Effect of Keto-Compounds from Essential Oils on the Growth and Reproductive Performance of *Tribolium castaneum* (Herbst)

DHARAMVIR KANDA[1], SANEHDEEP KAUR[2] AND OPENDER KOUL[1]*

[1]Insect Biopesticide Research Centre, 30 Parkash Nagar, Jalandhar-144003, India

[2]Department of Zoology, Guru Nanak Dev University, Amritsar-143005, India

KEY WORDS

Essential oils

Keto-compounds

Tribolium castaneum

Growth inhibition

Reproductive performance

ABSTRACT Keto-compounds that occur in essential oils of plants were evaluated for their impact on growth and reproductive performance of *Tribolium castaneum*. These compounds were carvone, fenchone, menthone, pulegone, and verbenone. The larval growth was reduced significantly by carvone, menthone and pulegone with an effective concentration to inhibit growth (EC_{50}) ranging between 1.4 to 3.1 mg/g of diet. Fenchone and verbenone were moderately active, with an EC_{50} of 25.6 and 18.7 mg/g, respectively. In the course of development larval mortality was significant under various treatments and surviving pupae ranged between 50 to 60% and adults between 44.4 to 53.3% in compound treatments compared to controls where 97.8% of adults survived. The experiments were carried out through F1 generation and the impact was evaluated from the adults obtained from rearing on different treatments at the EC_{50} dose for 30 days. In treatment groups, there were deformed larvae and pupae, and interestingly the number was significantly similar in all treatments. Deformed adults were also observed after some abnormal pupae were able to undergo eclosion. The data suggest that structures of the compounds contribute to biological activity, as the three most active compounds possess significant structural similarities compared to fenchone and verbenone.

INTRODUCTION

Essential oils (EOs) have many applications, including bactericidal, virucidal, fungicidal, antiparasitical, medicinal and cosmetic applications and are easily procedure extracted from aromatic plants that contain a variety of volatile molecules, such as terpenes and terpenoids, phenol-derived aromatic and aliphatic components (Bakkali *et al*., 2008). During the past two decades, essential oils, or the compounds that constitute these oils, have also been studied extensively as alternate strategies to control insect pests (Koul *et al*., 2008) in lieu of the conventional insecticides that have hazardous impacts on the environment, and also the problem of insecticide resistance . EOs have a wide spectrum of biological

*Corresponding author: E-Mail : okoul@koulresearch.org

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activities against insects (Adeyemi, 2010), and at the same time, they are low risk pesticides (Regnault-Roger *et al*., 2012; Kalita *et al*., 2013). Several studies show that EOs are active against Coleoptera species, and monoterpenoids are responsible for insecticidal activities (Nerio *et al*., 2010; Stefanazzi *et al*., 2011; Park *et al*. 2016).

Use of plant extracts against stored grain pests suggest that these products are toxic, repellent, deter feeding and affect growth and development (Rajashekar *et al*., 2012). Various modes of applications, like direct contact methods and grain coatings, show that they inhibit the development of insects, reduce survival rates, induce cuticle melanisation and cause subsequent death of insects (Boeke *et al*., 2004; Koul *et al*., 2008; Rajashekar *et al*. 2012). 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). Allelochemicals that have been reported as active ingredients are sulfur compounds from neem (Koul, 2004), carvone (Tripathi *et al*., 2003), *trans*-anethole (Koul *et al*., 2007) and some constituents from *Derris scandans* (Hymavathi *et al*., 2011) also have been studied in detail. At a global level, toxic effects of the 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) have been reported against several coleopteran storage pests, but most of the studies are based on fumigation studies in the laboratory. Our recent study with 10 compounds belonging to monoterpenoid and phenylpropanoid groups (Kanda *et al*., 2016) showed that these compounds from essential oils were mainly physiological toxins. However, some keto-compounds did not exhibit a direct contact toxicity or absolute antifeedant action but had impact on the development of coleopteran beetles infesting stored grains. Combined with other recent studies on the toxicity and repellency of essential oils against *Tribolium castaneum* (Lancelle *et al*., 2009; Kim *et al*., 2010; Olivero-Verbel *et al*., 2013; Wang *et al*., 2015), the objective of the present study was to determine the effect of such keto-compounds on the development and reproductive performance of this economically important flour beetle that not only attacks stored grains, but processed food products too like flour, cereals, biscuits, beans, nuts, etc.

