0.58 (I)
	4	0.92 ± 0.50 (I)	0.88 ± 0.30 (I)
	8	1.20 ± 0.32 (I)	0.76 ± 0.50 (I)
Myristica fragrans (Nutmeg)	1	1.32 ± 0.44 (I)	0.84 ± 0.65 (I)
	2	1.12 ± 0.44 (I)	1.08 ± 0.50 (I)
	4	1.28 ± 0.54 (I)	1.08 ± 0.50 (I)
	8	0.40 ± 0.35 (R)	0.32 ± 0.18 (R)
Pimenta racemosa (West Indian bay leaf)	1	0.84 ± 0.48 (I)	0.96 ± 0.43 (I)
	2	0.80 ± 0.63 (I)	0.76 ± 0.30 (I)
	4	0.88 ± 0.30 (I)	0.80 ± 0.14 (R)
	8	1.12 ± 0.48 (I)	0.60 ± 0.37 (R)
Syzygium aromaticum (Clove)	1	0.68 ± 0.33 (I)	1.00 ± 0.14 (I)
	2	0.72 ± 0.44 (I)	1.12 ± 0.23 (I)
	4	0.60 ± 0.20 (R)	0.60 ± 0.42 (I)
	8	0.60 ± 0.20 (R)	0.64 ± 0.26 (R)

[1] Repellent Index based on Kogan and Goeden (1970). A – Attractant (RI > 1 + SD), I – Indifferent (RI between 1 – SD and 1 + SD), R – Repellent (RI < 1 – SD).

Table 3: Percentage repellency of four concentrations of five essential oils against *Sitophilus zeamais* at 2 hours and 4 hours

Essential oil	Concentration (µL/mL)	% Repellency (Class)[1]	
		2 hours	4 hours
Cinnamomum verum (Cinnamon)	1	28 (II)	4 (I)
	2	48 (III)	40 (II)
	4	68 (IV)	48 (III)
	8	96 (V)	84 (V)
Cuminum cyminum (Cumin)	1	28 (II)	20 (I)
	2	32 (II)	28 (II)
	4	48 (III)	32 (II)
	8	56 (III)	32 (II)
Myristica fragrans (Nutmeg)	1	12 (I)	8 (I)
	2	28(II)	8 (I)
	4	32 (II)	16 (I)
	8	68 (IV)	48 (III)
Pimenta racemosa (West Indian bay leaf)	1	24 (II)	20 (I)
	2	40 (II)	32 (II)
	4	44 (III)	36 (II)
	8	52 (III)	40 (II)
Syzygium aromaticum (Clove)	1	50 (III)	40 (II)
	2	47 (III)	36 (II)
	4	40 (II)	20 (I)
	8	40 (II)	8 (I)

[1] Percentage Repellency based on Juliana and Su (1983) – Class 0 – 0-0.1%, Class I – 0.1 – 20%, Class II – 20.1-40%, Class III – 40.1-60%, Class IV – 60.1-80%, Class V – 80.1-100%

Mortality bioassay

Nutmeg oil gave a gradual increase in the mortality rate and at 50µL/mL produced 41% mortality of *S. zeamais* at the end of the day 1, whilst at 75 and 100µL/mL resulted in 81% and 87% of deaths respectively. By day 7, 97% mortality was observed for nutmeg oil at 50µL/mL, whereas at 75µL/mL, 98% mortality was recorded on day 6 and remained the same for day 7. At the highest concentration of 100µL/mL, 100% mortality was attained within two days of the trial.

Cinnamon oil exhibited the second highest mortality rate of the five oils tested. Mortalities of 10, 20 and 33% were observed for concentrations of 50, 75 and 100 µL/mL respectively after day 1. At a concentration of 50 µL/mL small increments in percentage mortality occurred until day 5, when there was 35% mortality until day 7. The 75 and 100µL/mL concentrations yielded a total of 78% and 97% mortalities respectively by day 7.

