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Article

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Effect Of Selected Volatiles On Two Stored Pests: The Fungus *Fusarium verticillioides* And The Maize Weevil *Sithophilus zeamais.*

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ABSTRACT

New agronomic practices and technology enabled Argentina a larger production of cereal grains, reaching a harvest yielded of 26.5 million metric tons of maize, of which, about 40% was exported. However, much of the maize production is lost annually by the attack of fungi and insects (2.6 million tons). In the study, the antifungal effect of selected volatiles on *Fusarium verticillioides,* its mycotoxin production, and repellent and insecticidal activities against weevill *S. zeamais,* insect vector of *F. verticillioides,* were evaluated. Compounds tested were (2E)-2-hexenal, (2E)-2-nonenal, (2E,6Z)-2,6-nonadienal, 1-pentanol, 1-hexanol, 1-butanol, 3-methyl-1-butanol, pentanal, 2-decanone and 3-decanone, which occur in the blend of volatile compounds emitted by various cereal grains. The most active antifungal were the aldehydes (2E)-2-nonenal, (2E)-2-hexenal and (2E,6Z)-2,6-nonadienal [Minimum Inhibitory Concentration (MIC) values of < 0.03mM, 0.06mM and 0.06mM, respectively]. The fumonisin B_1 (FB_1) occurrence also was prevented because these compounds completely inhibited the fungal growth. The best insecticidal fumigant activities against maize weevil were shown by 2-decanone and 3-decanone [Lethal Concentration (LC_{50}) $\leq$ 54.6 µl/L (<0.28 mM)]. Although, all tested compounds showed repellent activity against *S. zeamais* at a concentration of 4 µl/L, the (2E,6Z)-2,6-nonadienal was the most active repellent compound. These results demonstrate the potential of (2E,6Z)-2,6-nonadienal to be used as a natural alternative to synthetic pesticides on *F. verticillioides* and *S. zeamais*.

KEYWORDS: *Fusarium verticillioides* - Fumonisin B_1 - Volatile organic compounds – Kernels - *Sitophilus zeamais.*

INTRODUCTION

New agronomic practices and technology enabled Argentina a larger production of cereal grains, reaching a harvest yielded of 26.5 million metric tons of maize, of which, about 40% was exported.[1] However, much of the maize production is lost annually by the attack of fungi and insects (2.6 million tons).[2-4] *Fusarium verticillioides* (Sacc.) Niremberg (e.g. *F. moniliforme* Sheldon) is one of the most frequent fungal pathogens associated with maize worldwide. In addition, some isolates of this species are able to produce the mycotoxin fumonisin B_1 (FB_1) on the maize in the field and/or during the storage, that represents a considerable problem due to their immunotoxic, neurotoxic, hepatotoxic, nephrotoxic, and carcinogenic effects on animals.[5,6] The contamination of maize by *F. verticillioides* and fumonisin can occur in pre and post-harvest stages.[7, 8] However, fumonisin is mostly produced during grain storage, when the temperature, humidity and the presence of such as *S. zeamais* enable production of secondary metabolites by the fungus.

As a primary pest of stored maize, *Sitophilus zeamais* (Motschulsky) (Coleoptera: Curculionidae) contributes to the dispersal of fungal spores [9-11] and through feeding damage provides entry points for fungal infections.[12]

Synthetic pesticides are used to preserve maize grains from deterioration by stored pests. However, the development of resistant populations of fungi [13] and insects,[14-16] problems in the human health and other negative effects on the environment [12] have generated considerable interest in the preservation of grains by the use of natural compounds.[17] In recent years, semiochemicals have been of increasing interest in the search for natural control of stored grain pests.[18,19] Many volatile organic compounds (VOCs) emanating from kernels and seeds (e.g. maize, soybeans, barley, wheat) [19-23] are lipoxygenase (LOX)-derived products, affect both fungal growth [24] and the behavior of fungi-vectoring insects.[25] The antifungal effects of VOCs against *Aspergillus carbonarius*, *Fusarium proliferatum* and *Aspergillus flavus*, and the antimycotoxin activity against *Aspergillus* spp. have been previously reported.[26, 27] Nevertheless, to our knowledge, only the VOC (2E)-2-hexenal has been tested for its effect on *F. verticillioides* growth and FB_1 biosynthesis.[28] On the other hand, the insecticidal activity of the VOC components, alkyl ketones [29] and C6- and C9-aldehydes [30] against *Tribolium castaneum, Rhyzopertha dominica, Sitophilus granarius, Sitophilus oryzae* and *Cryptolestes ferrugineus* have been reported. However, no insecticidal studies against the main pest of stored maize, *S. zeamais*,[14] have yet been performed. The aim of this investigation was to determine the antifungal effect of ten recognized VOCs from cereal kernels on *F. verticillioides*, its mycotoxin production, and the insecticidal effects against its insect vector *S. zeamais*.

