Journal of Stored Products Research 69 (2016) 172–174

Contents lists available at ScienceDirect

Journal of Stored Products Research

journal homepage: www.elsevier.com/locate/jspr

Short communication

Toxicity of coumaran to stored products beetles

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ARTICLE INFO

Article history:
Received 20 November 2014
Received in revised form
1 July 2016
Accepted 27 July 2016

Keywords:
Coumaran
Fumigant toxicity
Mixed-age culture
Stored product beetles

ABSTRACT

The fumigant activity of coumaran was tested against mixed-age cultures of the rice weevil (*Sitophilus oryzae* L.), the lesser grain borer (*Rhyzopertha dominica* F.), the rust-red flour beetle (*Tribolium castaneum* Herbst), saw-toothed grain beetle (*Oryzaephilus surinamensis* L.) and flat grain beetle (*Cryptolestes ferrugineus* Stephens). *T. castaneum* was tolerant to coumaran, for total population extinction was achieved in 72 h exposure at 50 μg/L dose only. In contrast all developmental stages of *O. surinamensis*, *C. ferrugineus* and *R. dominica* were quite susceptible 100% mortality was attained at < 50 μg/L doses in 24 or 72 h exposure. Further results showed that *T. castaneum*, generally tolerant pest to fumigants and *R. dominica*, a predominant stored grain pest in India, could be controlled by coumaran. The findings indicate that, coumaran is good source for alternate to methylbromide for effective control of stored grain insect pests.

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1. Introduction

Stored product insect pests are the major cause of grain losses during post harvest storage, particularly in the tropical countries (Affognon et al., 2015; Rajashekar et al., 2012). It is estimated that more than one-third of the food products is lost to various pests during post-harvest storage (Oerke, 2006; Parfitt et al., 2010). For the control of stored product beetles, several insecticides are used mainly phosphine and methyl bromide. Although phosphine is generally effective, due to lack of persistence in the treated grain infestation may occur within a few weeks after treatment. Due to extensive use of fumigants, several stored products insects have developed different degrees of resistance to phosphine and hence, there is a problem in effective treatment of commodites using phosphine (Nayak et al., 2015; Phillips and Throne, 2009; Subramanyam and Hagstrum, 1995). In view of its ozone depleting potential, the use of methyl bromide is being restricted (Fields and White, 2002). In view of their health and environmental hazards several chemical fumigants or insecticides have either been banned or restricted for use in grain protection (Mohapatra et al., 2014; Subramanyam and Hagstrum, 1995). Consequently, intensive research is going on in developed and developing countries on alternative fumigants such as carbonyl sulfide, coumaran, ethyl formate, ethane dinitrile, propylene oxide, and sulfuryl fluoride (Jagadeesan et al., 2014; Rajashekar et al., 2013; Rajendran, 2001).

Coumaran (2,3-dihydrobenzofuran) is a non flammable, pale yellow liquid. It is slightly soluble in water, and is formed by reducing coumarone. Coumaran was isolated from the basal stem of *Cyperus nipponicus* French. & Savat. (Family: Cyperaceae) and *Cyperus distans* L.f. and has revealed insect antifeedants against the tobacco cutworm (*Spodoptera litura* Fabricius) (Morimoto et al., 1999). Scharf et al. (2006) reported that coumaran posses the fumigant activity against *Drosophila melanogaster* (fruit fly). Coumaran bioactive molecule isolated from the leaves of *Lantana camara* L. was also found toxic to various stored grain insect pests when used as a biofumigant (Rajashekar et al., 2013, 2014). All literature cited above indicates that coumaran has been used as a fumigant against stored grain pest, but there is a lack of information on toxicity (if any) on stored food commodites and on doses for 100% population extinction. Therefore, insecticidal efficacy or toxicity of synthetic coumaran to mixed-age cultures (all developmental stages) of the rust-red flour beetle, *Tribolium castaneum* (Herbst), the saw-toothed grain beetle, *Oryzaephilus surinamensis* (L.), the flat grain beetle, *Cryptolestes ferrugineus* (Stephens), the rice weevil, *Sitophilus oryzae* (L.), and the lesser grain borer, *Rhyzopertha dominica* (F.), was determined.