There were no deaths to *S. zeamais* when exposed to bay leaf oil at 50µL/mL dosage on day 1, while at 75 and 100 µL/mL mortalities of 8% and 10% respectively were recorded. Within 7 days, 50 and 75 µL/mL induced insect mortalities of 30% and 50% respectively, whereas the 100 µL/mL treatment achieved a maximum mortality of 54% within 5 days of exposure and remained constant thereafter.

Cumin oil was the fourth most toxic of all the oils in the mortality bioassay. The 50 µL/mL treatment reported no mortality for days 1 and 2,

however, on day 3 a mortality of 1% was observed which gradually increased to 3% on day 4 and remained constant thereafter. At a concentration of 75 µL/mL, 2% mortality was recorded on day 1 and gradually increased to 23% by day 7. For the 100 µL/mL concentration of cumin oil on day 1, 17% mortality was shown and a total of 55% by the day 7.

Clove oil demonstrated the lowest mortality to *S. zeamais* of the five oils used in the experiment. Death of *S. zeamais* began on day 3 with 3% for the 50 µL/mL concentration. Similarly 3% death was also recorded for the 75 µL/mL, but on day 2 of the trial, and a 5% death rate was observed on day 1 for the 100 µL/mL treatment. By day 7 a total of 5% mortality was documented for the 50 µL/mL concentration, while 16% and 21% deaths of the pest were detected for the 75 µL/mL and 100 µL/mL treatments respectively on day 6 and no further deaths were observed.

Nutmeg oil had the highest mortality (97%) of the five oils tested. Nutmeg oil at concentrations of 50, 75 and 100µL/mL demonstrated the same result of 97% mortality, which was the maximum when compared to the other five oils, whereas the clove oil displayed minimum deaths of 10 and 15% at concentrations of 75 and 100µL/mL respectively of all the oils.

The quantity of oil needed to kill 50% of the insects (LC_{50}) is shown in Table 4. Nutmeg oil (with a LC_{50} of 10.16µL/mL) was the most toxic of the five essential oils tested. Cinnamon oil exhibited the second lowest LC_{50} which was 56.47 µL/mL. Bay leaf oil and cumin oil LC_{50} values were: 86.66 µL/mL and 101.25 µL/mL respectively and were not significantly different ($P>0.05$) from each other. Lastly, clove oil was the least toxic oil to *S. zeamais* with an LC_{50} of 224.45 µL/mL which was significantly ($P<0.05$) lower than that of all the other oils tested. Table 5 provides the lethal time (LT_{50}) of the five essential oils when exposed to *S. zeamais*. The order of speed of mortality (LT_{50}) was: nutmeg oil> cinnamon oil> cumin oil, bay leaf oil> clove oil. Nutmeg oil took 1.44 days to kill 50% of the insects which was significantly ($P<0.05$) lower than all others tested. Cumin and bay leaf oils' lethal time were: 6.28 and 6.52 days respectively and were not significantly different ($P>0.05$) from each other. Clove oil took the longest to achieve 50% mortality (LT_{50} = 20.40 days). Nutmeg oil was the most toxic (LC_{50} = 10.16µL/mL) and also took the shortest time (LT_{50} = 1.44 days) to cause 50% mortality.

Table 4: Lethal concentration (LC_{50}) of five essential oils to *Sitophilus zeamais*

Essential oil	Probit equation	χ^2	LC_{50} (µL/mL) *	SE of LC_{50}	95% CI
Cinnamomum verum (Cinnamon)	y = 6.06x – 5.61	0.082	56.47[a]	1.05	51.65, 61.74
Cuminum cyminum (Cumin)	y = 5.72x – 6.48	0.006	101.25[b]	1.05	91.96, 111.47
Myristica fragrans (Nutmeg)	y = 2.48x + 2.50	0.238	10.16[c]	1.28	6.28, 16.44
Pimenta racemose (West Indian bay leaf)	y = 2.28x + 0.64	0.348	81.66[b]	1.11	66.43, 100.38
Syzygium aromaticum (Clove)	y = 2.35x – 0.51	0.044	224.45[d]	1.14	172.92, 291.34

*LC_{50} values followed by the same letter are not significantly different from each other based on Tukey test ($P>0.05$)

Table 5: Lethal time (LT_{50}) of *Sitophilus zeamais* when exposed to five essential oils