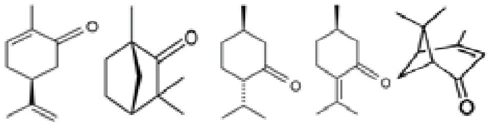
MATERIALS AND METHODS

Insects

Larvae and adults of *T. castaneum* were obtained from routine cultures in our laboratory. *T. castaneum* were bred in a mixture of wheat flour and yeast (12:1) at 32 ± 2°C and 70–75% R.H.

Test compounds

Five keto-compounds (Fig. 1) that occur in essential oils were used in the study. The compounds occur commonly in Lamiaceae and Lauraceae plants. These compounds were carvone, fenchone, menthone, pulegone, and verbenone. The pure compounds (97–99% purity) were procured from Sigma/Aldrich Chemie, GmbH, Germany, and Acros Organics, Morris Plains, NJ, USA.



Carvone Fenchone Menthone Pulegone Verbenone

Fig. 1. Compounds used for the study.

Bioassays

Two experimental designs were followed in the study. First, 30 freshly emerged larvae were transferred to treated 10 g wheat flour + yeast rearing medium in three replicates (n = 90). The compound treatment range was 1.25 to 30.0 mg/g of rearing medium. Each compound was dissolved in acetone to obtain the required concentration and subsequently thoroughly mixed with medium till the solvent was completely evaporated. In controls only solvent was used. The larvae were observed every week in order to determine the effect on growth, abnormalities and mortality, if any, until pupation. The effective concentration (EC_{50}) for inhibition of growth was calculated on the basis of larval duration versus subsequent death of larvae within ten days of treatment.

In the second experiment, neonates were treated with the EC_{50} dose and reared to pupae as in the first experiment. Pupae were separated by sex, with 15 males and 15 females selected for each treatment or control. After adults emerged, they were then paired accordingly into 5 pairs/replicate and transferred to fresh untreated rearing medium for oviposition. After one week, all adults were removed from both treated and control groups, and population counts were made for the F1 generation. After every 10 days, the larval, pupal and adult counts were recorded for all groups until 30 days. The effects of compounds on F1 population were regularly observed for numbers, deformities and mortality, if any, at various stages of development.

Statistical Analysis

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

RESULTS

The growth of *T. castaneum* larvae was impaired by various treatments of keto-compounds tested in the study. Carvone, menthone and pulegone reduced larval growth significantly with an effective concentration to inhibit growth (EC_{50}) ranging between 1.4 to 3.1 mg/g of diet (Table 1). These compounds had similar activity as was evident by the overlap of fiducial limits (Table 1). The other compounds, fenchone and verbenone, were moderately active, with an EC_{50} of 25.6 and 18.7 mg/g, respectively.

In the course of development there was significant mortality of larvae under various treatments (Fig. 2) in comparison to controls where no mortality was observed. Surviving pupae ranged between 50 to 60% and adults between 44.4 to 53.3% in compound treatments compared to controls where 97.8% of adults survived. Consequently, the percentage of emerged adults in the treated diets were significantly lower than the number in controls ($F = 33.6$; $df = 1,3$; $P < 0.01$).

Table 1. Effective concentrations (EC_{50}) of keto-compounds from essential oils in inhibiting the growth of *Tribolium castaneum*.

Compound	EC_{50} mg/g (Confidence limit of 95%)	χ^{2*}
Carvone	2.4 (1.7 – 3.2)	4.6
Fenchone	25.6 (21.7 – 28.8)	18.8
Menthone	3.1 (2.6 – 5.6)	3.8
Pulegone	1.4 (0.9 – 2.4)	4.2
Verbenone	18.7 (15.5 – 21.8)	17.6

*High χ^2 suggests steep growth inhibition values at higher concentrations.

