Research Application Summary

Fumigant toxicity of five essential oil constituents against major stored-product insect pests of food grains

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Abstract

Insect pests attacking stored food grains cause substantial losses that undermine food security in sub-Sahara Africa. Subsistence farmers predominantly use traditional plant-based products to protect their grains in storage. In an attempt to seek alternatives to synthetic fumigants, laboratory screening of essential oil constituents were conducted to evaluate their fumigant toxicity against five stored-product insects, *Sitophilus oryzae* L., *Rhyzopertha dominica* F., *Tribolium castaneum* (Herbst), *Oryzaephilus surinamensis* L. and *Callosobruchus chinensis* F. Five essential oil constituents, a- humulene, caryophyllene oxide, myrcene, R- (+)-a- pinene and R- (+)-â-pinene were each evaluated at four (0, 1, 5 and 10 µl/L air) rates in space fumigation chambers with four replicates per concentration. Results revealed a strong dose-, insect species- and time-dependent fumigant toxicity in which caryophyllene oxide, myrcene, a- humulene, R- (+)-a- pinene and R- (+)-â-pinene caused 18-100%, 49-100, 55.4-100, 47.3-100 and 32.5-100% adult mortalities of all test insects, except the most tolerant species, *T. castaneum*, at 10 µl/L air 168 h after treatment. Against all the test insects, except *T. castaneum*, Probit regression analysis produced end-point LC_{50} values of 0.03-8.5, 0.03-7.0, 0.01-4.82, 0.01-8.20 and 0.03-6.5 µl/L air for the five essential constituents, respectively. The observed fumigant potency levels could possibly be explained by variations in compound structure-insecticidal activity relationships that influenced their degree of penetration into the insect cuticle and neurotoxicity. These findings provide the scientific basis for using essential oils as fumigants against stored-product insect pests and hence, potential alternatives to synthetic fumigants in both subsistence and commercial agriculture. Further studies are recommended to evaluate the grain fumigation potency, biosafety and broad spectrum bioactivity of these essential constituents against insect pests of stored food commodities.

Key words: *Callosobruchus chinensis*, essential oil constituent, fumigant toxicity, *Oryzaephilus surinamensis, Rhyzopertha dominica, Sitophilus oryzae, Tribolium castaneum*

Résumé

Les insectes ravageurs attaquant les céréales stockées causent des pertes importantes qui compromettent la sécurité alimentaire en Afrique sub-saharienne. Les petits agriculteurs essentiellement emploient des produits traditionnels à base de plantes pour protéger leurs grains dans le stockage. Dans une tentative de chercher des alternatives aux fumigènes synthétiques, un dépistage au laboratoire des constituants essentiels d'huile a été mené pour évaluer leur toxicité fumigeante contre cinq insectes des denrées stockées : *Sitophilus oryzae* L., *Rhyzopertha dominica* F., *Tribolium castaneum* (Herbst), *Oryzaephilus surinamensis* L. et *Callosobruchus chinensis* F. Cinq constituants essentiels d'huile (a- humulène-, oxyde de caryophyllène, myrcène, R-(+)-a-pinène et R-(+)-A-pinène) ont chacun été évalués à quatre taux (0, 1, 5 et 10 µl/L air) dans les chambres de fumigation de l'espace avec quatre répétitions par concentration. Les résultats ont révélé une forte dose, des espèces d'insectes et de toxicité des fumigènes en fonction du temps dans lequel l'oxyde de caryophyllène, myrcène, a- humulène-, R-(+)-a-pinène et R-(+)-A-pinène ont causé 18-100 %, 49-100, de 55,4 à 100, de 47.3 à 100 et 32,5 à 100% de mortalité des adultes de tous les insectes d'essai, sauf les espèces les plus tolérantes, *T. castaneum*, à 10 µl/L air, 168 h après le traitement. Contre tous les insectes d'essai, sauf *T. castaneum*, l'analyse de régression de probité a produit les valeurs de LC50 de point final de 0.03 à 8.5, 0.03 à 7,0, de 0,01 à 4,82, de 0,01 à 8.20 0.03 à 6,5 µl/L air pour les cinq éléments essentiels, respectivement. Les niveaux observés d'activité fumigeante pourraient s'expliquer par des variations dans la structure complexe-les relations d'activité insecticide qui ont influencé leur degré de pénétration dans la cuticule de l'insecte et la neurotoxicité. Ces résultats fournissent la base scientifique pour l'utilisation des huiles essentielles comme fumigènes contre les insectes ravageurs des denrées alimentaires stockées et donc, des solutions de rechange potentielles aux fumigènes synthétiques dans l'agriculture de subsistance et l'agriculture commerciale. Des études complémentaires sont recommandées pour évaluer la puissance de fumigation du grain, la biosécurité et la bioactivité de large spectre de ces constituants essentiels contre les insectes ravageurs des denrées alimentaires stockées.