MATERIALS AND METHODS

Chemicals

The chemicals (2E)-2-hexenal (w256005, purity >95%), (2E)-2-nonenal (255653, 97%), (2E,6Z)-2,6-nonadienal (w337706, >96%), 1-pentanol (76929, >99%), 1-hexanol (471402, >99%), 1-butanol (281549, >99%), 3-methyl-1-butanol (309435, >99%), pentanal (w309818, >97%), 2-decanone (w510637, >98%), 3-decanone (268194, 98%) and propionic acid (101362192, 99.5%) were purchased from Sigma-Aldrich (Buenos Aires, Argentina). DDVP (dicholrvos, positive control, technical grade, >98 % purity) was purchased from Chemotécnica S.A (Buenos Aires, Argentina).

Fungal strain

An isolate of *Fusarium verticillioides* (Sacc) Niremberg (= *F. moniliforme* Sheldon teleomorph *G. fujikuroi* (Sawada) Ito in Ito & Kimura [31] strain M3125 (provided by Dr. Robert Proctor, United States Department of Agriculture, Agricultural Research Service, National Center for Agricultural Utilization Research, Peoria, IL, United States) was used for all experiments. This fumonisin-producing strain was isolated from maize in California.[32]

Inoculum preparation

F. verticillioides M3125 was grown in Czapek-dox agar Petri plates for 7 days at 28 ºC in the dark, to allow profuse sporulation. Then, sterile distilled water was added to each plate and a conidia suspension was obtained by scraping the colony surface with a sterile Drigalsky spatula, which was then filtered through a cheesecloth. The conidial concentration (1×10^{6} conidia/mL) was standardized using a haemocytometer.

Insects

Sitophilus zeamais (Motschulsky) were reared on sterilized whole maize grain in sealed containers. Insects were reared under controlled temperature and humidity (28 ºC and 60% - 70%) and a light/dark regime of 12:12.[33] Adults of a strain of *S. zeamais* were obtained from Metán, Salta province, Argentina. The colony was maintained in our laboratory for one year without exposure to insecticides. The male and female weevils used in all the experiments being approximately 2 weeks old. All experiments were conducted in complete darkness in a climatic chamber (28 °C and 60 -70% RH).

Effect of volatile organic compounds on fungal growth and fumonisin production

The antifungal activity of the VOCs was tested determining the radial growth of the fungal colony following a methodology proposed by Neri *et al.*[34] Briefly, a paper filter was placed on the inside cover of the maize meal extract agar (3%) Petri dish. The VOCs were added separately to 90-mm paper filter as pure liquid compounds, and the concentrations (0.03; 0.06; 0.13; 0.27; 0.53; 1.06; 2.12 and 4.24 mM) were expressed as 10^{-3} mol on filter paper per dish volume. A paper filter without VOCs was used as control. Then, 10 µL of a conidial suspension (1×10^6 conidia/mL) of *F. verticillioides* M3125 was added aseptically to the centre of the Petri dishes. The maize meal extract Agar (3%) Petri dishes were then covered, wrapped in parafilm and incubated in the dark at 28°C. The colony diameter of *F. verticillioides* was measured after 7 days of incubation, and the colony area calculated using the formula for the area of a circle ($\pi * r^2$). Minimum inhibitory concentration (MIC) was defined as the lowest concentration of the VOCs at which no fungal growth was observed. To study the effects of the VOCs on FB_1 production, the inoculated plates were incubated in the dark at 28°C for 28 days. After this incubation, the parafilm and filter papers were removed and agar in the experimental plates was dried for 96 h at 60°C in a forced-air oven before being ground to a fine dry powder. Finally, 5 mL of water was added to the dried agar from each disk, and FB_1 was extracted by shaking the dried dishes with water for 120 min on an orbital shaker, with the mixture then being centrifuged at 5000 rpm for 15 min. The experiments were repeated two times in triplicate.[35]