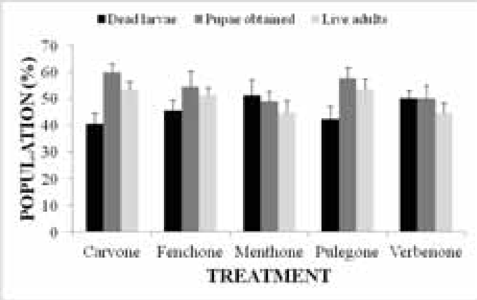


Fig. 2. Effect of rearing *T. castaneum* at EC_{50} dose (Table 1) of various keto-compounds from essential oils for 30 days, as determined by the number of dead larvae, or surviving pupae and adults. The number of dead pupae in different treatments were 6 (carvone), 5 (fenchone), 4 (menthone), 4 (pulegone) and 5 (verbenone). In controls no mortality was recorded and 97.8% adults were obtained after 30 days.

The impact on the F1 generation was evaluated from the adults obtained from rearing on different treatments at the EC_{50} dose for 30 days. In the control group, 100% F1 adults were recorded. However, in treatment groups, the range of surviving adults was 73.5 to 82.2% (Fig. 3). In treatement groups, there were deformed larvae and pupae (Fig. 4), and interestingly the number was significantly similar in all treatments ($F = 22{,}8$; $df = 1,3$; $P = 0.01$). In the carvone treatment, there were 10.4% deformed larvae and 14.0% deformed

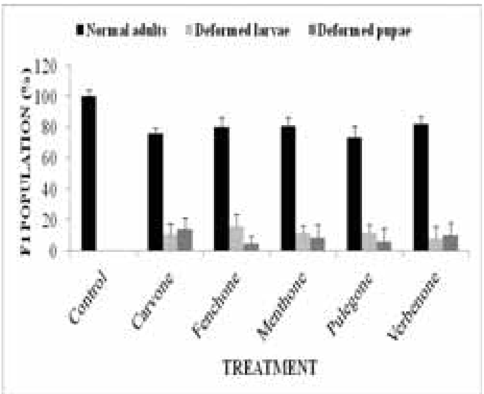
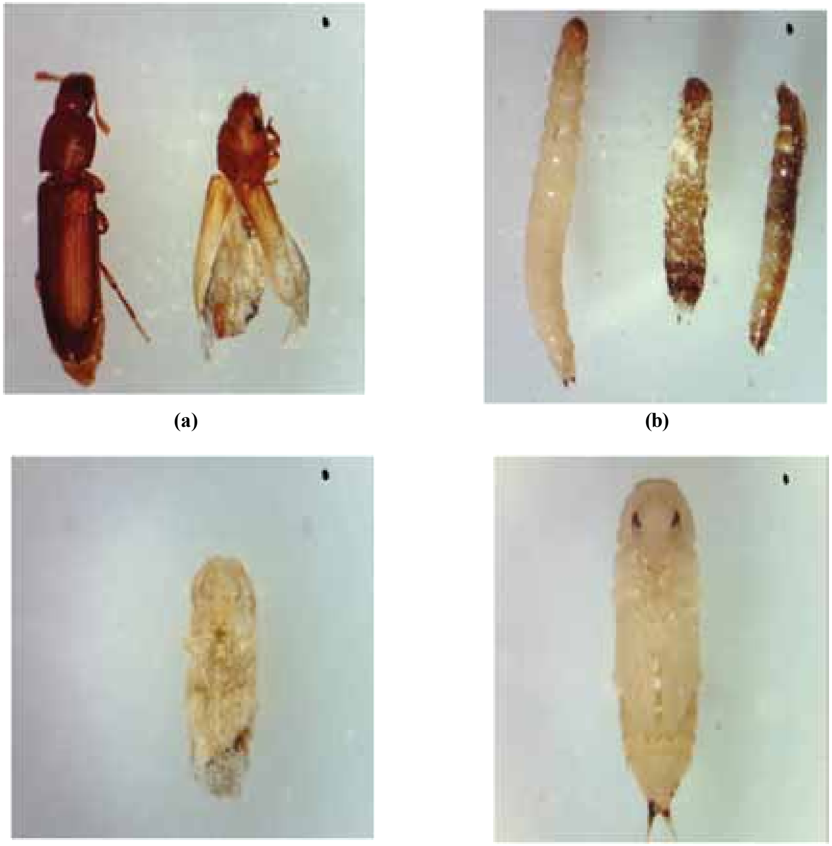