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http://dx.doi.org/10.1016/j.jspr.2016.07.006
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2. Materials and methods

2.1. Preparation of mixed-age-cultures

T. castaneum cultures were reared on whole wheat flour fortified with 5% dried yeast, *C. ferrugineus* and *O. surinamensis* on a 5:5:1 mixture of broken wheat, rolled oats and yeast, whereas *S. oryzae* and *R. dominica* were reared on whole wheat. *R. dominica* cultures was maintained at 30 ± 1 °C and 70% r.h. and cultures of other insects were maintained at 25 ± 1 °C and 65 ± 10% r.h. From these cultures, adults (1–2 weeks old) were taken for preparation of mixed-age cultures. About 300 adults of *T. castaneum, C. ferrugineus* and *O. surinamensis* were released into 1 kg of respective culture medium in 2 liter glass jars-Similarly, 300 adults of *S. oryzae* and *R. dominica* were allowed to breed separately in 1 kg of wheat in 2 litre glass jars. After one week, the adults were shifted from the cultures. A series of cultures of the respective species were thus maintained continuously. The insect culture jars were maintained at temperature 30 ± 1 °C for *R. dominica* and 25 ± 1 °C for other insects. Cultures in six successive weeks (containing 5–6, 4–5, 3–4, 2–3, 1–2, and 0–1 week old insects) were pooled such that the pooled populations contained all developmental stages of the respective species. The pooled mixture of individual species served as mixed-age cultures for toxicity tests (Leelaja et al., 2007).

Rearing media containing mixed-age cultures of individual species was weighed separately in 50 g aliquots and transferred into cloth bags of size 20 cm × 14 cm. These cloth bags were placed individually in 0.85 L desiccators that served as the fumigation chambers. The desiccators were provided with holes sealed by rubber septa for injecting coumaran (procured from Sigma-Aldrich Co (St. Louis MO, USA).

2.2. Fumigation

The insects were exposed to a range of doses of coumaran (0.3–50 μg/L) for 24 h and 72 h at 26 ± 2 °C. For each species, there were 5 replicates per dose with an equal number of untreated control replicates. Gas-tight micro syringes were used for injecting the fumigant. At the end of exposure, the fumigation was terminated and the test insect bags were taken out of the desiccators. The contents of the bags were transferred to individual bottles (12 cm × 5 cm size) and kept at the rearing temperature and humidity conditions for 8 weeks. The insects, which emerged from wheat (*S. oryzae* and *R. dominica*) or survived as adults (*T. castaneum*, *C. ferrugineus* and *O. surinamensis*) in their respective media were checked at weekly intervals for 8 weeks. Similarly, counts were made in untreated control batches every week. Percent kill was determined by taking the survival/emergence in the controls as 100%. In Each bioassay mortality were recorded and those insects that did not move when lightly probed or shaken in the light and mild heat were considered dead (Leelaja et al., 2007).

2.3. Statistical analysis

Mortality in coumaran was determined by taking the survival/emergence in the controls as 100%. Mortality data of the different insects were subjected to two-way ANOVA with time and dosage as factors and Post hoc testing was carried out using the Tukey's HSD test. A significance level of 0.01 was used for all statistical tests.

3. Results

Coumaran was highly toxic to *C. ferrugineus* and *O. surinamensis* that 100% mortality was recorded at the dose of 50 μg/L in 24 h exposure itself (Table 1). *S. oryzae, R. dominica* and *T. castaneum* were, slightly tolerant to the fumigant that 100% mortality of the insects was achieved only at the higher dose of 50 μg/L in 24 h exposures. Generally with the extended exposure period of 72 h, increased mortality was observed in all the five species ($P < 0.01$) (Table 1). The order of susceptibility of the tested species did not alter with the increased exposure period. The susceptibility of the insects with 24 or 72 h exposure was in the following decreasing order: *C. ferrugineus* > *O. surinamensis* > *R. dominica* > *S. oryzae* > *T. castaneum*. In untreated control, there was insignificant mortality in five species. The average number of survivors in untreated control batches of other species varied from 137 ± 5 (*T. castaneum)*, 148.2 ± 2.4 (*R. dominica*), 253.2 ± 2.8 (*C. ferrugineus*), 262 ± 6.5 (*O. surinamensis*) to 537 ± 4 (*S. oryzae*) in 24 h exposure and 140 ± 4 *(T. castaneum)* 172.2 ± 2.8 (*R. dominica*), 260.2 ± 2.8 (*C. ferrugineus*), 274.2 ± 4.4 (*O. surinamensis*) to 554 ± 5 *(S. oryzae)* in 72 h exposure, respectively ($P < 0.01$).