Mots clés: *Callosobruchus chinensis*, constituant essentiel de l'huile, toxicité fumigeante, *Oryzaephilus surinamensis, Rhyzopertha dominica, Sitophilus oryzae, Tribolium castaneum*

Background

Insect damage in stored food grains pose the greatest challenge to the provision of quality food with 10-60% losses in countries where modern storage technologies are yet to be fully adopted (Shaaya *et al*., 1997; Ogendo *et al*., 2003). The magnitude of loss is dependent upon the insect species involved, storage duration and pest control methods among other factors. In the tropics, the gelechiid moths and coleopteran beetles are the major insect pests of stored cereal and legume grains with *Sitotroga cerealella* Olivier, *Sitophilus* spp., *Prostephanus truncatus* Horn, *Rhyzopertha dominica* F., *Tribolium castaneum* Herbst, *Acanthoscelides obtectus* Say and *Callosobruchus* spp. being the most destructive species (Ogendo *et al*., 2003; Gressel *et al*., 2004). Currently, recommended pest control measures in durable stored food products rely heavily on use of synthetic insecticides which pose possible health hazards to warm-blooded animals, risk of environmental pollution, development of resistance by insects and pest resurgence (Shaaya *et al*., 1997).

Fumigation is one of the most successful and cost-effective methods used in the protection of durable stored food grains with negligible residues left on grains. Currently, phosphine and methyl bromide are the two principal fumigants used worldwide for the disinfestations of food grains. However, owing to the ozone-depleting properties of methyl bromide and reported development of resistance in many storage insect pests to phosphine (Shaaya *et al*., 1997), there is an urgent need to develop simple, convenient and safer alternatives to these toxic synthetic fumigants. In recent years, global research has focused on the possible use of plant secondary metabolites, especially essential oils, in protection of stored agricultural products. Essential oils and their major constituents, often monoterpenoids, are among the best known substances to have attracted research attention in recent years as potential alternatives to classical fumigants. Therefore, the study was aimed at evaluating the fumigant potency of selected plant essential oil constituents against major insect pests of stored food grains with special focus on adults of *T. castaneum, S. oryzae, O. surinamensis, R. dominica* and *C. chinensis*.

Literature Summary

The new scientific push for alternative insecticides is accelerated by the high cost-benefit ratio, residues on treated grains, development of pest resistance and serious health concerns posed to non-target organisms by synthetic pesticides. In addition to their flavouring, medicinal and cosmetic values, aromatic plants present potentially high level efficiency in post-harvest crop protection (Lamiri *et al*., 2001) and increasingly used as ingredients in foods, drinks, toiletries, medicine and cosmetics (Sacchetti *et al*., 2005). Additionally, essential oils and their constituents are increasingly gaining attention from crop protectionists owing to their relative safety status, wide acceptance by consumers and potential multi-purpose functional use.

Literature survey indicates that essential constituents, (at 0.1 µl/720 ml volume), eugenol, 1,8-cineole, camphor and linalool caused 85- 100, 80- 100 and 0- 13% mortality of adult *S. oryzae, R. dominica* and *T. castaneum* insects, respectively, 24 h after treatment (Rozman *et al*., 2006). Similarly, Bekele and Hassanali (2001) reported that the toxic effects of essential oils were due to the combined effects of different components, either with or without significant individual toxic action of their own against stored-product insects.

