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ORIGINAL PAPER

A comparative study of monoterpenoids and phenylpropanoids from essential oils against stored grain insects: acute toxins or feeding deterrents

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Abstract The relationship between the acute toxicity and feeding deterrent activity of ten compounds occurring commonly in essential oils was explored in order to determine whether they are acute toxins or antifeedants against stored-grain pests. Simultaneously, the objective was also to demonstrate the comparative efficacy against three post-harvest stored-grain pests. Thymol, carvacrol, eugenol and *trans*-anethole were specifically toxic, and linalool was a generalist feeding deterrent against all three species studied. Thymol was most toxic to *Tribolium castaneum* and *Rhyzopertha dominica* compared to carvacrol and eugenol but was least toxic to *Sitophilus oryzae*. Similarly, linalool deterred feeding of *S. oryzae* (FI_{50} = 0.025 mg/g of the wafer diet), *T. castaneum* (FI_{50} = 0.207 mg/g of the wafer diet) and *R. dominica* (FI_{50} = 0.482 mg/g of the wafer diet) at different concentrations; *R. dominica* beetles required about 20 times the concentration to deter feeding compared to *S. oryzae* and more than twice compared to *T. castaneum*. Comparison of toxicity and deterrent activity with respective artificial blends as binary mixtures revealed that synergism was not a generalized phenomenon, and the variations were both species as well as blend specific. Individual compound efficacy correlations were not ascertained, which suggests that artificial blends could be prepared to obtain potential mixtures for substantial control of stored-grain insect pests. The present study also implies that the compounds are mostly acute toxins, and whatever inhibition in feeding was obtained could be due to physiological toxicity rather than any interaction with gustatory receptors.

Keywords Essential oil compounds · Toxins, binary mixtures · Synergists · *Sitophilus oryzae* · *Tribolium Castaneum* · *Rhizopertha dominica*

Key message

- Compounds from essential oils are mostly acute toxins, and feeding inhibition is via physiological toxicity induced by these compounds.
- This mode of action has significance in playing a much greater role in the postharvest protection of food grains.
- Understanding the synergistic interaction of compounds as binary mixtures will help enhance the insecticidal spectrum of action, because various species have variable responses to individual compounds.

Introduction

It has been estimated that stored-grain losses are 5–10 % in developed countries and 35 % in developing countries (Boxall 1991). However, these losses can also go as high as 75 % because of insect consumption and contamination (Gorham 1991). In sub-Saharan Africa, losses in grain storage are valued to be about $ 4 billion a year (Mason and McDonough 2012). To manage these stored-grain pests, toxic fumigants such as methyl bromide, phosphine

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and sulfuryl fluoride have been used for decades; these are environmentally hazardous and have ozone-depleting effects (Makhijani and Gurney 1995). Accordingly, an endeavor has been to find alternatives, and one of them is the use of plant essential oils (EOs) (Koul et al. 2008), widely used as fragrances and flavors in the perfume and food industries. EOs are known to repel insects and also have contact and fumigant actions against specific insect pests (Koul et al. 2008; Germinara et al. 2015; Bedini et al. 2015; Abdelgaleil et al. 2016) and fungicidal actions against some of important plant pathogens (Al-Reza et al. 2010). The market for EOs as botanical insecticides has been growing in recent years, mostly because of relaxed safety and regulatory issues, and these are not always subject to rigorous testing or formal registration (Trumble 2002).

Due to the phytochemical diversity of EOs, they have potential as insecticides as they contain many biosynthetically different compounds and many analogs of one class. These compounds have been evaluated singly and in binary mixtures (Scott et al. 2002; Koul et al. 2013). Efficacy of monoterpenoid EOs against coleopterans such as *S. oryzae* (rice weevil), *Stegobium paniceum* (drugstore beetle), *T. castaneum* (red flour beetle), *Bruchus chinensis* (pulse beetle) and *Rhyzopertha dominica* (lesser grain borer) is well documented (Shaaya et al. 1991, 1994; Lee et al. 2001a, b; Kim et al. 2003; Tripathi et al. 2003; Rozman et al. 2007; Abdelgaleil et al. 2009; Yildirim et al. 2013; Park et al. 2016).

In India, most of the work done is related to general evaluation of vegetable oils and essential oils against various stored-product insects (Koul et al. 2008). The only detailed investigation available on active allelochemicals is on the sulfur compounds from neem (Koul 2004), carvone (Tripathi et al. 2003), *trans*-anethole (Koul et al. 2007) and some constituents of *Derris scandens* (Hymavathi et al. 2011). At the global level, toxic effects of essential oils (Shaaya et al. 1991; Sarac and Tunc 1995) and volatile terpenoid compounds (Karr and Coats 1988; Weaver et al. 1991; Shaaya et al. 1994; Ho et al. 1997; Huang and Ho 1998; Huang et al. 2000; Kim et al. 2016) are known for several coleopteran storage pests, but most of the research is based on fumigation studies at the bench scale against the major stored-product insects.

What is intriguing at this stage is whether these compounds are purely acute toxins or feeding inhibitors. While acute toxins could have diverse target sites, an insect antifeedant can specifically be defined as a behavior-modifying compound that acts directly on gustatory receptors and deters the feeding of an insect. Most studies suggest simple contact or fumigant toxicity of the compounds (Lee et al. 2001a, b; Koul et al. 2008; Abdelgaleil et al. 2009; Yildirim et al. 2013). However, many studies on botanicals also suggest that these are feeding-deterrent compounds against stored-product insect pests, and they were comprehensively reviewed recently (Nawrot and Harmatha 2012). Thus, the objectives of the present work were to determine the mode of action of EOs, specifically to know whether the toxicity is due to the modification of feeding behavior of the insects or something else, and also to determine the species specificity. Accordingly, the compounds chosen for the present study were common constituents of EOs of Laminaceae and Lauraceae plants. Some of the compounds such as thymol from *Thymus vulgaris* in Europe (Rice et al. 2002) and eugenol from clove oil in the USA (Wilson and Isman 2006) have already been commercialized. In this experiment, ten compounds belonging to monoterpenoid and phenylpropanoid groups were evaluated against three economically important stored-grain pests using various bioassays designed to meet the objectives of the study.

Materials and methods

Insects

Adults of *Sitophilus oryzae*, *Tribolium castaneum* and *Rhyzopertha dominica* (2–5 day old) were obtained from routine cultures in the laboratory. *T. castaneum* were bred in a mixture of wheat flour and yeast (12:1), and *S. oryzae* and *R. dominica* were cultured on whole wheat grain at 32 ± 2 °C and 70–75 % R.H.

Test compounds

Ten essential oil compounds (Fig. 1) were used in the study. The compounds occur commonly in Lamiaceae and Lauraceae plants. These were namely α-terpineol, thymol, carvacrol, *trans*-anethole, 1,8-cineole, linalool, eugenol, pulegone, verbenone and fenchone, representing aromatic, bicyclic, acyclic and keto- groups in essential oils. The pure compounds (97–99 % purity) were evaluated (procured from Sigma/Aldrich Chemie, GmbH, Germany, and Acros Organics, Morris Plains, NJ, USA).