Fig. 3. The effect of essential oil compounds on the percentage of *T. castaneum* deformed larvae and pupae obtained in F1 generation from the treated parental eggs (at EC_{50} level).



(a) (b) (c) (d)

Fig. 4. The images shown above are the abnormal adults, larvae and pupae obtained in F1 generation after various treatments in parental population. (a) Normal and deformed adult; (b) normal and deformed larvae; (c) deformed pupa and (d) normal pupa.

pupae. Similar numbers of deformed larvae and pupae were found in fenchone (15.4 and 4.6%), menthone (11 and 8%), pulegone (11 and 5.5%), and verbenone (7.8 and 10%), respectively. Deformed adults were also observed after some abnormal pupae were able to undergo eclosion (Fig. 4).

DISCUSSION

The exploration of natural products for insect pest control has been and is still being considered as a potential strategy for production of commercially available natural products that are effective against insect pests, non-toxic for the user, easily biodegradable, and are locally and economically produced, especially by farmers who are discriminately using expensive synthetic pesticides. Thus, essential oils or the compounds therein could play a significant role in controlling stored grain pests and specifically the red flour beetle, *T. castaneum*. Natural products have been compared to fumigant toxicity assays (Lee *et al*., 2002, Lee *et al*., 2003; Koul *et al*. 2008, Fang *et al*., 2010; Suthisut *et al*., 2011; Koul, 2016). The data obtained in the present study shows that after oral administration of the compounds used in the study, there was significant retardation of growth in larvae of *T. castaneum* due to carvone, menthone and pulegone (EC_{50} ranging between 1.4 to 3.1 mg/g of diet). However, to obtain similar level of control, the concentration required for verbenone and fenchone was 8 to 11-fold higher. The inhibition of growth could also be related to quality of diet impaired by the compounds that were mixed with the diet. Such a diet might reduce energy allocation to maintenance and contribute greater energy investment in growth and reproduction, as has been seen in *T. castaneum* provided with different qualities of nutritional diets (Ming and Cheng, 2012).

The impact on the F1 generation, after parental populations were subjected to the compounds, was more moderate, as the range of adult population obtained was 73.5 to 82.2%, suggesting the lack of severe carryover effect on the reproductive performance of *T. castaneum*. The only significant impact that was apparent was the impairment of growth of larvae and pupae, as deformed larvae and pupae were obtained in treated groups against none in controls. Some studies have demonstrated biological effects on *T. castaneum* larvae with anolides isolated from *Salpichroa origanifolia* that were incorporated into the larval diet at concentrations of 500 and 2000 ppm (Mareggiani *et al.*, 2002). Significant delay in the development of neonate larvae to adults, and development delays and lethal effects were produced by these compounds. Similarly, feeding of *Prganum*, *Ajuga*, *Aristolochia* and *Raphanus* extracts deterred growth of *T. castaneum* and disrupted the development cycle (Jbilou *et al.*, 2006). Even feeding *Carica papaya* and *Ricinus communis* extract-treated diets to *T. castaneum* has been shown to also reduce the percentages of larvae that pupated and of adults that emerged and the number of F1 progeny (Tatun *et al.*, 2014). This impairment been attributed to the reduction of α-amylase and glucose content of the insects. The impaired reproductive performance of *T. castaneum* pupae and adults in feeding medium surviving sublethal exposure to heat treatment has also been demonstrated (Mahroof *et al.*, 2005).

