

Acaricidal effects of natural six-carbon and nine-carbon aldehydes on stored-product mites

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Abstract The toxicities of three plant volatiles, (2*E*)-hexenal, (2*E*, 6*Z*)-nonadienal and (2*E*)-nonenal, intermediate products of the oxylipin biosynthesis pathway, were tested on three mites of importance for medical purposes and as pests. The aldehydes were diluted in hexane separately and incorporated into diets in ranges of 4–143 mg g⁻¹. The final density of mites in control and aldehyde-enriched diets was compared after 21 days. The aldehydes were toxic to the mites, whose final density showed an inverse correlation with aldehyde concentration. In addition to the effects of aldehyde concentration, the final density of mites was also influenced by the different aldehydes tested and the interaction among aldehyde concentration and chemical structure. In a functional combination of aldehydes and species, the doses calculated for growth inhibition and eradication of mites ranged from 4 to 35 mg g⁻¹ and from 36 to 314 mg g⁻¹, respectively. Due to the protective role displayed by natural six-carbon and nine-carbon aldehydes, these compounds are potential candidates for controlling stored-product mites in stored food and feed products.

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Introduction

Stored product mites are important pests of stored food commodities and animal feed in areas with humid climates (Sánchez-Ramos and Castañera 2005). In grain stores, the mites tend to remain in locations with high moisture such as the corners and the central area of stored rooms (Athanasios et al. 2003). Under these suitable abiotic conditions, their populations can increase to high numbers of individuals in dust of grain residues or in grain commodities (Radon et al. 2000; Athanasios et al. 2003, 2005; Hubert et al. 2006a), leading to a consistent reduction in food and feed quality (Solomon 1946) and safety due to acariosis (Farley et al. 1989; Li et al. 2003), allergenic reactions (Marx et al. 1993; Kondreddi et al. 2006) and the dissemination of toxigenic moulds (Hubert et al. 2004).

The treatment of mites is usually based on chemical pesticides (Collins 2006; Sánchez-Ramos and Castañera 2003; Hubert et al. 2007), but mites are more tolerant than stored product insects (Zdarkova and Horak 1974) and the extensive use of chemicals led to development of resistance from several mite species (Szlendak et al. 2000). Physical treatments proposed for mite control are expensive in some cases or not suitable for all commodities. Therefore, there is increasing interest in finding new natural acaricidal compounds that could reduce the massive application of chemical pesticides. Among plant natural compounds, several essential oils were reported to be repellent (Jaenson et al. 2006) or toxic for mites (Kim et al. 2004; Macchioni et al. 2002; Sánchez-Ramos and Castañera 2006; Lee et al. 2006).

Six-carbon (C6) and nine-carbon (C9) aldehydes and corresponding alcohols are the major components of volatile organic chemicals (VOC) produced by plants from polyunsaturated fatty acids via the lipoxygenase/hydroperoxide lyase pathway (Matsui 2006). VOC levels are normally low in intact plants but increase rapidly after wounding or insect/pathogen attack. In vivo, they function as direct antimicrobials/insecticides, but can also attract natural enemies of herbivores (Shiojiri et al. 2006).

C6 and C9 aldehydes are synthesized from 13- and 9-hydroperoxides produced by plant lipoxygenases (LOXs) from linoleic or linolenic acids. (2E)-nonenal and (2E, 6Z)-nonadienal are obtained from 9-hydroperoxy derivatives of linoleic and linolenic acids, whereas hexanal and (2E)-hexenal are originated from 13-hydroperoxy derivatives. Both C6 and C9 aldehydes can be further metabolized in vivo by alcohol dehydrogenase to form the corresponding alcohols. Together with their defense function, volatile aldehydes are also important contributors of the characteristic flavors of vegetables and fruits and are widely used as food additives in the agro-food industry to restore food flavor and aroma after sterilization or other industrial processes (Prost et al. 2005; Casey and Hughes 2004; Santino et al. 2005a, b).