Toxicity bioassay

The toxicity of compounds was determined against three stored-grain pests by a contact toxicity procedure. The test compounds were dissolved in acetone to reach the desired concentrations. To the insides of the bottom of glass petri dishes, 500 µl of each concentration of the compounds was applied. In the control treatment, only the solvent was used. The petri dishes were gently rotated to deposit compounds evenly (covering an approximately 65 cm^2 area) and kept

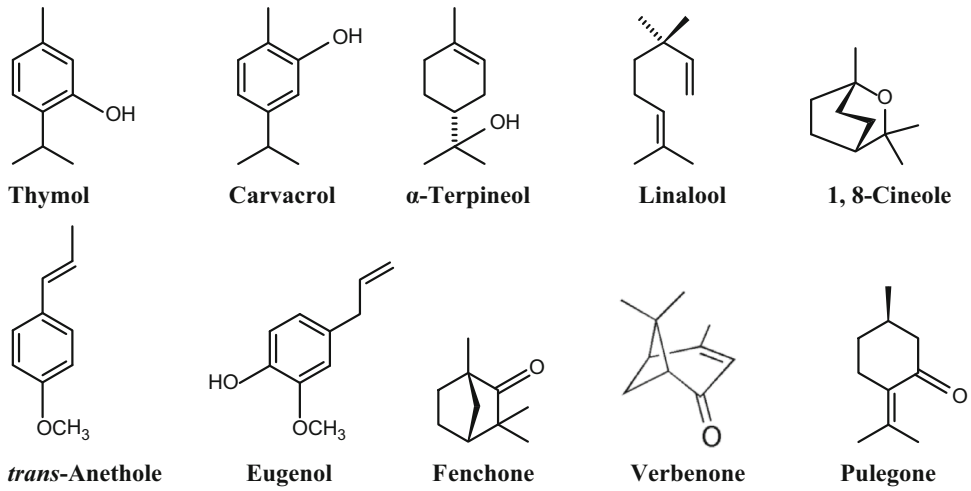

Fig. 1 Compounds used in the present study

as such to allow evaporation of the solvent. Concentrations ranged between 7.5 and 40.0 μg/cm^2 surface area. Adult beetles (2 to 5 days old) were used. The test insects ($n = 10$/replicate; 10 replicates) were transferred from the nucleus culture to petri dishes. The petri dishes were then covered with lids, and the edges were sealed with parafilm to prevent the escape of insects. Observations were recorded 24 and 48 h after treatment. LC_{50} and LC_{95} values were determined by probit analysis (Finney 1971).

Antifeedant evaluation

The 'wafer disc method' was used to determine the antifeedant activity using the previously described method (Paruch et al. 2000); 1-cm-diameter wafer discs weighing 17.75 ± 0.85 (mg ± SE) were impregnated with the test compounds. The weight of the discs was taken after the solvent had evaporated. For the antifeedant action, five insects per treatment in five replicates of unsexed adults (1 week old) were used. Various concentrations used for the treatment ranged between 0.05 and 1.0 mg/g of the disc, depending on the efficacy of the compound evaluated. Each disc was impregnated with 20 μl of treatment solution; however, in controls (CC) only solvent-impregnated discs were used. The duration of treatment was until 50 % of the disc had been consumed in the controls. The experiment was conducted with no-choice and choice tests. In the no-choice test, insects were forced to eat two test wafer disks (TT), and in the choice test, the insects were offered a choice between a control and test disk (CT). On the basis of the food consumed, a relative index of deterrence (when the insects were offered a choice of food) and an absolute index of deterrence (insects without the possibility of choice) were calculated for each concentration. The deterrence index was classified on the basis of the total coefficient of deterrence in order to determine the absolute antifeedance, if any, for each compound in comparison to azadirachtin, a known antifeedant compound (Koul 2005). From these data, three coefficients—relative (from choice tests), absolute (from no-choice tests) and total (the sum of two previous values)—were calculated. Classification of the total coefficients enabled a precise evaluation of compound activity. Azadirachtin was used as a positive control for comparison.

Feeding deterrence coefficients were calculated as follows:

$$\text{Absolute coefficient of deterrence} = [(CC - TT)/(CC + TT) \times 100] \quad \underline{A}$$

$$\text{Relative coefficient of deterrence} = [(C - T)/(C + T) \times 100] \quad \underline{R}$$

$$\text{Total coefficient of deterrence} = \sum \underline{A} + \underline{R}$$

Values below 0 = attractant

0 to 50 = poor deterrent

51 to 100 = medium deterrent

101 to 150 = good deterrent

151 to 200 = very good deterrent

The data obtained in these tests were also used to determine inhibition of feeding of 50 % (FI_{50}) and 90 % of the population (FI_{90}) by regression analysis.

Toxic effects of binary mixtures

The compounds that showed a toxicity effect in contact experiments were mixed in a 1:1 (w/w) ratio at half of the total concentration at the LC_{50} level. The experiments were conducted in similar fashion as described above. LC_{50} values were determined for the mixtures, and actual mortalities were compared to expected mortalities using a well-known procedure (Trisyono and Whalon 1999).

Antifeedant effects of binary mixtures

The compounds that exhibited antifeedant activity were mixed in a 1:1 (w/w) ratio such that each represented half of the total dose was tested in the feeding deterrence evaluation of individual compounds. Only choice tests were carried out and analyzed as described for individual compounds. FI_{50} values were determined for the mixtures.

Synergistic effects of a toxin and antifeedant mixture

The compounds that exhibited antifeedant and toxic activity individually were mixed in a 1:1 (w/w) ratio at two levels: (1) each represented half of the FI_{50} and LC_{50} values and (2) each represented by the levels of FI_{50} and LC_{50} values, respectively. Choice tests were carried out and analyzed as described for individual compounds. FI_{50} values were determined for the mixtures in order to determine the enhancement in activity, if any, in order to suggest synergistic effects.

Statistical analysis

Data were subjected to one-way ANOVA, and means were separated by Tukey's post hoc test. LC_{50} and LC_{95} were calculated by probit analysis. FI_{50} and FI_{90} were calculated by regression analysis using the StatPlus program 2008 (Analystsoft®).

Results

Acute contact toxicity

The toxicity of the test compounds was variable and species specific. The toxicity of various compounds to the adults of three different stored-grain pests, *Sitophilus oryzae*, *Tribolium castaneum* and *Rhyzopertha dominica*, varied among different species. While thymol, carvacrol, eugenol and *trans*-anethole were toxic to all three species (Tables 1, 2, 3), pulegone and verbenone were specifically toxic to *S. oryzae* and α-terpineol and verbenone to *R. dominica*. Thymol was most toxic to *T. castaneum* and *R. dominica* with an LC_{50} of 11.21 and 8.8 μg/cm^2, but in case of *S. oryzae*, eugenol was the best (LC_{50} = 16.08 μg/cm^2) and significantly similar to carvacrol (LC_{50} = 17.15 μg/cm^2) treatment, as shown by the overlap of confidence limits at the 95 % level. Carvacrol, eugenol and anethole were significantly similar in activity against *T. castaneum* (Table 2), and so were anethole and verbenone against *R. dominica* (Table 3). Other compounds were toxic in the range of 1–20 % at the highest concentrations used in the evaluation (Tables 1, 2, 3).