Essential oil	Probit equation	χ^2	LT_{50} (days)*	SE of LT_{50}	95% CI
Cinnamomum verum (Cinnamon)	y = 1.73x + 4.31	3.061	2.48[a]	1.10	2.06, 2.30
Cuminum cyminum (Cumin)	y= 1.41x + 3.87	3.056	6.28[b]	1.15	4.77, 8.27
Myristica fragrans (Nutmeg)	y = 2.14x + 4.66	2.340	1.44[c]	1.10	1.19, 1.75
Pimenta racemosa (West Indian bay leaf)	y = 1.54x + 3.75	1.787	6.52[b]	1.12	5.22, 8.15
Syzygium aromaticum (Clove)	y = 2.01x + 2.27	1.018	20.40[d]	1.17	15.02, 27.73

*LT_{50} values followed by the same letter are not significantly different from each other based on Tukey test (P>0.05)

Discussion

A total of 58 different compounds were identified from the essential oils of the five aromatic spices examined in this study. Both *P. racemosa* and *S. aromaticum* had eugenol as the main constituent which was similar to that reported previously (Pragadheesh et al. 2013). The major constituents of *C. verum* were (E)-Cinnamaldehyde and trans-cinnamic acid and were comparable to that obtained in the petiole of *C. verum* (Rao et al. 2007). Cuminaldehyde was the major constituent of *C. cyminum*, while elemicin and myristicin were the most abundant compounds of *M. fragrans* essential oil. The composition of essential oils from *C. cyminum* (Rana 2014) and *M. fragrans* (Maya et al. 2004) in other studies was similar to that of the current study.

The repellency test indicated that clove, nutmeg and cinnamon essential oils act as repellents against *S. zeamais* at concentrations of 4 and/or 8µL/mL for both 2 hours and 4 hours but exhibited no repellency at lower doses by the end of 4 hours. The efficacies of the essential oils demonstrate their dose dependence. Similar studies also indicate that increased repellent activity was observed with increasing concentrations of essential oils of *Laurelia sempervirens, Drimys winteri* and *Cinnamomum zeylanicum* tested (Zapata and Smagghe 2010; Fouad 2013; Kasim et al. 2014). Despite the fact that *M. fragrans, S. aromaticum* and *C. verum* essential oils demonstrated low repellent activity in this experiment, they are effective and efficient repellents and insecticides towards *S. oryzae, Tribolium castaneum, Callosobruchus maculatus* and *Rhyzopertha dominica* (Devi and Devi 2013; Ho 2000; Awojide et al. 2010; Shaytesteh and Ashouri 2010; Ojimelukwe and Adler 2002; Trongtokit et al. 2005). *P. racemosa* essential oil showed no repellency at the end of 2 hours however, by the end of 4 hours it exhibited repellent activity at the highest dosage. Likewise, cinnamon essential oil also showed an increase in repellent activity at the end of the 4th hour. These findings indicated that duration of exposure influenced the repellent effect of these essential oils. Similar results with cinnamon essential oil were obtained where there was maximum repellent activity against *S. zeamais* at 10mg/mL and exposed for a longer (2 hours) rather than a shorter (<2 hours) time period (Ishii, Matsuzawa, and Vairappan 2010). Additionally, cinnamon oil had a similar repellent effect against ants and house dust mites (Kasim et al. 2014; Huang and Ho 1998). Cinnamaldehyde is responsible for the potent flavour in cinnamon and has also been found to possess significant insecticidal properties (Ho 2000; Huanh and Ho 1998;

Mondal and Khalequzzaman 2006). These findings indicate that this active constituent may play a vital role in repellent activity against *S. zeamais*. Cumin essential oil displayed low repellent activity against *S. zeamais*. Data on the repellent activity of cumin oil against *S. zeamais* is limited, however, it exhibited 60.4% activity at the highest concentration tested against a related species, *Sitophilus oryzae* (Lashgari et al. 2013). Cumin oil has been listed as a Class III repellent (40.1 – 60% repellency) against *S. oryzae* (Juliana and Su 1983) which was also the same category as that obtained for *S. zeamais* at the highest concentration in the current study.