4. Discussion

Many organic compounds have fumigant or grain protectant properties. For example 1, 8 cineole to *S. oryzae* (L.), *T. castaneum* (Herbst) and *R. dominica* (F.) (Lee et al., 2004), Methyl salicylate to *Sitophilus zeamais* Motsch., and *R. dominica* (Jayasekara et al., 2005), three manoterpeniods, the ketones pulegone, *l*-fenchone, and the aldehyde perillaldehyde to *T. castaneum* (Lee et al., 2003), allyl acetate to five major stored beetles (Rajendran and Muralidharan, 2005), ethylformate to *T. castaneum* and *Sitotroga cerealella* (Olivier) (Muthu et al., 1984). Although comparison of toxicities between the above-mentioned fumigants is difficult, particularly monterpenes, because the have relatively low toxicity.

Insecticidal potency of coumaran to insects has been reviewed (Scharf et al., 2006; Rajashekar et al., 2013). In present study reveal that coumaran is toxic to *S. oryzae, R. dominica, T. castaneum, C.*

Table 1
Mortality (% ± SD) * of mixed-age cultures of *Sitophilus oryzae, Rhyzopertha dominica, Tribolium castaneum, Oryzaephilus surinamensis* and *Cryptolestes ferrugineus* exposed for 24 h and 72 h to coumaran at 28 ± 2 °C.

Dosage μg/L	*S. oryzae*		*R. dominica*		*T. castaneum*		*O. surinamensis*		*C. ferrugineus*	
	24 h	72 h	24 h	72 h	24 h	72 h	24 h	72 h	24 h	72 h
5	9.2 ± 3[a]	18.9 ± 2.1[a]	17.2 ± 2.1[a]	34.8 ± 4.7[a]	5.1 ± 1.2[a]	11.4 ± 3.2[a]	37.3 ± 9.1[a]	40.2 ± 4.4[a]	45.9 ± 2.8[a]	48.4 ± 4.2[a]
10	21.3 ± 2.1[b]	39.6 ± 7.4[b]	29.5 ± 7.2[b]	50.9 ± 4.2[b]	14.7 ± 2[b]	29.3 ± 5.4[b]	59.2 ± 10.4[b]	62.1 ± 10[b]	55.2 ± 6.2[b]	67.3 ± 4.2[b]
15	37 ± 2.4[c]	52.4 ± 1.2[c]	70.4 ± 12[c]	74.4 ± 3.2[c]	22.2 ± 1.4[c]	40.7 ± 4.2[c]	72.4 ± 4.2[c]	70.3 ± 2.2[c]	64.3 ± 5.2[c]	80.5 ± 4.8[c]
20	67.2 ± 1.5[d]	70.5 ± 3.6[d]	76.4 ± 9.5[cd]	83.2 ± 2.5[d]	43.8 ± 2.6[d]	50.2 ± 1.2[d]	75.8 ± 4.2[c]	85.5 ± 4.2[d]	73.5 ± 6.4[d]	88.2 ± 2.4[d]
25	83.1 ± 2.2[e]	80.5 ± 3.8[e]	87.8 ± 5.1[e]	90.4 ± 1.2[e]	57.4 ± 2.4[e]	65.5 ± 2.4[e]	86.2 ± 5.4[d]	96.4 ± 2.2[e]	76.5 ± 4.2[d]	94.5 ± 1.2[e]
30	87.6 ± 2.3[f]	90.6 ± 2.2[f]	83.4 ± 4.3[e]	95.1 ± 1[f]	71.3 ± 6.2[f]	83 ± 4.2[f]	93.6 ± 2.8[e]	100[f]	91.2 ± 2.2[e]	100[f]
40	90.2 ± 2.8[f]	98.2 ± 5.2[g]	93.6 ± 8[f]	100[g]	87.2 ± 3.4[g]	90.6 ± 6 [g]	97.4 ± 1[f]	100[f]	98.3 ± 3.2[f]	100[f]
50	97.4 ± 2.2[g]	100[gh]	100[g]	100[f]	93.8 ± 2.2[h]	100[h]	100[fg]	100[f]	100[f]	100[f]

*There were 5 replicates per dose and in untreated controls (50 g infested media per replicate tested). In untreated controls, the mean numbers of survivors per replicate given. Different letters within a column indicate that means are significantly different (Tukey's HSD, df = 4, $P < 0.01$).

ferrugineus and *O. surinamensis*. The results shows significant enhanced toxicity, particularly exposure period increase from 24 to 72 h. The results indicate that at the extended exposure period of 72 h, the mortality also inceased in all five species. In tests against all developmental stages of stored product insects such as *T. castaneum, S. oryzae,* and *Cryptolestes chenisis* Rajashekar et al. (2013) observed that coumaran was effective at a concentration-time (CT) product of 30 mg/m^3 x 7 d at 30 °C. Several report showed that essential oils and plant volatile organics compounds in *T. castaneum* was more susceptible than *S. zeamais* (Liu and Liu, 2014; Suthisut et al., 2011). But in the present study, for 100% population extinction of *T. castaneum* the target (CT) product was high at 50 mg/cm^3 x 72 h. This may be due to sorption of food commodities. For example, sorption of the fumigant phosphine is more in wheat flour rather than whole wheat (Reddy et al., 2007). This could be one of the major reasons for lower susceptibility of the *Tribolium* insects with 24 or 72 h exposure of coumaran in wheat flour. There is no previous report about the toxicity of synthetic coumaran to *T. castaneum*.