The direct effect of such compounds has been evaluated on various pathogens and pests (Hammond et al. 2000; Prost et al. 2005; Casey and Hughes 2004; Matsui 2006; Mita et al. 2007; Hubert et al. 2008). However, the effect of plant volatile aldehydes on mites has not been reported so far.

In this work, three different volatile aldehydes produced by the lipoxygenase pathway, (2E)-hexenal, (2E, 6Z)-nonadienal and (2E)-nonenal, were tested for their acaricidal activity in feeding tests against three stored product mite species (*Acarus siro*, *Aleuroglyphus ovatus* and *Tyrophagus putrescentiae*).

Materials and methods

Chemicals

Tested C6 and C9 aldehydes were: (2*E*, 6*Z*)-nonadienal (85+%) (Cat. No. 180556, Aldrich, Saint Louis, MO, USA), (2*E*)-nonenal (97%) (Cat. No. 255653, Aldrich), and (2*E*)-hexenal (98%) (Cat. No. 132659 Aldrich).

Mites

The specimens of stored product mites originated from the cultures kept at the Crop Research Institute, Prague. *Acarus siro* L. and *Tyrophagus putrescentiae* (Schränk) were collected in Bustehrad, Czech Republic, in April 1996. *Aleuroglyphus ovatus* (Troupeau) was obtained from the Central Science Laboratory, Sand Hutton, York, UK. The mites were mass-reared in frit-chambers (S0-frit, Kavalier Sazava, Czech R.) plugged with rubber. The rubber was pierced with a steal tube (5 mm diameter). Both ends of the glass tube were covered with muslin. The rearing diet (100 g) consisted of 44 g oat flakes, 44 g wheat germ, 10 g lyophilized yeast and 2 g dried *Daphnia* spp. All the ingredients were ground to a powder using ZN 200 Retch mill and sifted on AS 240 Retsch digit sieving machine (Retsch, Haan, Germany). Into every chamber, 1.5 ± 0.25 g of rearing diet was placed. The frits were placed into Secador desiccators (P-Lab, Praha, Czech R.) and kept at 85% RH and 25°C in darkness. Before the experiment, adults were collected with a brush from the surface of the rubber or the sides of the frit and counted under a Stemi 2000-C dissection-microscope (C. Zeiss, Jena, Germany).

Feeding tests

Glass tubes (20 ml volume, Kavalier, Sazava, Czech R.) were filled with 3 g of zeolite. The zeolite was moistened with 0.15 ml of 1% solution of Ajatin (Profarma, Praha, Czech R.) to prevent fungal contamination. About 0.2 g of meal supplemented with different amounts (141, 70.5, 35.3, 17.6, 8.8 and 4.4 mg) of aldehydes (diluted in *n*-hexane) were placed in each tube together with fifty mites. The tubes were covered with textile mesh and placed into the Secodar desiccator box at 85% RH and 25°C in the darkness. Ten replicates were prepared for each species, aldehyde and aldehyde concentration. The population growth was determined after 21 days. Mites were extracted using Berlese–Tullgren funnels, placed in a saturated solution of picric acid and counted under a stereomicroscope.

Data analysis

We tested the effects of different aldehydes, aldehyde concentrations, and mite species on the final number of mites. The distribution of the mite (*N*) density data was strongly skewed. We therefore transformed $[\log(N + 1)]$ the data to achieve normality. The analysis was performed using a generalized linear model assuming a normal distribution on transformed final density and aldehyde concentration, aldehyde chemical structure, mite species and their interaction as independent variables using S-Plus® software (Insightful, Corporation, Seattle, WA, USA). The doses rC_{50} , rC_{90} and rC_{100} , those aldehyde concentrations causing 50, 90 and 100% mortality of the population, and rC_{start} (aldehyde concentration required to obtain the same mite density at $t = 0$ and $t = 21$ days) were estimated from a

linear regression model. The standard error of each estimate was calculated to verify data reliability.