The essential oils of numerous plant species have been evaluated for their antifeedant, repellent and insecticidal activities against stored product pests (Huang et al. 1997; Liu and Ho 1999; Pugazhvendan et al. 2012). Moreover, spices and aromatic herbs in particular have been found to possess insecticidal properties (Huang et al. 1997). Freshly prepared garlic juice containing allicin has demonstrated insecticidal activity against *S. zeamais* (Nwachukwu and Asawalam 2014). Black pepper (*Piper nigrum*) seed powder caused high mortality in *S. zeamais* (Ishii et al. 2010). Spices are also frequently used in culinary preparations worldwide and are therefore considered safe for humans (if used as insecticides) as compared to non-edible plants (Huang et al. 1997). The essential oil from nutmeg was the most toxic of all essential oils tested with the lowest values of 10.16 µL/mL and 1.44 days for the LC_{50} and LT_{50} respectively. Nutmeg essential oil caused mortality at all life stages in both *S. zeamais* and *T. castaneum* as a result of contact and antifeedant activity (Huang et al. 1997). Similarly, nutmeg powder was very effective against *Dermestes maculatus* (Abdullahi et al. 2011). This insecticidal activity could be as a result of the presence of eugenol, limonene, tannic acid, asarone and citral in the oil (Golob and Webley 1980). Both elemicin and myristicin, present in high concentrations in nutmeg in the current study, have also been reported to possess insecticidal and acaricidal properties and cause high mortality in *S. zeamais* (Liu and Ho 1999; Huang et al. 2002; Muchtaridi et al. 2010; Ngamo et al. 2007).

Cinnamon essential oil was the second most toxic to *S. zeamais* after nutmeg, causing 50% mortality in 2.48 days at 56.47 µL/mL, analogous to total mortality of *S. oryzae* after 48 hours of exposure to cinnamon essential oil (Paranagama et al. 2004). The bioactivity of essential oils is mainly dependent on the volatile compounds they contain (Pugazhvendan et al. 2012). (E)-Cinnamaldehyde, the major constituent of *C. verum* in this study, has potent antifeedant and toxic effects to *T. castaneum* and *S. zeamais* (Ho 2000; Huang and Ho 1998; Mondal and Khalezuzzaman 2006). Studies on the efficiency of West Indian bay leaf essential oil against stored product insects are limited, however, numerous authors have reported on the toxicity of eugenol, the main constituent of *P. racemosa* to a wide array of insects (Enan 2001; Kim et al. 2008; Alitonou et al. 2012; Zhang et al. 2015). A moderately toxic effect was observed for cumin essential oil against *S. zeamais* which was also dose dependent. This mortality was attributed to the presence of large quantities of cuminaldehyde which causes mortality in several stored product pests including *T. confusum, S. granarius* and *C. chinensis* (Chaubey 2008; Martinez-Velazquez et al. 2011; Ziaee 2014).

The increasing evidence of the catastrophic effects on the environment and health due to the repeated use of conventional pesticides has necessitated research efforts in the development of safer pesticides (Perez et al. 2010). The essential oils tested in this study may be useful in production of these safe and eco-friendly insecticides for sustainable protection of stored products.

Conclusion

This study demonstrated the toxic and repellent activity of the essential oils from five spices against *S. zeamais* a serious pest of stored grain. The essential oils from clove, cinnamon, bay leaf and nutmeg oils were moderately repellent, while nutmeg oil exhibited maximum mortality of *S. zeamais* within a short duration. These essential oils thus make them ideal candidates for development as potential grain protectants against *S. zeamais* in a sustainable pest management programme.

Disclosure of interest

The authors report no conflict of interest.

References

Abbott, W.S. 1925. "A Method for Computing the Effectiveness of an Insecticide." *Journal of Economic Entomology* **18**:265-267.

Abdullahi, F., F. Auwal, B. Bello, and M. Yushau. 2011. "Laboratory Efficacy of Nutmeg (*Monodora myristica*) Powder against the Beetle *Dermestes maculatus* (Coleoptera: Dermestidae) on Treated Smoked Catfish (*Clarias gariepinus*) in Kano State of Nigeria." *International Journal of Pharmacology and Applied Science* **2**:52-55.