Fumonisin B_1 quantitation

The quantitation of the samples was performed following a methodology proposed by Shephard *et al.*[36] Briefly, samples (1000 µL) from the FB_1 extracts were diluted with acetonitrile: water (1:1), and then an aliquot (50 µL) was derivatized prior to injection;

during 3.5 min with 200 μL of a solution, which was prepared by adding 5 ml of 0.1 M sodium tetraborate and 50 μL of 2-mercaptoethanol to 1 mL of methanol containing 40 mg of o-phthaldialdehyde. Derivatized samples were analyzed using Perkin Elmer HPLC equipped with a fluorescence detector, with the wavelengths used for excitation and emission being 335 nm and 440 nm, respectively, and with an analytical reverse phase C18 column (150 mm × 4.6 mm internal diameter and 5 μm particle size) connected to a precolumn C_{18} (20 mm × 4.6 mm and 5 μm particle size). For the mobile phase, methanol and NaH_2PO_4 0.1 M (75:25) were used, with the pH being set at 3.35 ± 0.2 with orthophosphoric acid and a flow rate of 1.5 mL/min. The quantitation of FB_1 was carried out by comparing the peak areas obtained from samples with those corresponding to the analytical standards of FB_1 (PROMEC, Program on mycotoxins and experimental carcinogenesis, Tygerberg, Republic of South Africa).

Insecticidal assay

Insecticidal effect on *S. zeamais* was tested using fumigant toxicity assay described by Huang *et al*.,[37] with some modifications. Briefly, different amounts of pure VOCs at concentrations corresponding to 20- 600 μl/L air were placed onto Whatman filter paper disks of 2 cm diameter. Only the lowest concentrations were diluted in n-hexane, and in these cases each filter paper disk was air dried for 30s and placed on the underside of the screw cap of a glass vial (30 mL). Ten adult *S. zeamais* were placed into each vial, a nylon gauze piece was fitted 1cm under the screw cap of each glass vial, to avoid direct contact of the weevils with VOCs. The experiment was performed five replicates in two times per concentration, and control treatments were kept under same conditions without pure compounds. DDVP was used as a positive control due to its high vapor pressure and known insecticide activity. Insect mortality was checked after 24 h, with the mortality percentages and LC_{50} values being calculated according to Finney.[38]

Repellent/Attraction activity bioassay

The behavioral response of *S. zeamais* adults to individual VOCs was tested in two-choice olfactometer bioassay described by Herrera et al.[35] Briefly, two flasks (250 mL) were connected with a glass tube of 30 x 1 cm of diameter. In the middle (15 cm from the two flasks), a small hole was made of 1 x 1 cm. The connections between the two flasks and the tube were sealed with rubber plugs, which were covered with parafilm to prevent gas leakage. A filter paper of 2 cm diameter was placed within each flask where the compounds were added. Twenty insects, deprived of food for at least 4 h, were placed in the hole of the glass tube. These were then released and tested for 2 h in a climatic chamber, the experiments being carried out between 10:00 and 16:00 hours. The position of the flasks was changed at every replication, and insects that did not show any response in the experiment were not used to calculate response index. Insects were given a choice between a specific dose of the test compound and the solvent (n-hexane) used as a control. The experiments were performed five times for each assay, with insects only being used once. For each experiment, an independency control (without any compound) showed that the movement of the beetles towards either flask was random (RI= -2.1 ± 7.5). Propionic acid was used as positive control for repellent.[39]

In each trial, a response index (RI) was calculated by using the equation $RI = [(T-C)/Tot]\times 100$, where T is number of insects responding to the treatment, C is number of insects responding to the control, and Tot is the total number of insects released.[40] Positive values of RI indicate attraction to the treatment, while negative ones indicate repellence.