It has been determined that thymoquinone and dihydrocarvone were strongly repellent to *Sitophilus zeamais*. In fact, dihydrocarvone, menthone, α-thujone and thymoquinone have been reported to have direct relation between concentration and repellent action (Pizzolitto *et al.*, 2015). This implies that keto-compounds were significantly repellent, however, this cannot be ascertained in present experimental design where larvae were reared in the treated diet and thus forced to feed. However, the present study suggests that the compounds do induce physiological toxicity when orally administered, thereby either killing the insect or retarding the growth of insects significantly.

The data suggest that structures of the compounds contribute to biological activity, as the three most active compounds possess significant structural similarities compared to fenchone and verbenone. The steriochemistry of carvone, menthone and verbenone are similar, with variation in the keto group substitution at ortho- and meta-positions, and indicating that α,β-unsaturated carbonyl ketones act as potential anti-insect compounds. Another recent study found that carvone, menthone, pulegone and thujone were toxic to *Sitophilus zeamais* in fumigant assays, while E-Z-Ocimenone was moderately toxic with an open chain structure (Herrera *et al.*, 2014). In fact, carvones are well known bioactive compounds against several stored product insects (Tripathi *et al.*, 2003). The results presented here, therefore, demonstrate that naturally occurring plant toxins for insects (as the keto-compounds in present case) could show bioactivity in various ways in addition to being toxic to insects, i.e. they can retard growth and reproductive performance via impairment of physiological mechanisms. These compounds thus warrant further study to develop suitable application strategies in order to obtain potential control of stored grain pests under storage conditions.

REFERENCES

Adeyemi, M.H. (2010) The potential of secondary metabolites in plant material as deterents against insect pests: A review. *Afr. J. Pure Appl. Chem.*, **4**, 243–246.

Bakkali, F., Averbeck, S., Averbeck, D. and Idaomar, M. (2008) Biological effects of essential oils- A review. *Food Chem. Toxicol.*, **46**, 446–475.

Boeke, S.J., Baumgart, I.R., Van Loon, J.J.A., Huis, A. Van, Dicke, M. and Kossou, D.K. (2004)Toxicity and repellence of African plants traditionally used for the protection of stored cowpea against *Callosobruchus maculatus*. *J. Stored Prod. Res.*, **40**, 423–438.

Fang, R., Jiang, C.H., Wang, X.Y., Zhang, H.M., Liu, Z.L., Zhou, L., Du, S.S. and Deng, Z.W. (2010) Insecticidal activity of essential oil of *Carum carvi* fruits from China and its main components against two grain storage insects. *Molecules*, **15**, 9391–9402.

Herrera, J.M.; Zunino, M.P.; Massuh, Y.; Pizzollito, R.P.; Dambolena, J.S.; Gañan, N.; Zygadlo, J.A. (2014) Fumigant toxicity from five essential oils rich in ketones against *Sitophilus zeamais* (Motschulsky). *Agriscientia*, **31**, 35–41.

Ho, S.H., Ma, Y. and Huang, Y. (1997) Anethole, a potential insecticide from *Illicium verum* Hook f. against two stored product insects. *Int. J. Pest. Control*, **39**, 50–51.

Huang, Y. and Ho, S.H. (1998) Toxicity and antifeedant activity of cinnamaldehyde against the grain storage insects, *Tribolium castaneum* (Herbst) and *Sitophilus zeamais* Motsch.. *J. Stored Prod. Res.*, **34**, 11–17.

Huang, Y., Chen, S.X. and Ho, S.H. (2000) Bioactivities of methyl allyl disulfide and diallyl trisulfide from essential oil of garlic to two species of stored product pests, *Sitophilus zeamais* (Coleoptera: Curculionidae) and *Tribolium castaneum* (Coleoptera: Tenebrionidae). *J. Econ. Entomol.*, **93**, 537–543.

Hymavathi, A., Devanand, P., Suresh, B. K., Sreelatha, T., Pathipati, U.R., Madhusudana Rao, J. (2011) Vapor-phase toxicity of *Derris scandens* Benth.-derived constituents against four stored-product pests. *J. Agric. Food Chem.*, **59**, 1653–1657.