Alitonou, G.A., J.P. Noudogbessi, P. Sessou, A. Tonouhewa, F. Avlessi, C. Menut, and D.C.K. Sohounhloue. 2012. "Chemical Composition and Biological Activities of *Pimenta racemosa* (Mill). J.W Moore from Benin." *International Journal of Bioscience* **2**:1-12.

Awojide, S., H. Fayemiwo, and A. Kehinde. 2010. "Bioefficacy of Vapour Effect of Essential Oil Formulation from *Syzygium aromaticum* against *Callosobruchus maculatus*." *Nature Science* **8**:244-249.

Bakkali, F., S. Averbeck, D. Averbeck, and M. Idaomar. 2008. "Biological Effects of Essential Oils." *Food Chemistry and Toxicology* **46:**446-475.

Brahmi, F., A. Abdenour, M. Bruno, P. Silvia, P. Alessandra, F. Danilo, Y.G. Drifa, E.M. Fahmi, M. Khodir, and C. Mohamed. 2016. "Chemical Composition and *In Vitro* Antimicrobial, Insecticidal and Antioxidant Activities of the Essential Oils of *Mentha pulegium* L. and *Mentha rotundifolia* (L.) Huds Growing in Algeria." *Industrial Crops and Products* **88:**96-105.

Caneppele, M.A.B., C. Caneppele, F.A. Lazzari, and S.M.N. 2003. "Correlation between the Infestation Level of *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) and the Quality Factors of Stored Corn *Zea mays* L. (Poaceae). *Revista Brasileira de Entomologia* **47**:625-630.

Carvalho, M.O., I. Pires, A. Barbosa, G. Barros, J. Ruidavets, A.C. Garcia, C. Brites, and S. Navarro. 2012. "The Use of Modified Atmospheres to Control *Sitophilus zeamais* and *Sitophilus oryzae* on Stored Rice in Portugal." *Journal of Stored Products Research* **50**:49-56.

Chaubey, M.K. 2008. "Fumigant Toxicity of Essential Oils from Some Common Spices against Pulse Beetle, *Callosobruchus chinensis* (Coleoptera: Bruchidae)." *Journal of Oleo Science* **57**:171-179.

Cherry, A.J., P. Abalo, and K. Hell. 2005. "A Laboratory Assessment of the Potential of Different Strains of the Entomopathogenic Fungi *Beauveria bassiana* (Balsamo) Vuilleum and *Metarhizium anisopliae* (Metschnikoff) to Control *Callosobruchus maculatus* (F.) (Coleoptera:Bruchidae) in Stored Cowpea." *Journal of Stored Products Research* **41**:295-309.

Chu, S.S., J. Feng Hu, and Z.L. Liu. 2011. Composition of Essential Oil of *Chenopodium ambrosoides* and Insecticidal Activity against Maize Weevil, *Sitophilus zeamais. Pest Management Science* **67:**714-718.

Conti, B., A. Canale, P.L. Cioni, and G. Flamini. 2010. "Repellence of Essential

Oils from Tropical and Mediterranean Lamiaceae against *Sitophilus zeamais*." *Bulletin of Insectology* **63**:197-202.

Cosimi S, Rossi E, Cioni PL, Canale A. 2009. "Bioactivity and Qualitative Analysis of Some Essential Oils from Mediterranean Plants against Stored-Product Pests: Evaluation of Repellency against *Sitophilus zeamais* Motschulsky, *Cryptolestes ferrugineus* (Stephens) and *Tenebrio molitor* (L.)." *Journal of Stored Products Research* **45:**125-132.

Danho, M., C. Gaspar, and E. Haubruge. 2002. "The Impact of Grain Quantity on the Biology of *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae): Oviposition, Distribution of Egg, Adult Emergence, Body Weight and Sex Ratio." *Journal of Stored Products Research* **38**: 259-266

Devi, K.C., and S.S. Devi. 2013. "Insecticidal and Oviposition Deterrent Properties of Some Spices against the Coleopteran Beetle, *Sitophilus oryzae*." *Journal of Food Science and Technology* **50**:600-604.