Feeding-deterrent effects

The antifeedant effects of various compounds on the adults of three different stored-grain pests in a choice assay again varied among different species with an exception of eugenol being active against all three species (Tables 4, 5, 6). In case of *S. oryzae*, linalool was the best feeding deterrent (FI_{50} = 0.025 mg/g of the wafer disc) followed by eugenol (FI_{50} = 0.041 mg/g of the wafer disc), and these values are less than the lowest testing concentration (Table 4). In case of *T. castaneum*, only eugenol, anethole and linalool were active deterrents and significantly different in activity (Table 5) as no overlap of confidence intervals was observed. On the contrary, eugenol, α-terpineol, linalool and pulegone were active feeding deterrents against *R. dominica* but apparently similar in activity (Table 6) as all confidence intervals overlapped at the 95 % level. Other compounds were either totally inactive or the range of activity was <40 % at the highest concentrations used in the evaluation (Tables 4, 5, 6).

A wafer disc method used for the calculation of three coefficients of antifeedance—relative (from choice tests), absolute (from no-choice tests) and total (the sum of two previous values)—enabled a precise evaluation of the antifeedant activity of the compounds. In this experiment, it was clear that none of the compounds were absolute antifeedants as compared to the positive control azadirachtin (total antifeedant coefficient of 200), a known antifeedant compound from neem. In case of *S. oryzae*, eugenol, anethole, linalool and pulegone exhibited moderate total antifeedant coefficients (about 100) (Fig. 2) compared to only eugenol and linalool in case of *T. castaneum* (Fig. 3) and none in case of *R. dominica* (Fig. 4).

Acute contact toxicity of mixtures

The compounds that were found toxic to the respective insect species were combined to determine their toxicity in mixtures. The data obtained suggest that for all those combinations that contained eugenol or *trans*-anethole as one of the components, the LC_{50} values obtained were significantly lower than the expected values (Table 7). However, in case of *T. castaneum*, a combination of thymol and carvacrol was also more effective than individual compounds as the LC_{50} obtained was 9.16 μg/cm^2 as compared to the expected value (11.2–21.2 μg/cm^2), which was quite significant as no overlap of confidence interval at the 95 % level was recorded (Table 7). This was also true for *R. dominica* treatments, where a thymol + carvacrol combination gave an LC_{50} of 7.16 μg/cm^2 against the expected value of (8.8–9.2 μg/cm^2) (Table 7).

Table 1 Toxicity of various compounds (μg/cm^2) against *Sitophilus oryzae* in the contact assay

Compound	Slope ± SE	LC_{50} (confidence limits at 95 %)	LC_{90} (confidence limits at 95 %)	χ^2
Thymol	9.17 ± 0.58	24.07 (23.09–25.08)	33.23 (31.31–35.31)	1.24
Carvacrol	4.41 ± 0.26	17.15 (15.92–18.46)	33.46 (30.15–37.23)	1.78
Eugenol	4.80 ± 0.46	16.08 (14.84–17.23)	29.54 (26.85–32.46)	4.89
trans-Anethole	7.05 ± 3.41	22.77 (17.01–30.69)	34.69 (22.31–53.77)	102.7[a]
α-Terpineol	10 % mortality at 40 μg/cm^2			
Linalool	18 % mortality at 40 μg/cm^2			
Pulegone[b]	8.74 ± 5.44	42.85 (27.92–65.77)	60.08 (29.23–123.08)	122.4[a]
Verbenone	10.06 ± 1.05	35.77 (34.54–37.11)	47.92 (45.07–50.92)	2.87
Fenchone	2 % mortality at 40 μg/cm^2			
1,8-Cineole	4 % mortality at 40 μg/cm^2			

[a] High χ^2 suggests steep mortality values at higher concentrations

[b] LC_{50} >40 μg/cm^2 and the value given for pulegone are extrapolated from the probit

Table 2 Toxicity of various compounds (μg/cm^2) against *Tribolium castaneum* in the contact assay

Compound	Slope ± SE	LC_{50} (confidence limits at 95 %)	LC_{90} (confidence limits at 95 %)	χ^2
Thymol	5.38 ± 0.37	11.21 (10.35–12.14)	19.38 (17.57–21.39)	1.31
Carvacrol	5.53 ± 0.95	21.16 (18.58–24.09)	36.08 (29.71–43.82)	13.92[a]
Eugenol	4.12 ± 0.43	18.88 (17.18–20.74)	38.59 (33.68–44.22)	1.56
trans-Anethole	9.97 ± 2.32	20.06 (17.85–22.41)	26.89 (22.89–31.58)	6.67
α-Terpineol	18 % mortality at 40 μg/cm^2			
Linalool	2 % mortality at 40 μg/cm^2			
Pulegone	18 % mortality at 40 μg/cm^2			
Verbenone	12 % mortality at 40 μg/cm^2			
Fenchone	1 % mortality at 40 μg/cm^2			
1,8-Cineole	5 % mortality at 40 μg/cm^2			

[a] High χ^2 suggests steep mortality values at higher concentrations

Table 3 Toxicity of various compounds (μg/cm^2) against *Rhyzopertha dominica* in the contact assay

Compound	Slope ± SE	LC_{50} (confidence limits at 95 %)	LC_{90} (confidence limits at 95 %)	χ^2
Thymol	3.85 ± 0.76	8.80 (6.94–11.15)	18.91 (14.84–24.09)	7.30
Carvacrol	4.27 ± 0.83	9.18 (7.38–11.42)	18.32 (14.52–23.13)	8.15[a]
Eugenol	10.75 ± 0.38	15.67 (14.92–16.46)	20.62 (19.25–22.07)	1.17
trans-Anethole	6.97 ± 1.12	25.25 (23.02–27.70)	38.56 (33.08–44.95)	7.09
α-Terpineol[b]	8.71 ± 0.59	43.18 (41.64–44.78)	60.59 (56.35–65.14)	1.84
Linalool	20 % mortality at 40 μg/cm^2			
Pulegone	4 % mortality at 40 μg/cm^2			
Verbenone	4.62 ± 0.45	23.63 (22.12–25.24)	44.72 (39.55–50.55)	3.81
Fenchone	1 % mortality at 40 μg/cm^2			
1,8-Cineole	2 % mortality at 40 μg/cm^2			

[a] High χ^2 suggests steep mortality values at higher concentrations

[b] LC_{50} >40 μg/cm^2 and the value given for α-terpineol are extrapolated from the probit