Results

The results of the analysis show that the final density of mites is strongly influenced by all of the following considered factors: mite species, aldehyde concentration and aldehyde chemical structure. Significant interactions were also evident among all of these variables (Table 1). Analyses carried out on each mite species using different amounts of aldehydes indicated a significant relationship between concentration and final density of mites, with the exception of the strong suppressive effect of (2*E*, 6*Z*)-nonadienal on *T. putrescentiae*. The steepest relationship was found with the toxic effect of (2*E*)-nonenal towards *A. siro* and *A. ovatus*, while (2*E*, 6*Z*)-nonadienal towards *A. siro* (Table 2). The predicted concentrations of each aldehyde necessary to obtain 50, 90 and 100% reductions of the mite population are shown on Table 3. With the exception of (2*E*)-nonenal, which shows a lower effect on *T. putrescentiae* and a relatively high standard error (for this reason these data were not included on Table 3), all of the volatile aldehydes tested in this work showed a powerful acaricidal effect on all three mite species.

Discussion

In a previous study (Hubert et al. 2008), we showed that the C6 and C9 aldehydes tested in this study showed a strong insecticidal effect on five different species of stored product beetles, either in fumigation or in feeding tests. Results presented here further expand the spectrum of their toxicity to mites, i.e., *A. siro*, *A. ovatus* and *T. putrescentiae*. All the tested aldehydes were effective in the reduction of the growth of mites, with the exception of (2*E*)-nonenal, which showed a limited effect on *T. putrescentiae* (see Table 3). The predicted concentrations volatile aldehydes necessary for the eradication of mites ranged from 36 to 314 mg g⁻¹ (Table 3).

Our results also indicated a remarkable difference in terms of the sensitivity of mite species towards the tested aldehydes. For example, in the case of (2*E*)-hexanal, the amount required to obtain 90% growth inhibitions (rC₉₀) of *A. siro* and *T. putrescentiae* populations ranged from 18 (*A. siro*) to 110 mg g⁻¹ (*T. putrescentiae*), thus showing about one

Table 1 Effects of mite species (*Acarus siro*, *Aleuroglyphus ovatus* and *Tyrophagus putrescentiae*), aldehyde type and aldehyde concentration on the final density of mites after 21 days of experiment

Effect	Df	<i>P</i>	<i>R</i> ²
Species	2	<0.001	0.32
Concentration	1	<0.001	0.22
Aldehyde type	2	0.04	0.00
Species*concentration	2	<0.001	0.06
Species*aldehyde type	4	<0.001	0.04
Concentration*aldehyde type	2	<0.001	0.01
Species*concentration*aldehyde type	4	<0.001	0.03
Residuals	612		

Data on mite density were log-transformed before the analyses, Df error = 612

Table 2 Relationship between final density of stored product mites and aldehyde concentration, Df error = 68

Species	Aldehyde	Linear regression				
		<i>m</i>		<i>b</i>		<i>P</i>
		Value	SE	Value	SE	
<i>Acarus siro</i>	Hexenal	−0.0120	0.0010	1.84	0.06	<0.001
	Nonadialenal	−0.0118	0.0016	2.17	0.10	<0.001
	Nonenal	−0.0170	0.0016	2.29	0.10	<0.001
<i>Aleuroglyphus ovatus</i>	Hexenal	−0.0040	0.0009	2.06	0.06	<0.001
	Nonadialenal	−0.0174	0.0018	2.35	0.12	<0.001
	Nonenal	−0.0166	0.0021	1.84	0.13	<0.001
<i>Tyrophagus putrescentiae</i>	Hexenal	−0.0053	0.0006	2.77	0.03	<0.001
	Nonadialenal	nd.	nd.	nd.	nd.	0.96
	Nonenal	−0.0018	0.0005	2.94	0.03	0.001

The results are based on linear regression: $\log(y + 1) = mx + b$; x is the concentration of aldehyde (mg g^{-1}), y is the final density of mites (individuals). Nonadialenal: (2*E*, 6*Z*)-nonadialenal; Nonenal: (2*E*)-nonenal; Hexenal: (2*E*)-hexenal. SE: standard errors

Table 3 Aldehyde concentration (mg g^{-1}) needed to obtain a 50 (rC_{50}), 90 (rC_{90}) and 100% (rC_{100}) reductions of the mite population.