Enan, E. 2001. "Insecticidal Activity of Essential Oils: Octopaminergic Sites of Action." *Comparative Biochemistry and Physiology – Part C: Toxicology & Pharmacology* **130**:325-337.

Finney, D.J. 1971. *Probit analysis*, 3rd ed., 8-48. New York: Cambridge University Press.

Fouad, H.A. 2013. "Bioactivity of Five Essential Oils against *Bruchidius incarnatus* (Bohemann, 1833)." *Notulae Scientia Biologicae* 5:354-359.

Gnonlonfin, G.J.B., K. Hell, A.B. Siame, and P. Fandohan. 2008. "Infestation and Population Dynamics of Insects on Stored Cassava and Yam Chips in Benin, West Africa." *Journal of Economic Entomology* **101:**1967-1973.

Golob, P., and D.J. Webley. 1980. "The Use of Plants and Minerals as Traditional Protectants of Stored Products." Tropical Products Institute G138. Post-Harvest Pest and Quality Section, Natural Resources Institute, Chatham, United Kingdom.

Ho, S.H. 2000. "Response of *Tribolium castaneum* and *Sitophilus zeamais* to Potential Fumigants Derived from Essential Oils of Spices." In *Proceedings of the International Conference on Controlled Atmosphere and Fumigation in Stored Products, 29 October – 3rd November 2000*, edited by E.J. Donahaye, S. Navarro and J.G. Leesch, 119-124. Fresno, California.

Huang, Y., and S.H. Ho. 1998. "Toxicity and Antifeedant Activities of Cinnamaldehyde against the Grain Storage Insects, *Tribolium castaneum* (Herbst) and *Sitophilus zeamais* Motsch." *Journal of Stored Products Research* **34**:11-17.

Huang, Y., S.H. Ho, H. Chieh-Lee, and Y.L. Yap. 2002. "Insecticidal Properties of Eugenol, Isoeugenol and Methyleugenol and Their Effects on Nutrition of *Sitophilus zeamais* Motsch. (Coleoptera: Curculionidae) and *Tribolium castaneum* (Herbst) (Coleoptera: *Tenebrionidae*). *Journal of Stored Products Research* **38**: 403-412.

Huang, Y., J.M.W. Tan, R.M. Kini, and S.H. Ho. 1997. Toxic and Antifeedant Action of Nutmeg Oil against *Tribolium castaneum* (Herbst) and *Sitophilus zeamais* Motsch. *Journal of Stored Products Research* **33**:289-298.

Ileke, K.D., and M.O. Oni. 2011. "Toxicity of Some Plant Powders to Maize Weevil, *Sitophilus zeamais* (Coleoptera: Curculionidae) on Stored Wheat Grains." *African Journal of Agricultural Research* **6**:3043-3048.

Ishii, T., H. Matsuzawa, and C.S. Vairappan CS. 2010. "Repellent Activity of Common Spices against *Sitophilus zeamais* Motsch (Coleoptera: Curculionidae). *Journal of Tropical Biology and Conservation* **7:**75-80.

Julianna, G., and H.C.F. Su. 1983. "Laboratory Studies on Several Plant Materials as Insect Repellents for Protection of Cereal Grains." *Journal of Economic Entomology* **76**:154-157.

Kasim, N.N., S.N.A.S. Ismail, A.S. Masdar, F.A. Hamid, and W.I. Nawawi. 2014. "Extraction and Potential of Cinnamon Essential Oil towards Repellency and Insecticidal Activity." *International Journal of Scientific and Research Publications* **4**:1-6.

Kim, J., Y.S. Lee, S.G. Lee, S.C. Shin, and I.K. Park. 2008. "Fumigant Antifungal of Plant Essential Oils and Components of West Indian Bay (*Pimenta racemosa*) and Thyme (*Thymus vulgaris*) Oils against Two Plant Phytopathogenic Fungi." *Flavour and Fragrance Journal* **23**:272-277.