Study Description

The fumigant toxicity of five major essential constituents of indigenous aromatic Kenya plants, a-humulene, caryophyllene oxide, myrcene, R-(+)-a- pinene and R-(+)-b-pinene, were evaluated in space fumigation according to Shaaya *et al*. (1991). The essential oil constituents (standards) used for the fumigant toxicity studies were obtained from Sigma-Aldrich Israel Ltd. Twenty unsexed adults of five stored-product insects, *Callosobruchus chinensis, Sitophilus oryzae, Oryzaephilus surinamensis, Rhyzopertha dominica* and *Tribolium castaneum* were introduced into meshed metallic cages with small amount of food and suspended by hook in 3.4 L flat bottom glass space fumigation chambers. Each essential oil was applied, at four rates (0, 1, 5 and 10 ìl/L air) with four replicates, to a small piece of filter paper (Whatman No. 1) and suspended together with test insects by hook in the glass chamber slightly below the cage (Fig. 1). Magnetic stirrers were used over a 24 h exposure time to ensure even distribution of fumigant in a room maintained at 30±2^{o}C, 68±2% RH and 12D: 12D (total darkness). The numbers of dead (N_D) insects were recorded 24, 72, 120 and 168 h from onset of space fumigation and the actual percent adult mortality computed according to Asawalam *et al*. (2006) and corrected for natural mortality using Abbott's formula (Abbott, 1925). Data on corrected percent mortality were homogenised for treatment variances using arcsine-transformation (Ogendo *et al*., 2008) before being subjected to one-way analysis of variance (ANOVA) and treatment means separated using Tukey's studentized (HSD) test (Gomez and Gomez, 1984; Talukder and Howse, 1995). The dose-response relationship between concentration of essential oil constituent and percent mortality was determined using Probit regression analysis of transformed data to estimate LC_{50} values. Any two LC_{50} values in a column whose 95% confidence limits did not overlap were significantly different (Talukder and Howse, 1995).

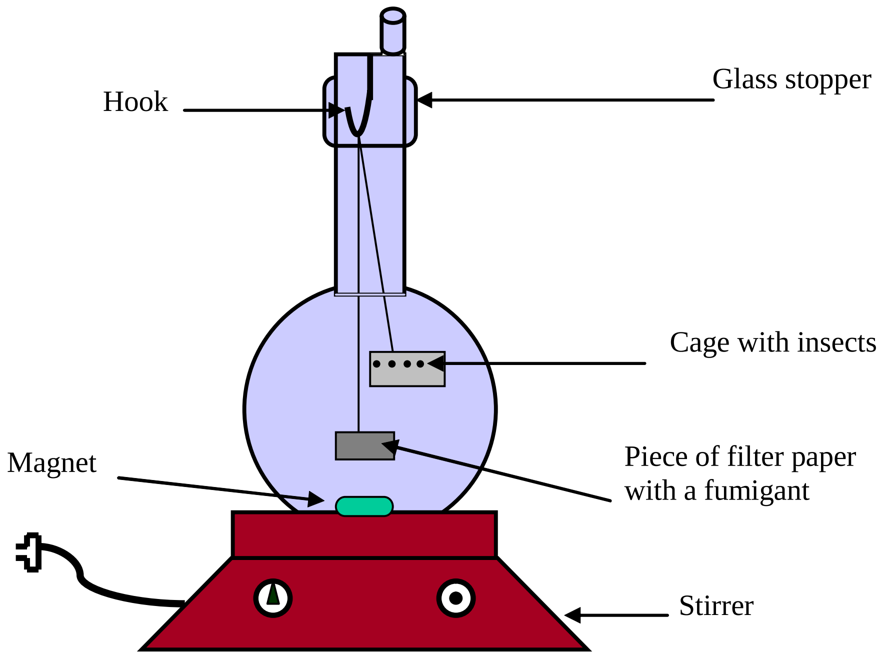

Figure 1. A 3.4 litre flat bottom glass (space) fumigation chamber.