Species	Aldehyde	Predicted doses from linear regression model							
		rC_{100}		rC_{90}		rC_{50}		rC_{Start}	
		Predicted	SE	Predicted	SE	Predicted	SE	Predicted	SE
<i>Acarus siro</i>	Hexenal	115	7	18	4	—	—	20	4
	Nonadialenal	105	10	38	4	12	6	40	4
	Nonenal	100	7	35	4	9	4	36	3
<i>Aleuroglyphus ovatus</i>	Hexenal	146	25	49	5	11	8	51	6
	Nonadialenal	94	7	38	4	15	5	38	4
	Nonenal	74	6	24	5	4	6	25	5
<i>Tyrophagus putrescentiae</i>	Hexenal	314	29	110	8	35	4	131	10
	Nonadialenal	39	40	40	13	40	6	40	16
	Nonenal	nd.	nd.	nd.	nd.	nd.	nd.	nd.	nd.

rC_{start} stands for the aldehyde concentration required to obtain the same mite density at $t = 0$ and $t = 21$ days. N.d. stands for not determined since the linear model is not significant. The calculated values fitted the linear regression model to a certain degree

order difference. (2*E*, 6*Z*)-nonadialenal and (2*E*)-nonenal, which differ by a single double bond, had similar effects on *A. siro* and *A. ovatus*, but had quite different effects on *T. putrescentiae*. Indeed, (2*E*, 6*Z*)-nonadialenal was highly effective, whereas (2*E*)-nonenal had only a limited effect (Table 3). Similar trends in sensitivity of *A. siro* and *T. putrescentiae* were observed in previous study. *T. putrescentiae* was more sensitive than *A. siro* in experiments using bean flour-enriched diet (Hubert et al. 2006b) and the acaricide Alleroff 175 CS (Hubert et al. 2007).

At present, the mechanism of aldehyde toxicity is unknown. Under the experimental conditions tested here, one can hypothesize combined actions as antifeedants and fumigant molecules (Hubert et al. 2008). In the case of the volatile aldehydes in this study, they could interact with some ingredients of the rearing diet used in the experiments, thus influencing their taste and acting as antifeedants. However, most of the C6 green leaf volatiles

produced in the lipoxygenase pathway act as feeding stimulants to insect pests (Halischke et al. 2004). At the moment, it is unclear if they can have similar effects on mites.

Alternatively, volatile aldehydes could function as nonionic surfactants, thus disrupting and disorganizing hydrogen bonds and the fluidity of cell membranes (Kubo et al. 2003).

A wide variety of effects of plant volatile aldehydes on insects were reported as a result of co-evolution within specific plant–insect interactions (Matsui 2006). A synergistic effect of volatile aldehydes on the aggregation and on the action of sex pheromones of some insects was reported by Reddy and Guerrero (2004). Further studies are needed to verify if plant volatile aldehydes can have similar effects on mites.

The mite species considered here are frequent pests in stored grain (Marx et al. 1993; Athanassiou et al. 2003, 2005; Hubert et al. 2006a) and are the main allergen producers (Puerta et al. 1993; Ferrera et al. 2004; Yadav et al. 2006). Our study is the first report showing an acaricidal effect of plant volatile aldehydes on different mite species.

On the basis of the results presented here, the tested aldehydes could be potential candidates in the control of the main mite species in food and feed commodities. However, for the practical use of natural compounds such as acaricidal agents, further research should be done on the safety of using these compounds and the potential effects on human and animal health, on the acaricidal mode of action, and on new formulations that improve their efficacy and stability.

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