Table 4 Feeding deterrence (mg/g of wafer disc) due to various compounds in *Sitophilus oryzae* in the wafer disc choice assay

Compound	Slope ± SE	FI_{50} (confidence limits at 95 %)	FI_{90} (confidence limits at 95 %)	χ^2
Thymol	26.9 % Feeding deterrence at maximum concentration of 1.0 mg/g used			
Carvacrol	No feeding deterrence at maximum concentration of 1.0 mg/g used			
Eugenol	2.39 ± 0.36	0.041 (0.034–0.047)	0.138 (0.110–0.174)	4.06
trans-Anethole	2.19 ± 0.43	0.104 (0.079–0.137)	0.399 (0.245–0.651)	6.32
α-Terpineol	2.24 ± 0.23	0.071 (0.062–0.082)	0.267 (0.214–0.332)	4.84
Linalool	1.43 ± 0.32	0.025 (0.017–0.033)	0.197 (0.131–0.294)	4.25
Pulegone	2.34 ± 0.27	0.058 (0.051–0.067)	0.203 (0.166–0.249)	5.71
Verbenone	1.42 ± 0.38	0.059 (0.039–0.088)	0.466 (0.165–1.089)	7.0[a]
Fenchone	No feeding deterrence at maximum concentration of 1.0 mg/g used			
1,8-Cineole	1.46 ± 0.28	0.051 (0.035–0.075)	0.383 (0.204–0.718)	8.73[a]

[a] High χ^2 suggests steep mortality values at higher concentrations

Table 5 Feeding deterrence (mg/g of wafer disc) due to various compounds in *Tribolium castaneum* in the wafer disc choice assay

Compound	Slope ± SE	FI_{50} (confidence limits at 95 %)	FI_{90} (confidence limits at 95 %)	χ^2
Thymol	19.3 % Feeding deterrence at maximum concentration of 1.0 mg/g used			
Carvacrol	No feeding deterrence at maximum concentration of 1.0 mg/g used			
Eugenol	2.67 ± 0.39	0.010 (0.003–0.026)	0.055 (0.027–0.091)	1.5
trans-Anethole	3.06 ± 0.59	0.468 (0.389–0.562)	1.021 (0.778–1.932)	16.8[a]
α-Terpineol	6.67 % Feeding deterrence at maximum concentration of 1.0 mg/g used			
Linalool	3.59 ± 0.58	0.207 (0.187–0.229)	0.471 (0.381–0.582)	1.9
Pulegone	18.22 % Feeding deterrence at maximum concentration of 1.0 mg/g used			
Verbenone	No feeding deterrence at maximum concentration of 1.0 mg/g used			
Fenchone	No feeding deterrence at maximum concentration of 1.0 mg/g used			
1,8-Cineole	No feeding deterrence at maximum concentration of 1.0 mg/g used			

[a] High χ^2 suggests steep mortality values at higher concentrations

Table 6 Feeding deterrence (mg/g of wafer disc) due to various compounds in *Rhyzopertha dominica* in the wafer disc choice assay

Compound	Slope ± SE	FI_{50} (confidence limits at 95 %)	FI_{90} (confidence limits at 95 %)	χ^2
Thymol	38.4 % Feeding deterrence at maximum concentration of 1.0 mg/g used			
Carvacrol	30.0 % Feeding deterrence at maximum concentration of 1.0 mg/g used			
Eugenol	2.35 ± 0.58	0.449 (0.341–0.593)	1.572 (0.722–2.339)	12.07[a]
trans-Anethole	38.6 % Feeding deterrence at maximum concentration of 1.0 mg/g used.			
α-Terpineol	2.94 ± 0.92	0.503 (0.456–0.544)	1.373 (0.912–2.068)	5.06
Linalool	2.46 ± 0.88	0.482 (0.397–0.614)	1.407 (0.767–2.124)	4.16
Pulegone	2.36 ± 0.58	0.371 (0.286–0.480)	1.292 (0.625–2.088)	14.09[a]
Verbenone	35.6 % Feeding deterrence at maximum concentration of 1.0 mg/g used			
Fenchone	21.9 % Feeding deterrence at maximum concentration of 1.0 mg/g used			
1,8-Cineole	17.1 % Feeding deterrence at maximum concentration of 1.0 mg/g used			

[a] High χ^2 suggests steep mortality values at higher concentrations

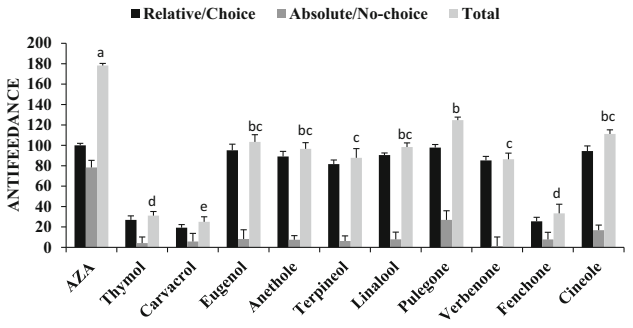

Fig. 2 Relative (from choice tests), absolute (from no-choice tests) and total (the sum of the values) coefficients of antifeedance calculated for various compounds against *Sitophilus oryzae* at 1.0 mg/g dose applied to wafer discs. Azadirachtin (AZA) was used as positive control for comparison. *Bars* with the *same letter* indicate no significant differences in total feeding deterrence (Tukey HSD test, $P < 0.05$)

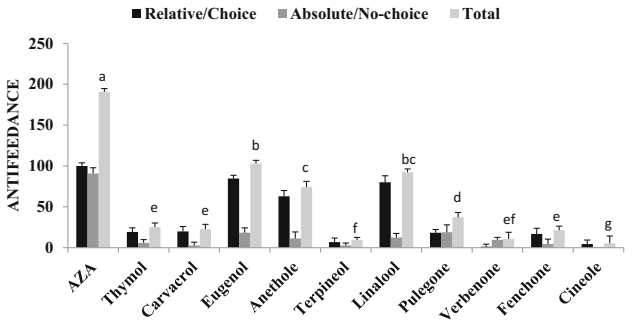

Fig. 3 Relative (from choice tests), absolute (from no-choice tests) and total (the sum of the values) coefficients of antifeedance calculated for various compounds against *Tribolium castaneum* at 1.0 mg/g dose applied to wafer discs. Azadirachtin (AZA) was used as positive control for comparison. *Bars* with the *same letter* indicate no significant differences in total feeding deterrence (Tukey HSD test, $P < 0.05$)