Research Application

The fumigant toxicity of plant essential oil constituents against adult insects were significantly (P<0.001) influenced by time after application, concentration, insect species and corresponding factor interactions. The cumulative percent mortality of all insects tested were highest 168 h after treatment with clear dose-dependent inter-species variations in susceptibility by adult *S. oryzae, R. dominica, O. surinamensis, C. chinensis* and

T. castaneum. At the highest concentration (10 µl/L air), alpha humulene was the most toxic essential oil constituent against adult *S. oryzae* (96.3% kill) and *O. surinamensis* (69% kill) whereas R-(+)-alpha pinene, caryophyllene oxide, myrcene and R-(+)- beta pinene were equally the most toxic against adult *R. dominica* (97-100% kill) 168 h after treatment (Fig. 2). Adult *T. castaneum* and *C. chinensis* were the most tolerant and susceptible insect species, respectively. At the highest concentration (10 µl/L air), only 10.0 and 15% adult *T. castaneum* mortalities were observed 168 h after treatment for caryophyllene oxide and myrcene, respectively compared to 58 and 68% mortality of adult *C. chinensis* 72 h after treatment (Fig. 2). Results of the probit regression analyses revealed that the lethal concentration that killed 50% of test insects (LC_{50}) varied with plant essential oil constituent and the insect species. All test essential oil constituents showed weak or no response (no LC_{50} values) against *O. surinamensis* and *T. castaneum* whereas *C. chinensis* had LC_{50} values of 29.6, 0.48, 5.11, 2.94 and 42.7 µl/L air 24h after treatment for caryophyllene oxide, alpha humulene, R-(+)-alpha pinene, R-(+)-beta pinene and myrcene, respectively. These results have revealed the existence

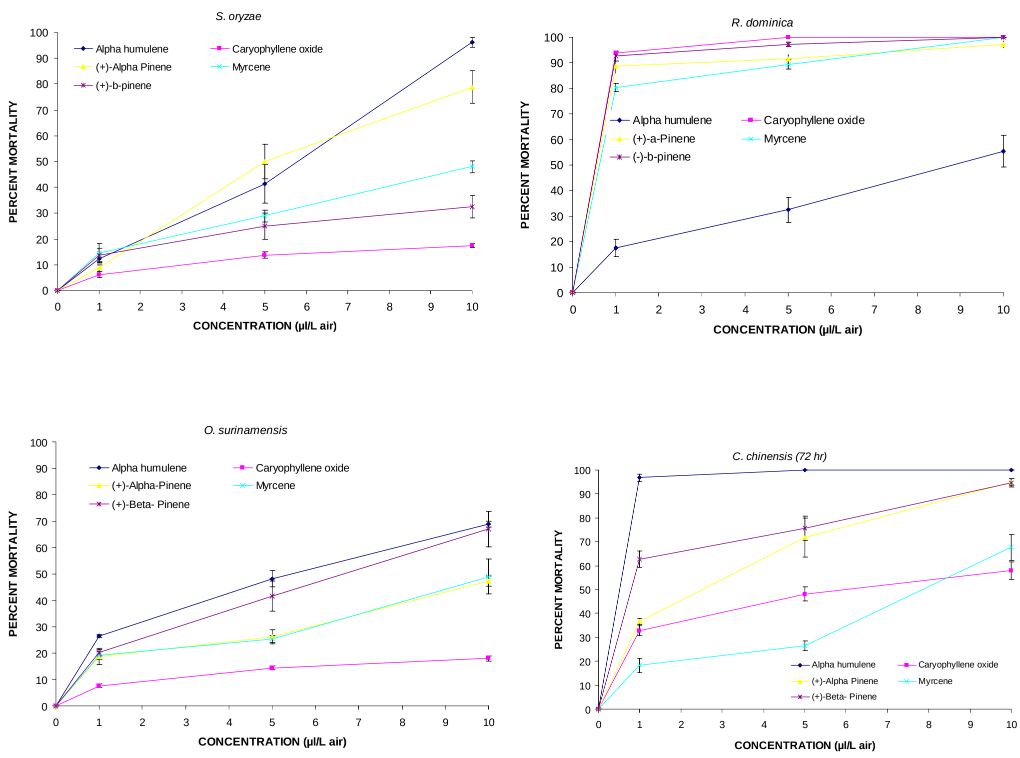

Figure 2. Percent adult mortality (mean ± *SE*, n= 4) of four stored-product insect pests 168 h after treatment with five essential oil constituents in space fumigation chambers

of plant essential oil constituent by insect species interaction effects on adult mortality of test insects.