Desmarchelier (1994) noted that the immature stages of *S. oryzae* were highly tolerant to carbonyl sulfide fumigant. At the dosage of 60 mg/L exposed for a period of 24 h at 25 °C he achieved 99.8% mortality and at 40 mg/L for 48 h, 100% mortality of *S. oryzae* was observed. Evidently, the present study shows coumaran is more toxic than carbonyl sulfide. Rajendran et al. (2000) performed the laboratory tests with the mixed-age cultures of phosphine-resistant field strain of *S. oryzae* in 7-day exposures, and determined an effective phosphine concentration of 0.8 mg/L (CT product 0.8 g/m^3 x 168 h - 134 g h/m^3). However, unlike phosphine, coumaran is rapidly toxic to insect pests so that 24–72 h exposure is adequate to achieve the 100% mortality of the insects. Rajashekar et al. (2013) demonstrated that coumaran is more toxic than liquid fumigants such as ethyl format and allyl acetate to stored product insects.

The study shows that coumaran has the advantage of its high toxicity to secondary pests, such as *C. ferrugineus* and *O. surinamensis* which are generally tolerant to other fumigant (Rajendran and Muralidharan, 2005; Kaur and Nayak, 2015). Also, these insects are serious pest of cereals, cereal products, oil seeds, dried fruits, copra, dried herbs and cocoa beans. Like phosphine, coumaran may be less toxic to the egg stage of insects. It has to be examined whether stored grain insects will develop resistance to coumaran quickly (following exposure to sub-lethal doses in 3–5 generations) as in the case of phosphine.

It has clear from the findings of this study that the coumarn has potential as a fumigant for five major insect pests of stored food commodities and is therefore ideal applicant fumigants of stored durable agricultural products. As with any fumigant based on plant products, registration and adoption of synthetic coumaran will depend on the outcome of research on potential negative effects on human health and the environment, and socio-economic factors (Isman, 2006; Weaver and Subramanyam, 2000).

Acknowledgements

The author wish to thank the Director, IBSD for his keen interest in this study. The author acknowledges the financial support by the Department of Biotechnology (DBT), New Delhi, India.