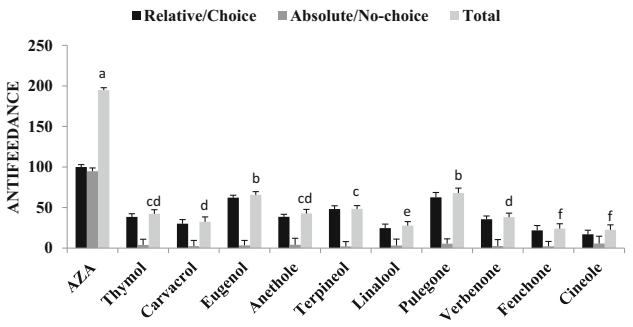

Fig. 4 Relative (from choice tests), absolute (from no-choice tests) and total (the sum of the values) coefficients of antifeedance calculated for various compounds against *Rhyzopertha dominica* at 1.0 mg/g dose applied to wafer discs. Azadirachtin (AZA) was used as positive control for comparison. *Bars* with the *same letter* indicate no significant differences in total feeding deterrence (Tukey HSD test, $P < 0.05$)

Feeding deterrent effects of mixtures

The compounds that were found to be feeding deterrents to respective insect species were combined to determine their activity in mixtures. The data obtained suggest that for all those combinations that contained eugenol or linalool as one of the components, the FI_{50} values obtained were significantly lower than the expected values (Table 8). In all the treatments, the values for the combination of eugenol with linalool were significantly better than expected. In case of *S. oryzae, the* FI_{50} value for this combination was 0.018 mg/g of the wafer disc against the expected value of 0.025–0.041 mg/g (Table 8). In case of *T. castaneum* (Table 8), the FI_{50} obtained for a similar combination was 0.005 mg/g as compared to the expected value (0.010–0.207 mg/g), and for *R. dominica* the FI_{50} for the eugenol + linalool combination was 0.287 against the expected value of 0.449–0.482 mg/g (Table 8). However, in *R. dominica* treatments, the linalool + pulegone combination was the best where FI_{50} was 0.217 mg/g against the expected value of 0.371–0.482 mg/g. (Table 8).

Combined effects of a toxin and an antifeedant

The effect of a toxin and an antifeedant in combination, when evaluated at half the concentration of the LC_{50}/FI_{50} values obtained or at LC_{50}/FI_{50} levels, gave variable results for the three beetles. There was an overall decrease in mortality of *S. oryzae* beetles in all combinations (Table 9). However, increased feeding deterrence was observed in various combinations of anethole, carvacrol or linalool under both treatment conditions. Similar results were recorded for *T. castaneum* as well (Table 10), and the only difference was that a combination with thymol also showed increased antifeedant activity against this insect. A specific increase in mortality was observed when anethole and thymol were used in combination (Table 10). The treatment to *R. dominica* showed a specific increase in both toxicity and feeding deterrence when linalool was combined with eugenol. There was a significant decrease in toxicity but increase in antifeedant activity of thymol when combined with linalool (Table 11). Comparatively both activities were lower than the individual compound activities in linalool + carvacrol combinations. Combination of anethole, α-terpineol and verbenone showed an antagonistic effect on the antifeedant activity of pulegone against *R. dominica* adults (Table 11).

Discussion

Fumigation by conventional chemical pesticides has been the main strategy to control stored-grain insect pests for decades, and only methyl bromide and phosphine remain in

Table 7 Enhanced toxicity of specific mixtures of toxic compounds (μg/cm^2) against various stored-grain insects in the contact assay

Compound	Expected LC_{50}	Observed LC_{50} (confidence limits at 95 %)	Slope ± SE	χ^2
Sitophilus oryzae				
Thymol + anethole	(22.8–24.1)	18.23 (16.31–20.42)	6.54 ± 0.38	2.34
Thymol + eugenol	(16.1–24.1)	12.44 (10.72–15.32)	5.34 ± 0.33	2.89
Carvacrol + anethole	(17.2–22.8)	14.55 (12.66–16.78)	4.56 ± 0.45	2.45
Carvacrol + eugenol	(16.1–17.2)	11.22 (9.23–14.23)	4.66 ± 0.34	2.34
Anethole + eugenol	(16.1–22.8)	10.34 (8.78–13.25)	4.78 ± 0.43	2.88
Anethole + verbenone	(22.8–35.8)	19.44 (15.36–21.87)	5.02 ± 0.45	3.12
Anethole + pulegone	(22.8–42.8)	20.23 (16.24–22.27)	4.67 ± 0.55	4.77
Pulegone + eugenol	(16.1–42.8)	13.78 (11.34–15.78)	6.25 ± 0.71	5.27
Eugenol + verbenone	(16.1–35.8)	11.56 (9.87–14.23)	3.27 ± 0.43	3.21
Tribolium castaneum				
Thymol + anethole	11.2–20.1	8.21 (6.34–10.45)	4.53 ± 0.48	3.14
Thymol + carvacrol	11.2–21.2	9.16 (7.35–10.97)	6.21 ± 0.53	4.78
Thymol + eugenol	11.1–18.9	8.14 (6.32–10.72)	5.64 ± 0.38	3.89
Carvacrol + anethole	20.1–21.2	16.51 (12.60–19.28)	3.36 ± 0.46	3.55
Carvacrol + eugenol	18.9–20.1	13.27 (11.27–16.29)	4.62 ± 0.44	3.34
Anethole + eugenol	18.9–20.1	15.24 (11.78–17.20)	6.78 ± 0.47	3.45
Rhyzopertha dominica				
Thymol + anethole	6.9–8.8	4.23 (3.37–6.42)	3.54 ± 0.18	2.84
Thymol + carvacrol	8.8–9.2	7.16 (6.25–9.33)	5.21 ± 0.43	2.78
Thymol + eugenol	8.8–15.7	5.44 (4.72–7.12)	5.14 ± 0.73	2.19
Carvacrol + anethole	6.9–9.2	5.85 (4.16–6.88)	5.26 ± 0.35	3.15
Carvacrol + eugenol	9.2–15.7	7.82 (6.13–9.03)	4.46 ± 0.44	4.34
Anethole + eugenol	6.9–15.7	4.54 (3.28–6.15)	5.18 ± 0.63	3.88
Anethole + verbenone	6.9–23.6	4.84 (3.36–6.37)	5.72 ± 0.55	4.23
Anethole + α-terpineol	6.9–43.2	5.11 (3.24–6.77)	8.67 ± 0.35	2.67
α-Terpineol + eugenol	15.7–43.2	10.68 (8.64–13.28)	5.25 ± 0.71	2.27
Eugenol + verbenone	15.7–23.6	12.36 (10.57–14.83)	2.27 ± 0.63	5.21

use today (Rajendran 2016). Even these products are now on the verge of being discontinued because of regulatory (Anonymous 1997), environmental (EPA 1993), human health (Garry et al. 1989, 1990) and pest resistance (Zettler 1991) concerns. Because of this, there is a renewed interest in developing new, alternative methods that will fit in integrated pest management (IPM) programs for the control of stored-grain insect pests.