The fact that the five plant essential oil constituents achieved a 50% kill of the test insects 24 h after treatment, except *T. castaneum* and *O. surinamensis*, at fairly low concentrations of 0.48- 145 µl/L air, demonstrates the existence of strong fumigant toxicity and potential use as grain fumigants.

Recommendation

It is recommended that further bioactivity (space fumigation, repellence and growth inhibitions) and grain fumigation studies be conducted with these essential oil constituents, either individually or blends, against a wider spectrum of stored-product insects. These volatile constituents, together with their parent essential oils, hold good promise for a cost-effective and environment-friendly alternatives to synthetic fumigants.

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References

Abbott, W.W. 1925. A method for computing the effectiveness of an insecticide. *Journal of Economic Entomology* 18:265-267.

Asawalam, E.F., Emosairue, S.O. and Hassanali, A. 2006. Bioactivity of *Xylopia aetiopia* (Dunal) A. Rich essential oil constituents on maize weevil *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae). *Electronic Journal of Environmental, Agricultural and Food Chemistry* 5(1):1195-1204.

Gomez, K.A., Gomez, A.A. 1984. Statistical Procedures for Agricultural Research. 2nd Edition, Wiley Interscience Publications, New York.

Gressel, J., Hanafi, A., Head, G., Marasas, W., Obilana, A.B., Ochanda, J., Souissi, T. and Tzotzos, G. 2004. Major heretofore intractable biotic constraints to African food security that may be amenable to novel biotechnological solutions. *Crop Protection* 23:661-689.

Lamiri, A., Lhaloui, S., Benjilali, B. and Berrada, M. 2001. Insecticidal effects of essential oils against Hessian fly, *Mayetiola destructor* (Say). *Field Crops* 71:9-15.

Ogendo, J.O., Kostyukovsky, M., Ravid, U., Matasyoh, J.C, Deng, A. L., Omolo, E.O., Kariuki, S.T. and Shaaya, E. 2008. Bioactivity of *Ocimum gratissimum* L. oil and two constituents against five insect pests attacking stored food products. *Journal of Stored Products Research* 44:328-334.

Ogendo, J.O., Deng, A.L., Omolo, E.O., Matasyoh, J.C. and Tabu, I.M. 2003. Grain pests management practices in subsistence agriculture: A case of farmers in Suba District, Nyanza Province, Kenya. Farm Survey Report. (AICAD Grant No. AICAD/RD-07/SED/02-001).

Rozman, V., Kalinovic, I. and Korunic, Z. 2007. Toxicity of naturally occuring compounds of Lamiaceae and Lauraceae to three stored-product insects. *Journal of Stored Products Research* 43(4):347-355.

Sacchetti, G., Maietti, S., Muzzoli, M., Scaglianti, M., Manfredini, S., Radice, M. and Bruni, R. 2005. Comparative evaluation of 11 essential oils of different origin as functional antioxidants, antiradicals and antimicrobials in foods. *Food Chemistry* 91: 621-632.

Shaaya, E., Kostjukovsky, M., Berg, J.E.H. and Sukprakarn, C. 1997. Plant oils as fumigants and contact insecticides for the control of stored-product insects. *Journal of Stored Products Research* 33(1): 7-15.

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. *Journal of Chemical Ecology* 17:499-504.

Talukder, F.A. and Howse, P.E. 1995. Evaluation of *Aphanamixis polystachya* as repellents, antifeedants, toxicants and protectants in storage against *Tribolium castaneum* (Herbst). *J. of Stored Products Research* 31: 55-61.