Statistical analysis

Data were analyzed using InfoStat/Professional 2010p. [41] at p = 0.05. Randomized complete block design (RCBD) was used to the experimental designs and a one-way analysis of variance (ANOVA) to study the experimental data. The Shapiro-Wilk test was utilized to test the normality of the experimental data, and comparisons between treatments were carried out using the Duncan test. Experimental data without a normal distribution were statistically analyzed by the Kruskal-Wallis non-parametric test (at $p<0.05$). The pairwise comparison was used to compare means among treatment ranges. The lethal concentrations (LC_{50} and LC_{95}) were calculated from dose-mortality values, using probit regression analysis by POLO-PLUS Software.[42] The significance of the mean RI in each treatment of the two-choice olfactometer bioassay was evaluated by the Student's t-test for paired comparisons.[40] The chemical properties lipophilicity (Log P: Logarithm of the octanol/water partition coefficient) and vapour pressure, of the VOCs compounds, were obtained from ChemSpider database.[43]

RESULTS

Antifungal and antimicotoxicogenic activities

The inhibitory effects mediated by the VOCs on *F. verticillioides* growth was dose-dependent, with the most active compounds being the aldehydes: (2E)-2-nonenal, (2E)-2-hexenal, (2E, 6Z)-2,6-nonadienal and pentanal, which exhibited MIC values of <0.03 mM, 0.06 mM, 0.06 mM and 0.53 mM, respectively (Table 1). Of the alcohols tested, 1-hexanol revealed the highest activity, while of the alkyl ketones, 3-decanone had a greater inhibitory effect on fungal growth than 2-decanone, at several concentration. Treatments such as (2E)-2-hexenal (0.06mM), 1-pentanol (4.24mM), 1-hexanol (2.12 and 4.24 mM), pentanal (0.53 and 1.06 mM), 2-decanone (4.24 mM) and 3-decanone (2.12 and 4.24mM) all caused a delay in the fungal growth, with no growth being observed on the seventh day. However, on the 28th day post-inoculation fungal growth was apparent and the FB_1 concentration was determined**.** On the other hand, 1-pentanol showed a slight stimulatory effect on fungal growth at lower concentrations. The VOC effects on FB_1 production are presented in Table 2, where it can be observed that (2E)-2-hexenal, (2E)-2-nonenal and (2E, 6Z)-2,6-nonadienal caused a total inhibition of mycelium growth, implying an absence of FB_1 production. The 1-hexanol (4.24 mM) and 1-butanol (0.53 mM and 4.24 mM) effectively inhibited fumonisin production by *F. verticillioides*.

Insecticidal and repellent/attraction effects

The results of fumigant insecticidal activity of VOCs tested on *S. zeamais* are shown in Table 3. After 24 h exposure, the most active compounds were 2- and 3-decanone, with LC_{50} values of 50.4µL/L and 54.6 µL/L, respectively. 1-hexanol, 1-pentanol, 1-butanol and (2E)-2-hexenal showed insecticide LC_{50} values between 224.1 µl/L and 306.6 µl/L, while (2E)-2-nonenal and pentanal did not show any insecticidal activity in the range of the evaluated concentrations (20 to 600 µl/L). The LC_{50} values of (2E, 6Z)-2,6-nonadienal and 3-methyl-1-butanol could not be determined because they did not show a dose-dependent relationship. However, at dose 150 µl/L the mortality was 98.0% (± 4.5) for 3-methyl-1-butanol and 28.7% (± 19.4) for (2E, 6Z)-2,6-nonadienal (data not shown).

The behavioral responses of *S. zeamais* adults to VOCs are shown in Figure 1. All the compounds showed repellent effect at 4 µl/L. Moreover, only (2E, 6Z)-2,6-nonadienal showed repellent effects at 0.05 µl/L (0.31µM), with a response index of -37.3 ± 14.0. On the other hand, 3-methyl-1-butanol and 1-butanol showed attractant effects at 0.4 µl/L.