Jbilou, R., Ennabili, A. and Sayah, F. (2006) Insecticidal activity of four medicinal plant extracts against *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae). *Afr. J. Biotechnol.*, **5**, 936–940.

Kalita, B., Bora, S. and Sharma, A.K. (2013) Plant essential oils as mosquito repellent-A review. *Int. J. Res. Dev. Pharm. Life Sci.*, **3**, 741–747.

Kanda, D., Kaur, S. and Koul, O. (2016) A comparative study of monoterpenoids and phenylpropanoids from essential oils against stored grain insects: acute toxins or feeding deterrents. *J. Pest Sci.* (DOI 10.1007/s10340-016-0800-5).

Karr, I. and Coats, R.J. (1988) Insecticidal properties of d-limonene. *J. Pestic. Sci.*, **13**, 287–290.

Kim, S.-W., Lee, H.-R., Jang, M.-J., Jung, C.-S. and Park, I.-K. (2016) Fumigant toxicity of Lamiaceae plant essential oil and blends of their constituents against rice weevil, *Sitophilus oryzae*. *Molecules*, **21**, 361 (doi:10.3390/molecules21030361), 10 pp.

Kim, S.-I., Yoon, J.-S., Jung, J.W., Hong, K.-B., Ahn, Y.-J. and Kwon, H.W. (2010) Toxicity and repellency of origanum essential oil and its components against *Tribolium castaneum* (Coleoptera: Tenebrionidae) adults. *J. Asia-Pacific Entomol.*, **13**, 369–373.

Koul, O. (2004) Bioactivity of volatile di-n-propyl disulfide from seeds of neem, *Azadirachta indica* (Meliaceae) to two species of stored grain pests, *Sitophilus oryzae* (L.) and *Tribolium castaneum* (Herbst). *J. Econ. Entomol.*, **97**, 1142–1147.

Koul, O. (2016) *Handbook of Naturally Occurring Insecticidal Toxins*. CAB International, Wallingford, UK.

Koul, O., Singh, G., Singh, R. and Singh, J. (2007) Mortality and reproductive performance of *Triboilum castaneum* exposed to anethole vapours at high temperature. *Biopestic. Int.*, **3**, 126–137.

Koul, O., Walia, S. and Dhaliwal, G.S. (2008) Essential oils as green pesticides: Potential and constraints. *Biopestic. Int.*. **4**, 63–84.

Lancelle, H.G., Giordano, O.S., Sosa, M.E. and Tonn, C.E. (2009) Chemical composition of four essential oils from *Eupatorium* spp. Biological activities toward *Tribolium castaneum* (Coleoptera: Tenebrionidae). *Rev. Soc. Entomol. Argent.*, **68**, 329–338.

Lee, B.H., Lee, S.E., Annis, P.C., Pratt, S.J., Park, B.S. and Tumaalü, F. (2002) Fumigant toxicity of essential oils and monoterpenes against the red flour beetle, *Tribolium castaneum* Herbst. *J. Asia-Pacific Entomol.*, **5**, 237–240.

Lee, S., Peterson, C.J. and Coats, J.R. (2003) Fumigation toxicity of monoterpenoids to several stored product insects. *J. Stored Prod. Res.*, **39**, 77–85.

Mahroof, R., Subramanyam, B. and Flinn, P. (2005) Reproductive performance of *Tribolium castaneum* (Coleoptera: Tenebrionidae) exposed to the minmum heat treatment temperature as pupae and adults. *J. Econ. Entomol.*, **98**, 626–636.

Mareggiani, G., Picollo, M.I., Veleiro, A.S., Tettamanzi, M.C., Benedetti-Doctorovich, M.O., Burton, G. and Zerba, E. (2002) Response of *Tribolium castaneum* (Coleoptera: Tenebrionidae) to *Salpichroa origanifolia* withanolides. *J. Agric. Food Chem.*, **50**, 104–107.

Ming, Q-L. and Cheng, C. (2012) Influence of nutrition on male development and reproduction in *Tribolium castaneum*. *J. Econ. Entomol*., **105**, 1471-1476.