One approach that has made some headway is the use of essential oil compounds obtained by steam distillation of aromatic plants. These compounds are safe as they are being used as fragrances and flavorings in the perfume and food industries and also as herbal medicines (Coppen 1995; Buckle 2003). Plant essential oils have huge commercial potential as they are produced from various aromatic plants belonging to over 60 families, mostly from Lauraceae, Myrtaceae, Umbelliferae, Labiatae and Compositae. The oils are generally composed of complex mixtures of monoterpenes, biogenetically related phenols and sesquiterpenes (Koul et al. 2008). The terpenoids and phenylpropanoids from these oils are considered safe because they are moderately toxic or mostly nontoxic to mammals, birds and fish (Stroh et al. 1998; Isman 2000). These compounds are also cheaper, which makes them favorable for the development of botanical insecticides. In fact, EOs have been used traditionally to protect stored grains in Asia and Africa; however, since the 1990s research has been done to demonstrate the potential of these oils in pest control (Isman 2000). Although much work has been or is being done using essential oil compounds to demonstrate their efficacy against a variety of pests (Koul et al. 2008), the objective of the present study was to determine whether these compounds are generalist toxins or specific feeding deterrents and also to show the inter-species variations vis-à-vis the treatments given to three stored-grain insects using various bioassays designed to meet the objectives of the study.

Table 8 Enhanced feeding inhibition due to specific antifeedant compound mixtures (mg/g wafer disc) in various stored-grain insects in the wafer disc assay

Compound	Expected FI_{50}	Observed FI_{50} (confidence limits at 95 %)	Slope ± SE	χ^2
Sitophilus oryzae				
Eugenol + anethole	0.041 to 0.104	0.023 (0.019–0.038)	1.54 ± 0.28	1.85
Eugenol + α-terpineol	0.041 to 0.071	0.033 (0.027–0.039)	2.21 ± 0.43	1.78
Eugenol + linalool	0.025 to 0.041	0.018 (0.014–0.021)	2.14 ± 0.71	2.12
Eugenol + verbenone	0.041 to 0.059	0.035 (0.028–0.035)	4.18 ± 0.26	3.72
Eugenol + pulegone	0.041 to 0.058	0.029 (0.026–0.033)	3.22 ± 0.45	9.62[a]
Eugenol + 1,8-cineole	0.041 to 0.051	0.031 (0.022–0.037)	5.23 ± 0.55	3.45
Anethole + linalool	0.025 to 0.104	0.013 (0.010–0.021)	5.12 ± 0.38	4.47
α-Terpineol + linalool	0.025 to 0.071	0.020 (0.018 –0.022)	5.67 ± 0.45	4.47
Linalool + pulegone	0.025 to 0.058	0.017 (0.013–0.020)	2.28 ± 0.33	1.47
Linalool + Verbenone	0.025 to 0.059	0.019 (0.016–0.022)	3.21 ± 0.46	2.12
Linalool + 1,8-cineole	0.025 to 0.051	0.019 (0.014–0.024)	3.24 ± 0.27	2.72
Tribolium castaneum				
Eugenol + anethole	0.010 to 0.468	0.006 (0.004–0.009)	1.64 ± 0.23	1.75
Eugenol + α-terpineol	0.010 to >1.0	0.033 (0.027–0.054)	2.43 ± 0.53	2.18
Eugenol + linalool	0.010 to 0.207	0.005 (0.004–0.009)	2.18 ± 0.22	2.32
Anethole + α-terpineol	0.468 to >1.0	0.097 (0.075–0.114)	7.65 ± 0.64	13.64[a]
Anethole + linalool	0.207 to 0.468	0.113 (0.092–0.188)	5.32 ± 0.48	4.57
Anethole + verbenone	0.468 to >1.0	0.298 (0.277–0.331)	5.46 ± 0.64	13.54[a]
Anethole + pulegone	0.458 to >1.0	0.286 (0.246–0.312)	4.68 ± 0.33	4.78
Anethole + 1,8-cineole	0.458 to >1.0	0.318 (0.287–0.402)	3.71 ± 0.35	12.13[a]
α-Terpineol + linalool	0.207 to >1.0	0.128 (0.118 –0.222)	6.68 ± 0.55	5.27
Linalool + pulegone	0.207 to >1.0	0.117 (0.093–0.178)	2.68 ± 0.43	2.41
Linalool + verbenone	0.207 to >1.0	0.129 (0.112–0.160)	3.27 ± 0.56	2.18
Linalool + 1,8-cineole	0.207 to >1.0	0.126 (0.114–0.158)	3.38 ± 0.37	2.42
Rhyzopertha dominica				
Eugenol + anethole	0.449 to >1.0	0.248 (0.215–0.386)	1.86 ± 0.29	2.18
Eugenol + linalool	0.449 to 0.482	0.287 (0.265–0.312)	2.65 ± 0.44	2.42
Anethole + α-terpineol	0.503 to >1.0	0.397 (0.335–0.514)	6.55 ± 0.44	13.42[a]
Anethole + linalool	0.482 to >1.0	0.273 (0.212–0.388)	5.38 ± 0.61	6.17
Anethole + pulegone	0.371 to >1.0	0.286 (0.246–0.342)	5.22 ± 0.92	4.38
α-Terpineol + linalool	0.482 to 0.503	0.321 (0.318 –0.402)	4.92 ± 0.55	5.46
Linalool + pulegone	0.371 to 0.482	0.217 (0.193–0.278)	2.65 ± 0.48	2.27
Linalool + verbenone	0.482 to >1.0	0.329 (0.278–0.460)	4.31 ± 0.56	2.82
Linalool + 1,8-cineole	0.482 to >1.0	0.315 (0.284–0.418)	3.38 ± 0.22	3.02

[a] High χ^2 suggests steep mortality values at higher concentrations

Thymol, carvacrol, eugenol and *trans*-anethole were specifically toxic, and linalool was a generalist feeding deterrent against all three species studied, though linalool has been reported as 100 % toxic to *S. oryzae* adults via fumigation (Kim et al. 2016). However, the present study demonstrates that the potency for respective efficacies was species specific. For instance, thymol was most toxic to *T. castaneum* and *R. dominica* compared to carvacrol and eugenol, but was least toxic to *S. oryzae* compared to the other two species. Similarly, linalool deterred feeding of *S. oryzae*, *T. castaneum* and *R. dominica* at different concentrations; *R. dominica* beetles required about 20 times the concentration to deter feeding compared to *S. oryzae* and more than twice that of *T. castaneum*. However, the toxicity or feeding deterrence due to other compounds was variable and species specific. Pulegone and verbenone were toxic to *S. oryzae* but not to *T. castaneum*, and only verbenone showed toxicity against *R. dominica*. Similarly,

Table 9 Combined effect of toxin (μg/cm^2 contact) + antifeedant (mg/g wafer disc) compounds in a binary mixture against *Sitophilus oryzae*