DISCUSSION

The results obtained in the present work show the capacity of 10 natural VOCs present in the headspace volatiles of several cereal kernels [26, 27, 29, 39, 44] to affect the fungal growth of *F. verticillioides* and FB_1 production. Besides, these VOCs showed insecticidal and repellent effects against its insect vector *S. zeamais*. Our findings revealed that aldehydes had higher levels of antifungal activity than alcohols or alkyl ketones. In agreement, a previous report by Mita *et al.* [24] showed antifungal activity of C6 and C9 aldehydes against *Aspergillus carbonarius* and *Fusarium proliferatum*, with (2E)-2-nonenal being the most effective compound. In addition, other studies reported high antifungal activity of (2E)-2-hexenal, (2E)-2-nonenal and (2E,6Z)-2,6-nonadienal.[26-28, 45] Moreover, the results presented here suggest that a relationship between the antifungal activity and molecular properties, such as lipophilicity (Log P) and vapour pressure may exist in compounds with the same functional group. In the present work, the most active compound against *F. verticillioides* was (2E)-2-nonenal, which is the aldehyde with the highest lipophilic property. The relationship between Log P and antifungal activity of plant phenolic compounds against *F. verticillioides* has been previously reported by Dambolena *et al.*[46] In the present study, (2E)-2-hexenal, (2E)-2-nonenal and (2E, 6Z)-2,6-nonadienal, also prevented FB_1 production because these compounds inhibited completely the fungal growth, at the tested concentrations.

Previous investigations have reported aflatoxin B_1 being inhibited by (2E)-2-hexenal.[26,27] However, this compound did not have any effect on FB_1 production.[28]

Kernels fed on by insects provide a favorable environment for *F. verticillioides* growth and FB_1 production,[43] and contribute to the dispersal of fungal spores. Hence, insect control could be considered a key strategy for controlling fungal growth in stored maize kernels. So, the repellent and insecticidal effects of VOCs against *S. zeamais*, an insect vector of *F. verticillioides* in stored maize, were also determined. The VOCs emitted by cereal grains are detected by the antennal sensila of the granary weevil and induce behavioral responses at different doses.[39] All the evaluated VOCs show repellent effects against *S. zeamais* at 4 µL/L, however at very low concentrations (0.4 µL/L and 0.05 µL/L) the repellent effect was only shown by (2E, 6Z)-2,6-nonadienal (one of the most antifungal compound). In agreement with our results, Germinara *et al*.[39] demonstrated a repellent effect of diolefinic aldehydes, alkyl ketones and the aliphatic alcohol 1-hexanol, and the attractive effects of butyl alcohols on *S. granarius*. 2-decanone and 3-decanone revealed strong fumigant activities against *S. zeamais*. On the other hand, the most antifungal and repellent compounds, (2E, 6Z)-2,6-nonadienal and (2E)-2-nonenal, did not show strong fumigant toxicity against *S. zeamais*, at the tested concentrations. However, Hubert *et al.* [30] reported insecticidal activity of (2E, 6Z)-2,6-nonadienal and (2E)-2-nonenal (LD_{50} ranging from 0.44 to 2.76 mg g^{-1}) against *Sitophilus granarius* and *Sitophilus oryzae*, in fumigant assays.

Summing up, our results demonstrate that the different biological activities are mainly related with the functional group of the compounds tested, with the most active antifungal and insect repellent compounds being the aldehydes, while the most insecticide compounds were the ketones. (2E, 6Z)-2,6-nonadienal demonstrated a complete inhibition of *F. verticillioides* growth and a repellent activity against its insect vector *S. zeamais,* thus preventing FB_1 occurrence, dispersion of fungal spores and broken grains. These results reveal the strong potential for this compound to be used as a natural alternative to synthetic fungicides. In addition, lethal dosis (LD_{50}) values of aldehyde VOCs show slight toxicity (≤ 5g/kg) in rats.[48] On the other hand, other evaluated VOCs showed a potential capacity to be used as a natural insecticidal (ketones) or as a lure for *S. zeamais* (alcohol). The future use of VOC therefore opens up possibilities for a safer and economically viable option for the conservation of stored kernels and pest management.