Kogan, M., and R.D. Goeden. 1970. "The Host Plant Range of *Lema tritineata daturaphila* (Coleoptera:Chrysomelidae)." *Annals of the Entomological Society of America* **62:**1175-1180.

Lashgari, A., S. Mashayekhi, M. Javadzadeh, and R. Marzban. 2013. "Effect of *Menthe Piperita* and *Cuminum cyminum* Essential Oil on *Tribolium castaneum* and *Sitophilus oryzae*." *Archives of Phytopathology and Plant Protection* **47**:324-329.

Liu, Z.L., and S.H. Ho. 1999. "Bioactivity of the Essential Oil Extracted from *Evodia rutaecarpa* Hook Thomas against the Grain Storage Insects, *Sitophilus zeamais* Motsch. and *Tribolium castaneum* (Herbst)." *Journal of Stored Products Research* **35**:317-328.

Liu, Z.L., Q.R. Liu, S.S. Chu, and G.H. Jiang GH. 2010. "Insecticidal activity and Chemical Composition of the Essential Oils of *Artemisia lavandulaefolia* and *Artemisia sieversiana* from China." *Chemistry and Biodiversity* **7:**2040-2045.

Markham, R.H., N.A. Bosque-Perez, C. Borgemeister, and W.G. Weikle. 1994. "Developing Pest Management Strategies for the Maize Weevil, *Sitophilus zeamais* and the Larger Grain Borer, *Prostephanus truncatus*, in the Humid and Sub-Humid Tropics." *FAO Plant Protection Bulletin* **42**:97-116.

Martinez-Velazquez, M., G.A. Castillo-Herrera, R. Rosario-Cruz, J.M. Flores-Fernandez, J. Lopez-Ramirez, R. Hernandez-Gutierrez, and E.D.C. Lugo-Cervantes. 2011. "Acaricidal Effect and Chemical Composition of Essential Oils Extracted from *Cuminum Cyminum, Pimenta dioica* and *Ocimum basilicum* Against Cattle Tick *Rhipicephalus* (*Boophilus*) *microplus* (Acari: Ixodidae)." *Parasitology Research* **108**:481-487.

Maya, K.M., T.J. Zachariah, and B. Krishnamoorthy. 2004. "Chemical Composition of Essential Oil of Nutmeg (*Myristica fragrans* Houtt.) Accessions. *Journal of Spices and Aromatic Crops* **13**:135-139.

Mondal, M., and M. Khalequzzaman. 2006. "Toxicity of Essential Oils against Red Flour Beetle, *Tribolium castaneum* (Herbst.) (Coleoptera: Tenebrionidae). *Journal of Biological Sciences* **14**:3-48.

Muchtaridi, S.A., A. Apriyantono, and R. Mustarichie. 2010. "Identification of Compounds in the Essential Oil of Nutmeg Seed (*Myristicia fragrans* Houtt.) that Inhibit Locomotor Activity in Mice. *International Journal of Molecular Sciences* **11**:4771-4781.

Mwololo, J.K., S. Mugo, P. Okori, T. Tefera, M. Otim, and S.W. Munyiri. 2012. "Sources of Resistance to the Maize Weevil *Sitophilus zeamais* in Tropical Maize." *Journal of Agricultural Science* **4:**206-215.

Ngamo, T.L.S., A. Goudoum, M.B. Ngassoum, L.G. Mapongmetsem, F. Malaisse, and T. Hance. 2007. "Chronic Toxicity of Essential Oils of Local Aromatic Plants towards *Sitophilus zeamais* Motsch. (Coleoptera: Curculionidae)." *African Journal of Agricultural Research* **2:**164-167.

Nwachukwu, I.D., and E.F. Asawalam. 2014. "Laboratory Evaluation of Freshly Prepared Juice from Garlic (*Allium sativum* L.) Liliaceae as Protectants against the Maize Weevil, *Sitophilus zeamais* (Motsch.) (Coleoptera: Curculionidae)." *African*

Journal of Biotechnology **13**:1123-1130.

Ojimelukwe, P., and C. Adler. 2002. "Potential of Cinnamaldehyde and Methylchavicol as Grain Protectants against Four Insect Pests of Stored Products." *Integrated Protection of Stored Products* **25**:147-152.