Compound (FD + toxin)	Treatment at		Mortality (%) at		FD (%) at		Change in mortality (½)C/1C[a]	Change in FD (½)C/1C[a]
	(½) FI_{50}/LC_{50}	FI_{50}/LC_{50}	(½) FI_{50}/LC_{50}	LC_{50}/FI_{50}	(½) FI_{50}/LC_{50}	LC_{50}/FI_{50}		
Linalool + thymol	0.013/12.04	0.025/24.07	8.0	52.0	94.73	96.15	dec/same	inc/inc
Linalool + carvacrol	0.013/8.58	0.025/17.15	6.0	42.0	76.44	100.0	dec/same	inc/inc
Linalool + verbenone	0.013/17.88	0.025/35.77	2.0	0.0	4.19	87.33	dec/dec	dec/inc
Anethole + thymol	0.052/12.04	0.104/24.07	10.0	92.0	80.44	87.56	dec/inc	inc/inc
Anethole + carvacrol	0.052/8.58	0.104/17.15	14.0	54.0	79.44	92.49	dec/same	inc/inc
Anethole + verbenone	0.052/17.88	0.104/35.77	2.0	16.0	3.71	94.04	dec/dec	dec/inc
Pulegone + thymol	0.029/12.04	0.058/24.07	6.0	34.0	67.73	92.83	dec/dec	inc/inc
Pulegone + carvacrol	0.029/8.58	0.058/17.15	10.0	16.0	98.77	100.0	dec/dec	inc/inc
Pulegone + verbenone	0.029/17.88	0.058/35.77	0.0	0.0	21.85	45.81	dec/dec	dec/dec
α-Terpineol + thymol	0.035/12.04	0.071/24.07	4.0	34.0	23.85	41.71	dec/dec	dec/dec
α-Terpineol + carvacrol	0.035/8.58	0.071/17.15	16.0	26.0	29.51	36.85	dec/dec	dec/dec
α-Terpineol + verbenone	0.035/17.88	0.071/35.77	0.0	10.0	9.41	21.66	dec/dec	dec/dec
Eugenol + thymol	0.020/12.04	0.041/24.07	0.0	54.0	50.26	60.23	dec/same	same/inc
Eugenol + carvacrol	0.020/8.58	0.041/17.15	2.0	46.0	15.79	86.69	dec/same	same/inc
Eugenol + verbenone	0.020/17.88	0.041/35.77	2.0	50.0	38.60	66.51	dec/same	same/inc
1,8-Cineole + thymol	0.025/12.04	0.051/24.07	12.0	36.0	16.71	70.54	dec/dec	dec/inc
1,8-Cineole + Carvacrol	0.025/8.58	0.051/17.15	0.0	40.0	26.86	28.66	dec/dec	dec/dec
1,8-Cineole + verbenone	0.025/17.88	0.051/35.77	0.0	6.0	11.14	48.88	dec/dec	dec/same

FD feeding deterrence, *dec* decrease in activity, *same* no change, *inc* increase in activity

[a] (½)C/1C = effect at (½)/equal to FI_{50}/LC_{50} concentrations

Table 10 Combined effect of toxin (μg/cm^2 contact) + antifeedant (mg/g wafer disc) compounds in a binary mixture against *Tribolium castaneum*

Compound (FD + toxin)	Treatment at		Mortality (%) at		FD (%) at		Change in mortality (½)C/1C[a]	Change in FD (½)C/1C[a]
	(½) FI_{50}/LC_{50}	FI_{50}/LC_{50}	(½) FI_{50}/LC_{50}	LC_{50}/FI_{50}	(½) FI_{50}/LC_{50}	LC_{50}/FI_{50}		
Linalool + thymol	0.103/5.60	0.207/11.21	0.0	4.0	50.16	84.45	dec/dec	inc/inc
Linalool + carvacrol	0.103/10.58	0.207/21.16	2.0	16.0	78.60	84.60	dec/dec	inc/inc
Anethole + thymol	0.234/5.60	0.468/11.21	62.0	86.0	72.07	100.0	inc/inc	inc/inc
Anethole + carvacrol	0.234/10.58	0.468/21.16	66.0	76.0	53.0	58.09	inc/inc	inc/inc

FD feeding deterrence, *dec* decrease in activity, *same* no change, *inc* increase in activity

[a] (½)C/1C = effect at (½)/equal to FI_{50}/LC_{50} concentrations

Table 11 Combined effect of toxin (μg/cm^2 contact) + antifeedant (mg/g wafer disc) compounds in a binary mixture against *Rhyzopertha dominica*

Compound (FD + toxin)	Treatment at		Mortality (%) at		FD (%) at		Change in mortality (½)C/1C[a]	Change in FD (½)C/1C[a]
	(½) FI_{50}/LC_{50}	FI_{50}/LC_{50}	(½) FI_{50}/LC_{50}	LC_{50}/FI_{50}	(½) FI_{50}/LC_{50}	LC_{50}/FI_{50}		
Linalool + thymol	0.241/4.40	0.482/8.80	14.0	24.0	89.53	82.38	dec/dec	inc/inc
Linalool + carvacrol	0. 241/4.59	0.482/9.18	12.0	42.0	18.59	32.82	dec/dec	dec/dec
Linalool + eugenol	0. 241/7.83	0.482/15.67	34.4	90.0	62.69	63.55	inc/inc	inc/inc
Linalool + anethole	0. 241/12.62	0.482/25.25	14.0	88.0	35.65	78.32	dec/inc	inc/inc
Linalool + α-terpineol	0. 241/21.59	0.482/43.18	94.0	100.0	20.3	36.29	inc/inc	dec/dec
Linalool + verbenone	0. 241/11.81	0.482/23.63	26.0	100.0	13.67	11.66	same/inc	dec/dec
Pulegone + thymol	0.185/4.40	0.371/8.80	76.0	96.0	21.32	32.13	inc/inc	same/dec
Pulegone + carvacrol	0.185/4.59	0.371/9.18	86.0	98.0	19.66	73.33	inc/inc	dec/inc
Pulegone + eugenol	0.185/7.83	0.371/15.67	12.0	96.0	69.81	76.95	dec/inc	inc/inc
Pulegone + anethole	0.185/12.62	0.371/25.25	58.0	90.0	4.59	13.0	inc/inc	dec/dec
Pulegone + α-terpineol	0.185/21.59	0.371/43.18	10.0	90.0	16.84	19.27	dec/inc	dec/dec
Pulegone + verbenone	0.185/11.81	0.371/23.63	18.0	98.0	8.05	35.62	dec/inc	dec/dec

FD feeding deterrence, *dec* decrease in activity, *same* no change in activity, *inc* increase in activity