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Figure captions

Figure 1. Behavioural responses of *S. zeamais* adults to VOCs.

Footnote:

$*P \le 0.05$; $**P<0.01$; $***P<0.001$ (significant response to experimental stimulus; paired-sample T test).
Values having different letters in the same column are significantly different from each other according to Duncan's multiple range test at $P \le 0.05$ (n=5).
(+) values of RI indicate attraction.
(-) values of RI indicate repellence.

Table 1. Antifungal activity of VOCs against *Fusarium verticillioides* M3125 in maize meal extract agar (3%) at 28°C.

Compounds	**MIC**[A]		**Inhibition of fungal growth**[B] (%)							
	mM	**µl/L**	**0.03 mM**	**0.06 mM**	**0.13 mM**	**0.27 mM**	**0.53 mM**	**1.06 mM**	**2.12 mM**	**4.24 mM**
(2E)-hexenal	0.06	7.8	46.4 ± 2.6[b]	100[*a]	100[*a]	100[*a]	100[*a]	100[*a]	100[*a]	100[*a]
(2E)-nonenal	<0.03	<5.6	100[*a]	100[*a]	100[*a]	100[*a]	100[*a]	100[*a]	100[*a]	100[*a]
(2E,6Z)-nonadienal	0.06	10.6	53.9 ± 3.0[b]	100[*a]	100[*a]	100[*a]	100[*a]	100[*a]	100[*a]	100[*a]
pentanal	0.53	56.2	5.7 ± 4.3[b]	26.3 ± 6.1[b]	39.1 ± 0.2[b]	42.3 ± 7.5[b]	100[*a]	100[*a]	100[*a]	100[*a]
1-pentanol	4.24	460.7	2.8 ± 2.8 [b]	-42.0 ± 0.2[b]	-42.0 ± 0.1[b]	-64.7 ± 0.1[b]	11.4 ± 0.2[b]	49.6 ± 13.3[b]	81.1 ± 8.8[b]	97.8 ± 0.9[*a]
1-hexanol	2.12	264.0	-0.1 ± 1.5 [b]	18.8 ±3.8[b]	21.3 ± 6.3[b]	34.6 ± 2.3[b]	54.2 ± 0.2[b]	89.5 ± 0.5[b]	99.8 ± 0.3[*a]	100[*]
3-methyl-1-butanol	>4.24	>460.7	-9.0 ± 4.6[b]	1.4 ± 0.2[b]	1.4 ± 0.1[b]	6.9 ± 5.5[b]	21.3 ± 2.7[b]	18.6 ± 0.1[b]	69.7 ±5.8[b]	73.9 ± 1.5[b]
1-butanol	>4.24	>393.2	-0.1 ± 4.4[b]	28.8 ± 1.2[b]	30.0 ± 2.4[b]	26.4 ± 1.2[b]	31.6 ± 0.2[b]	32.8 ± 3.7[b]	46.6 ± 7.7[b]	78.3 ± 1.4[b]
2-decanone	>4.24	>797.7	0.1± 1.5[b]	42.8 ± 5.8[b]	3.0 ± 1.5[b]	13.3 ± 2.8[b]	62.3 ± 9.3[b]	81.9 ± 7.1[b]	92.9 ± 0.4[*a]	93.4 ± 2.7[*a]
3-decanone	>4.24	>797.7	-7.8 ± 3.2[b]	42.9 ± 1.2[b]	20.2 ± 4.1[b]	53.8 ± 1.0[b]	61.8 ± 0.9[b]	91.98 ± 1.3[*a]	95.2 ± 2.6[*a]	96.6 ± 1.1[*a]

Values are expressed as means ± SD. [A]MIC: minimum inhibitory concentration. [B]Inhibition of fungal growth was determined after 7 days of incubation.
(-): Indicate fungal growth stimulation.
[*] Indicate significant difference with the control according to Kruskal-Wallis non parametric test (H= 249.27. P < 0.0001). All pairwise comparison was used to compare the means among treatments ranges.
[a, b] Values having different letters are significantly different from each treatments. The experiments were performed twice in triplicate.