References

Affognon, H., Mutungi, C., Sanginga, P., Borgemeister, C., 2015. Unpacking postharvest losses in sub-Saharan Africa: a meta-analysis. World Dev. 66, 49–68.
Desmarchelier, J.M., 1994. Carbonyl sulfide as a fumigant for control of insects and mites. Stored Product Protection. In: Highley, E., et al. (Eds.), Proceedings of the 6th International Working Conference on Stored -product Protection, 17-23 April, 1994, Canberra, Australia. CAB International, Wallingford, UK, pp. 78–82.
Fields, P.G., White, N.D., 2002. Alternatives to methyl bromide treatments for stored-product and quarantine insects 1. Annu. Rev. Entomology 47, 331–359.
Isman, M.B., 2006. Botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world. Annu. Rev. Entomology 51, 45–66.
Jagadeesan, R., Nayak, M.K., Pavic, H., Chandra, K., Collins, P.J., 2014. Susceptibility to sulfuryl fluoride and lack of cross-resistance to phosphine in developmental stages of the red flour beetle, Tribolium castaneum (coleoptera: tenebrionidae). Pest Manag. Sci. 71 (10), 1379–1386.
Jayasekara, T.K., Stevenson, P.C., Hall, D.R., Belmain, S.R., 2005. Effect of volatile constituents from *Securidaca longipedunculata* on insect pests of stored grain. J. Chem. Ecol. 31 (2), 303–313.
Kaur, R., Nayak, M.K., 2015. Developing effective fumigation protocols to manage strongly phosphine resistant *Cryptolelestes ferrugineus* (Stephens) (Coleoptera: laemophloeidae). Pest Manag. Sci. 71 (9), 1297–1302.
Lee, S., Peterson, C.J., Coats, J.R., 2003. Fumigation toxicity of monoterpeniods to several stored product beetles. J. Stored Prod. Res. 39 (1), 77–85.
Lee, B.-H., Annis, P.C., Tumaali, F., Choi, W.-S., 2004. Fumigant toxicity of essential oils from the Myrtaceae family and 1, 8-cineole against 3 major stored-grain insects. J. Stored Prod. Res. 40 (5), 553–564.
Leelaja, B., Rajashekar, Y., Reddy, P.V., Begum, K., Rajendran, S., 2007. Enhanced fumigant toxicity of allyl acetate to stored-product beetles in the presence of carbon dioxide. J. Stored Prod. Res. 43, 45–48.
Liu, X.C., Liu, Z.L., 2014. Evaluation of insecticidal activity of *Nardostachys jatamansi* essential oil against some grain storage insects. J. Entomology Zool. Stud. 2 (4), 335–340.
Mohapatra, D., Kar, A., Giri, S.K., 2014. Insect pest management in stored pulses: an overview. Food Bioprocess Technol. 1–27.
Morimoto, M., Fujii, Y., Komai, K., 1999. Antifeedants in Cyperaceae: coumaran and quinones from Cyperus spp. Phytochemistry 51, 605–608.
Muthu, M., Rajendran, S., Krishnamurthy, T.S., Narasimhan, K.S., Rangaswamy, J.R., Jayaram, M., Majumder, S.K., 1984. Ethylformate as a safe general fumigant. Controlled atomsphere and fumigation in grain storage. In: Ripp, R.E. (Ed.), Proceedings of an International Sysmposium " Practical Aspects of Controlled Atomsphere and Fumigation in Grain Storages" Held from 11 to 22 April 1983 in Perth, Westeran Australia, pp. 369–393.
Nayak, M.K., Daglish, G.J., Phillips, T.W., 2015. Managing resistance to chemical treatments in stored products pests. Stewart Postharvest Rev. 11, 1–6.
Oerke, E.-C., 2006. Crop losses to pests. J. Agric. Sci. 144, 31–43.
Parfitt, J., Barthel, M., Macnaughton, S., 2010. Food waste within food supply chains: quantification and potential for change to 2050. Philos. Trans. R. Soc. B Biol. Sci. 365, 3065–3081.
Phillips, T.W., Throne, J.E., 2009. Biorational approaches to managing stored-product insects. Annu. Rev. Entomology 55, 375.
Rajashekar, Y., Rao, L.J., Shivanandappa, T., 2012. Decaleside: a new class of natural insecticide targeting tarsal gustatory sites. Naturwissenschaften 99, 843–852.
Rajashekar, Y., Vijay Kumar, H., Ravindra, K.V., Bakthavatsalam, N., 2013. Isolation and characterization of biofumigant from leaves of *Lantana camara* for control of stored grain insect pests. Ind. Crops Prod. 51, 224–228.
Rajashekar, Y., Ravindra, K., Bakthavatsalam, N., 2014. Leaves of *Lantana camara* Linn. (Verbenaceae) as a potential insecticide for the management of three species of stored grain insect pests. J. Food Sci. Technol. 51, 3494–3499.
Rajendran, S., 2001. Alternatives to methyl bromide as fumigants for stored food commodities. Pestic. Outlook 12, 249–253.
Rajendran, S., Muralidharan, N., 2005. Effectiveness of allylacetate as fumigant against five stored grain beetles. Pest Manag. Sci. 61 (1), 97–101.
Rajendran, S., Shrilatha, B., Rekha-Savanth, T.L., Nayak, K., 2000. Response of field strains of stored grain insect pests to phosphine fumigant. Pestic. Res. 12, 63–67.
Reddy, P.V., Rajashekar, Y., Begum, K., Leelaja, B.C., Rajendran, S., 2007. The relation between phosphine sorption and terminal gas concentrations in successful fumigation of food commodities. Pest Manag. Sci. 63, 96–103.
Scharf, M.E., Nguyen, S.N., Song, C., 2006. Evaluation of volatile low molecular weight insecticides using *Drosophila melanogaster* as a model. Pest Manag. Sci. 62, 655–663.
Subramanyam, B., Hagstrum, D.W., 1995. Resistance measurement and management. Integrated Management of Insects in Stored Products, pp. 331–397.
Suthisut, D., Fields, P.G., 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.
Weaver, D., Subramanyam, B., 2000. Botanicals. In: Subramanyam, B., Hagstrum, D. (Eds.), Alternatives to Pesticides in Stored-product IPM. Springer, US, pp. 303–320.