[a] (½)C/1C = effect at (½)/equal to FI_{50} and LC_{50} concentrations

both these compounds were also feeding deterrents for *S. oryzae* but not for *T. castaneum*. Only pulegone was a feeding deterrent to *R. dominica*. Similarly, α-terpineol was toxic to *R. dominica* adults but not to *S. oryzae* or *T. castaneum*, but this compound deterred feeding of *S. oryzae* beetles. This shows that the compounds behave differently against different insect species. For instance, another isomer of terpineol, terpinen-4-ol, has been reported as a contact and fumigant toxicant against *T. castaneum* (Wang et al. 2015). Various studies imply that EO compounds act as acute toxins or feeding deterrents against insects (Tripathi et al. 2003; Koul et al. 2008; Phillips and Throne 2010; Koul 2012; Regnault-Roger et al. 2012), but whether these should be called toxins or feeding deterrents is a question that cannot be summarily ignored. Therefore, the comparative evaluation of the compounds used in the present study was done to determine their absolute antifeedance with the well-known antifeedant compound azadirachtin (Koul 2005). The data obtained showed reasonably significant relative feeding deterrence, but all the compounds were quite moderate in exhibiting absolute antifeedance against all three insects studied. While the total coefficients of antifeedance calculated for azadirachtin were 178.3, 190.4 and 194.8 for *S. oryzae*, *T. castaneum* and *R. dominica*, respectively, these values were too moderate for all the monoterpenoids evaluated (Figs. 2, 3, 4), and only linalool, eugenol and pulegone exhibited a total coefficient of around 100, that too being species specific. This suggests that the compounds evaluated might not be true antifeedants as per the definition given above, and whatever inhibition in feeding was obtained could be due to physiological toxicity rather than any interaction with gustatory receptors. Obviously, the more than 200 botanicals that have been shown to deter feeding of stored-

grain pests (Nawrot and Harmatha 2012) cannot be generalized as feeding deterrent compounds as azadirachtin is, which interacts at gustatory receptor sites of insects (Koul, 2008), though it also has other modes of action at the molecular level, having different binding sites (Mordue 2004).

Compounds were also evaluated as binary mixtures for their toxic as well as feeding deterrent action, and it was obvious that any combinations that contained anethole or eugenol were significantly more toxic than the individual compounds with lower LC_{50} values than expected (showing no overlap of fiducial limits). Similarly, any combination that contained anethole or linalool deterred feeding more than the individual compounds with FI_{50} values lower than the expected values. This implies that enhancement of activity in a mixture was not a generalist characteristic of the compounds, and only specific combinations were responsible for the synergism. That the combination of the compounds obtained from essential oils can be additive, antagonistic or synergistic has been observed previously (Hummelbrunner and Isman 2001; Koul et al. 2013; Kumrungsee et al. 2014), though evaluated against lepidopterans. This also suggests that selected blends are required to induce synergism as in the case of synergistic toxic action reported between 1,8-cineole and (±) camphor against *Trichoplusia ni* (Tak et al. 2015), the boosting effect of camphor with other terpenoids against *Spodoptera littoralis* (Pavela 2014), the combinations of eugenol and isoeugenol and some other monoterpenoids against *Culex quinquefasciatus* (Pavela 2015) or linalool, *p*-cymene and myrcene act synergistically as fumigants against rice weevils (Kim et al. 2016). This obviously emphasizes the need to evaluate compounds from an essential oil individually and also as blends in a mixture. However, this may not apply to feeding deterrents per se because the effects of an individual constituent within an essential oil and the overall activity of essential oil as a whole are not always correlated (Akhtar et al. 2012). Such variations could also be relative to the potential of a compound to penetrate the cuticle of an insect. In a recent study with essential oil compounds against *T. ni*, a reversed order of toxicity to camphor and 1,8-cineole between injection and topical application was observed and enhanced activity seen if the cuticular barrier was bypassed (Tak and Isman 2015).

The combined effect of a toxin and an antifeedant was variable too. All combinations used were less toxic to *S. oryzae and T. castaneum* beetles, but an increase in feeding deterrence was recorded when anethole, carvacrol or linalool was combined even at a reduced concentration to half of the LC_{50} and FI_{50} values obtained for individual compounds. Surprisingly, thymol, which acts as an acute toxin against many insect species (Koul et al. 2008), also showed increased antifeedant activity against *T. castaneum* and *R. dominica* when used in combination with anethole, carvacrol or linalool. Similarly, both toxic action and antifeedant activity increased when *R. dominica* adults were treated with linalool + eugenol together. The increase in antifeedant activity in combination with a toxin, therefore, must be an outcome of physiological toxicity induced by the acute toxins that synergize the activity of other compounds in a blend but at the same time the dose may not be sufficient to kill the insect. However, constituents together must be severely interfering with the physiological processes that cause insects to avoid feeding vis-à-vis the chemical interaction between the constituents in a mixture.

The present results, therefore, suggest that on one hand the compounds are not generalized acute toxins but the effects are species specific, and on the other none of the compounds evaluated were absolute antifeedants (i.e., like azadirachtin), and the activity seems to be oriented more toward physiological toxicity. This conclusion could be further substantiated by earlier observations in which monoterpenoids interacting with octopamine receptors (Bischof and Enan 2004; Kostyukovsky et al. 2002) like eugenol have an octopaminergic site of action (Enan 2001). Similarly, thymol, carvacrol and α-terpineol interact with tyramine (a precursor of octopamine) in *D. melanogaster* (Enan, 2005). GABA-gated neurons are also targeted by monoterpenes contained in EOs. For instance, thymol binds to GABA receptors associated with chloride channels located on the membrane of postsynaptic neurons and disrupts the functioning of GABA synapses (Priestley et al. 2003). This implies that monoterpenoids and phenylpropenes in essential oils are basically acute toxins, and it is quite apparent that the induced feeding deterrence is an outcome of the physiological toxicity. These modes of action also suggest that these compounds are safer for mammalian systems (as such receptors are absent in mammals), and development of insecticidal products based on these compounds and their blends would be well suited for use in stored-food commodities because of their natural origin and biodegradable characteristics with diverse physiological targets within insects. This consequently may also delay the evolution of insect resistance. However, stability of compounds and detailed field evaluations are necessary on a larger scale to allow these products to compete with conventional products for inclusion in any IPM module for stored-grain pests.

Conclusions

The relationship between the acute toxicity and feeding deterrent activity of ten compounds that commonly occur in essential oils was determined. Thymol, carvacrol, eugenol and *trans*-anethole were specifically toxic, and

linalool deterred feeding of all three beetles studied. Comparative efficacy studies showed that the compounds were acute toxins rather than feeding deterrents, which is a behavioral response from gustatory receptor sites, and cannot be classified as antifeedants. Comparison of toxicity and deterrent activity with respective artificial blends as binary mixtures revealed that synergism was not a generalized phenomenon, and the variations were both species specific as well as blend specific.

A combined effect of a toxin and an antifeedant showed an overall decrease in the mortality of beetles but an increase in feeding deterrence in various combinations of anethole, carvacrol or linalool. The increased feeding deterrence in a combination of a toxin with a feeding deterrent compound suggests a physiological toxicity induced by the acute toxins that synergizes the activity of a deterrent compound in a mixture, and the dose may not be sufficient to kill. However, the constituents together must be interfering with physiological processes in a way that would determine the response of the insect to decide whether or not to feed.

Author contributions

DK conducted experiments designed by him and OK. OK and SK carried out the statistical analysis and wrote parts of the manuscript.

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Compliance with ethical standards

Conflict of interest The authors, DK, SK and OK, declare that they have no conflict of interests.

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