Table 2. Effects of VOCs on FB_1 production in maize meal extract agar (3%) at 28°C.

Compounds	Inhibition of FB_1 production (%)							
	0.03 mM	0.06 mM	0.13 mM	0.27 mM	0.53 mM	1.06 mM	2.12 mM	4.24 mM
(2E)-hexen*l	19.3 ± 60.7^{b}	- 5.7 ± 82.1^{b}	ND	ND	ND	ND	ND	ND
(2E)-nonenal	ND	ND	ND	ND	ND	ND	ND	ND
(2E, 6Z)-nonadienal	57.5 ± 16.4^{b}	ND	ND	ND	ND	ND	ND	ND
pentanal	- 25.2 ± 17.2^{b}	47.8 ± 21.5^{b}	- 10.2 ± 15.6^{b}	9.1 ± 15.9^{b}	65.0 ± 10.5^{b}	50.0 ± 27.7^{b}	ND	ND
1- pentanol	38.3 ± 51.7^{b}	3.4 ± 32.4^{b}	63.6 ± 214.9^{b}	- 81.9 ± 79.6^{b}	- 3.8 ± 19.2^{b}	12.5 ± 15.5^{b}	- 0.4 ± 20.0^{b}	- 110.6 ± 23.4^{b}
1-hexanol	52.9 ± 86.0^{b}	59.9 ± 31.9^{b}	40.4 ± 51.6^{b}	8.1 ± 18.9^{b}	58.2 ± 37.4^{b}	57.5 ± 20.0^{b}	59.2 ± 8.2^{b}	100.0 ± 14.13^{*a}
3-methyl-1-butanol	64.3 ± 32.8^{b}	21.1 ± 13.7^{b}	34.2 ± 10.3^{b}	58.9 ± 4.7^{b}	64.3 ±13.0^{b}	36.3 ± 15.4^{b}	39.5 ± 59.2^{b}	- 39.3 ± 24.0^{b}
1-butanol	23.3 ± 9.5^{b}	8.5 ± 19.3^{b}	29.6 ± 11.8^{b}	55.2 ± 4.8^{b}	78.1 ± 4.1^{*a}	57.6 ± 7.5^{b}	26.5 ± 15.9^{b}	73.8 ± 3.1^{*a}
2-decanone	- 71.0 ± 40.9^{b}	16.1 ± 74.6^{b}	22.3 ± 9.6^{b}	27.1 ± 12.9^{b}	38.7 ± 14.1^{b}	- 241.6 ± 64.2^{b}	- 8.2 ± 12.5^{b}	- 56.3 ± 27.3^{b}
3-decanone	25.1 ± 5.4^{b}	56.9 ± 9.3^{b}	18.8 ± 27.3^{b}	37.7 ± 8.1^{b}	38.0 ± 23.8^{b}	30.1 ± 27.0^{b}	- 85.9 ± 30.5^{b}	- 169.1 ± 79.1^{b}