Paranagama, P.A., K.H.T. Abeysekera, L. Nugaliyadde, and K.P. Abeywickrama. 2004. "Repellency and Toxicity of Four Essential Oils to *Sitophilus oryzae* (Coleoptera: Curculionidae)." *Journal of the Natural Science Foundation* **32**:127-128.

Perez, S.G., M.A. Ramos-Lopez, M.A. Zavala–Sanchez, and N.C. Cardenas-Ortega. 2010. "Activity of Essential Oils as a Bio-Rational Alternative to Control Coleopteran Insects in Stored Grains." *Journal of Medicinal Plants Research* **4:**2827-2835.

Pragadheesh, V.S., A. Yadav, S.C. Singh, N. Gupta, and C.S. Chanotiya. 2013. "Leaf Essential Oil of Cultivated *Pimenta racemosa* (Mill.) J.W. Moore from North India: Distribution of phenylpropanoids and chiral terpenoids." *Medicinal and Aromatic Plants* **2**:118.

Pugazhvendan, S.R., P.R. Ross, and K. Elumalai. 2012. "Insecticidal and Pepellent Activities of Plant Oils against the Stored Grain Pest, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae). *Asian Pacific Journal of Tropical Diseases* S412-S415.

Rana, V.S. 2014. "Chemical Composition of the Essential Oil of *Cuminum cyminum* L. Seeds from Western India." *Journal of Medicinal Plants and By-products* **2**:207-210.

Rao, B.R.R., D.K. Rajput, and A.K. Bhattacharya. 2007. "Essential Oil Composition of *Cinnamomum verum* Bercht. and Presl." *Journal of Spices and Aromatic Crops* **16:**38-41.

Regnault-Roger, C. 1997. "The Potential of Botanical Essential Oils for Insect Pest Control." *Integrated Pest Management Reviews* **2:**25-34.

Shaytesteh, N., and S. Ashouri. 2010. "Effect of Four Powdered Spices as Repellents against Adults of *Rhyzopertha dominica* (F.), *Sitophilus granarius* (L.) and *Tribolium castaneum* (Herbst) in Laboratory Conditions." In *Proceedings of 10th International Working Conference on Stored Product Protection, 27 June - 2 July 2010*, edited by M.O. Carvalho, P.G. Fields, C.S. Adler, F.H. Arthur, C.G. Athanassiou, J.F. Campbell, F. Fleurat-Lessard, P.W. Flinn, R.J. Hodges, A.A. Isikber, S. Navarro, R.T. Noyes, J. Riudavets, K.K. Sinha, G.R. Thorpe, B.H. Timlick, P. Trematerra, and N.D.G. White. Berlin, Germany **425:**799-804.

Throne, J.E. 1994. "Life History of Immature Maize Weevils (Coleoptera: Curculionidae) on Corn Stored at Constant Temperatures And Relative Humidities in the Laboratory." *Environmental Entomology* **23**:1459-1471.

Trongtokit, Y., Y. Rongsrivam, N. Komalamisra, and C. Apiwathnasorn. 2005. "Comparative Repellency of 38 Essential Oils against Mosquito Bites." *Phytotherapy Research* **19**:303-309.

Zapata, N., and G. Smagghe. 2010. "Repellency and Toxicity of Essential Oils from the Leaves and Bark of *Laurelia sempervirens* and *Drimys winteri* against *Tribolium castaneum.*" *Industrial Crops and Products* **32:**405-410.

Zhang, W.J., K. Yang, C.X. You, C.F. Wang, Z.F. Geng, Y. Su, Y. Wang, S.S. Du, and Z.W. Deng. 2015. "Contact Toxicity and Repellency of the Essential Oil from *Mentha haplocalyx* Briq. against *Lasioderma serricorne.*" *Chemistry and Biodiversity* **12**:832-839.

Ziaee, M. 2014. "The Effects of Topical Applications of Two Essential Oils against *Sitophilus granaries* (Coleoptera: Curculionidae) and *Tribolium confusum* (Coleoptera: Tenebrionidae)." *Journal of Crop Protection* **3**:589-595.