Nerio, L.S., Olivero-Verbel, J. and Stashenko, E. (2010) Repellent activity of essential oils: A review. *Bioresour. Technol.*, **101**, 372–378.

Olivero-Verbel, J., Tirado-Ballestas, I., Caballero-Gallardo, K. and Stashenko, E.E. (2013)

Essential oils applied to the food act as repellents toward *Tribolium castaneum*. *J. Stored Prod. Res.*, **55**, 145–147.

Park, C.G., Shin, E. and Kim, J. (2016) Insecticidal activities of essential oils, *Gaultheria fragrantissima* and *Illicium verum*, their components and analogist against *Callosobruchus chinensis* adults. *J Asia-Pacific Entomol*., **19**, 269–273.

Pizzolitto, R.P., Herrera, J.M., Zaio, Y.P., Dambolena, J.S., Zunino, M.P., Gallucci, M.N. and Zygadlo, J.A. (2015) Bioactivities of ketones terpenes: Antifungal effect on *f. verticillioides* and repellents to control insect fungal vector, *S. zeamais*. *Microorganisms*, **3**, 851-865.

Rajashekar, Y., Bakthavatsalam, N. and Shivanandappa, T. (2012) Botanicals as grain protectants. *Psyche*, **2012**, 1–13.

Regnault-Roger, C., Vincent, C. and Arnason, J.T. (2012) Essential oils and insect control: Low-risk products in a high-stakes world. *Annu. Rev. Entomol*., **57**, 405–424.

Sarac, A. and Tunc, I. (1995) Toxicity of essential oil vapours to stored-product insects. *J. Plant Dis. Prot.*, **102**, 69–74.

Shaaya, E., Ravid, U., Paster, N., Juven, B., Zisman, U. and Pissarev, V. (1991) Fumigant toxicity of essential oils against four major stored-product insects. *J. Chem. Ecol*., **17**, 499–504.

Shaaya, E., Kostjukovsky, M. and Ravid, U. (1994) Essential oils and their constituents as effective fumigants against stored product insects. *Israel Agres.*, **7**, 133–139.

Stefanazzi, N., Stadler, T. and Ferrero, A. (2011) Composition and toxic, repellent and feeding deterrent activity of essential oils against the stored-grain pests *Tribolium castaneum* (Coleoptera: Tenebrionidae) and *Sitophilus oryzae* (Coleoptera: Curculionidae). *Pest Manag. Sci.*, **67**, 639–646.

Suthisut, D., Fields, P and Chandrapatya, A. (2011) Fumigant toxicity of essential oils from three Thai plants (Zingiberaceae) and their major compounds against *Sitophilus zeamais*, *Tribolium castaneum* and two parasitoids. *J. Stored Prod. Res.*, **47**, 222-230.

Tatun, N., Vajarasathira, B., Tunjitwitayakul, J. and Sakurai, S. (2014) Inhibitory effects of plant extracts on growth, development and α-amylase activity in the red flour beetle *Tribolium castaneum* (Coleoptera: Tenebrionidae). *Eur. J. Entomol.*, **111**, 181–188.

Tripathi, A.K., Prajapati, V. and Kumar, S. (2003) Bioactivities of l-carvone, d-carvone, and dihydrocarvone towards three stored product beetles. *J. Econ. Entomol.*, **96**, 1594–1601.

Wang, C.-F., Yang, K., You, C.-X., Zhang, W.-J., Guo, S.-S., Geng, Z.-F., Du, S.-S. and Wang, Y.-Y. (2015) Chemical composition and insecticidal activity of essential oils from *Zanthoxylum dissitum* leaves and roots against three species of storage pests. *Molecules*, **20**, 7990–7999.

Weaver, D.K., Dunkel, F.V., Ntezurubaza, L., Jackson, L.L. and Stock, D.T. (1991) The efficacy of linalool a major component of freshly milled *Ocimum canum* Sims (Lamiaceae) for protection against post-harvest damage by certain stored product Coleoptera. *J. Stored Prod. Res.*, **27**, 213–220.

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