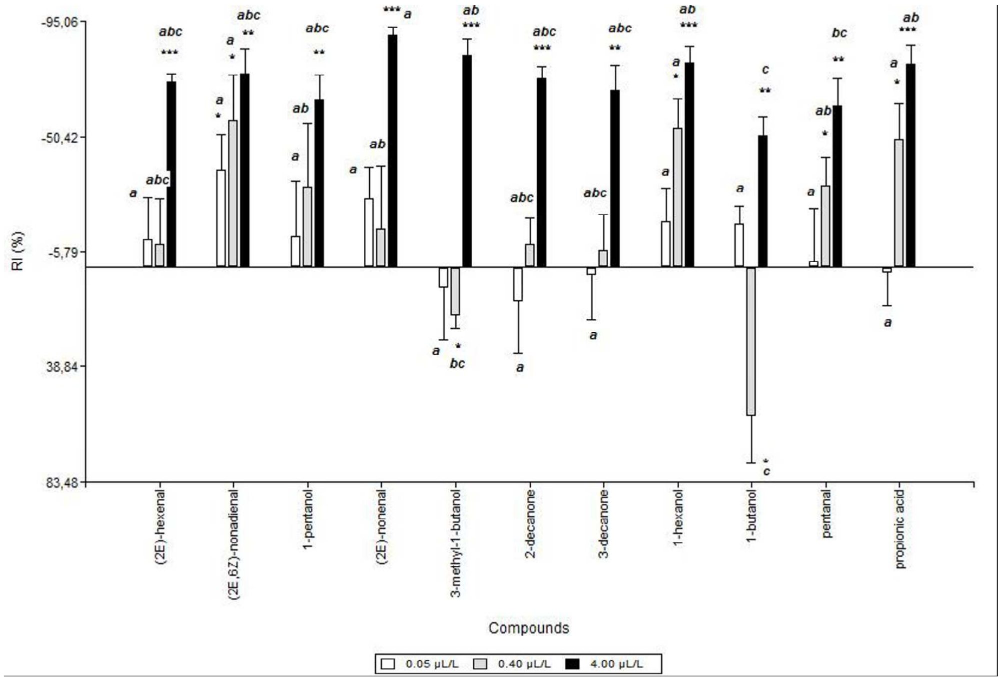
Values are expressed as medians ± SE. ND: No determined. FB_1 inhibition was not determined due to there was no fungal growth(-): Indicate FB_1 stimulation.
[*] Indicate significant difference with the control according to Kruskal-Wallis non parametric test (H= 249.27. P < 0.0001). All pairwise comparison was used to compare the means among treatments ranges.
[a, b] Values having different letters are significantly different from each treatments. The experiments were performed twice in triplicate.

Table 3. Fumigant toxicity of VOCs against *S. zeamais* adults after 24 h exposure[a].

Compounds	LC_{50} (mM)	LC_{50} (µl/L)	95% confidence interval (µl/L)	LC_{95} (mM)	LC_{95} (µl/L)	95% confidence interval (µl/L)	Slope ± SE	$(\chi^2)^b$	Log P[a]	VP (Pascal) 25°C[a]
(2E)-2-hexenal	2.64	306.6	263.7 - 612.0	3.95	458.2	361.4 - 1678.2	5.44 ± 0.89	38.44	1.58	613.2
(2E)-2-nonenal	>3.62	>600							3.17	39.99
(2E. 6Z)-2.6-nonadienal	ND	ND							2.6	39.99
pentanal	>5.64	>600							1.44	4239.6
1-pentanol	2.49	271.2	241.8 – 321.4	3.71	403.2	343.6 - 572.5	7.32 ± 1.02	23.48	1.41	373.3
1-hexanol	1.78	224.1	199.0 - 252.6	3.44	431.6	375.6 – 531.1	2.53 ± 0.85	3.29	1.94	119.99
3-methyl-1-butanol	ND	ND							1.22	559.95
1-butanol	3.18	291.6	260.9 - 354.9	5.21	477.0	394.9 - 727.6	5.33 ± 1.08	1.49	0.88	1133.2
2-decanone	0.26	50.4	46.4 – 55.5	0.35	66.2	59.8 – 80.7	13.53 ± 1.95	16.95	3.56	26.6
3-decanone	0.28	54.6	49.9 – 59.6	0.46	86.6	78.6 – 99.2	5.76 ± 0.84	1.46	3.56	26.6
DDVP		<0.06								

ND: not determined. Each value represents the mean of five times/ concentration, each set up with 10 adults. [a]Values obtained from Chemspider 2013, Log P (Logarithm of the octanol/water partition coefficient) and VP (Vapor pressure). $^bX^2$: chi-square value, significant at $P < 0.05$ level. LC: lethal concentration.

Figure 1.



RI (%)
-95,06
-50,42
-5,79
38,84
83,48
(2E)-hexenal
(2E,6Z)-nonadienal
1-pentanol
(2E)-nonenal
3-methyl-1-butanol
2-decanone
3-decanone
1-hexanol
1-butanol
pentanal
propionic acid
Compounds
0.05 µL/L
0.40 µL/L
4.00 µL/L

TOC graphic