Insecticidal effect of anisaldehyde against *Acanthoscelides obtectus* and *Callosobruchus maculatus* (Coleoptera: Bruchidae)

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Abstract

In the present study, anisaldehyde, a compound found in the essential oil of *Clausena anisum-olens*, was tested for its insecticidal activities against *Acanthoscelides obtectus* and *Callosobruchus maculatus*. The amounts of anisaldehyde applied were 0, 0.5, 1, 2 and 4 µL diluted in 1mL of acetone and applied to 40 g of either beans or cowpeas corresponding to the doses of 0, 0.008, 0.016, 0.033 and 0.066 µL/g of seed. Additionally, adsorbent clay was used as a carrier of this product in order to increase the persistence of its insecticidal activity over time. This clay was mixed with the aforementioned volumes of anisaldehyde to form a powder formulation. Furthermore, to assess the insecticidal effect over time, the F_1 progeny production was also evaluated. These two products caused significant mortality in the two tested insects. Nevertheless, *C. maculatus* was more susceptible than *A. obtectus* at tested doses. The progeny production decreased with the increasing doses of anisaldehyde and ACP with 0 % at the highest dose (0.066 µL/g). According to the LD_{50}, LD_{95} and their confidence intervals, the toxicity of ACP was significantly different (P<0.05) to anisaldehyde at the tested doses towards *A. obtectus* adults. However, there was no significant difference observed between the effects of these two products towards *C. maculatus*. These preliminary results suggest that anisaldehyde and ACP could be used in stored-product protection, but this needs further research. Research is also needed to determine its toxicity on rats in order to assess its potential hazards for workers and consumers.

Keywords: Anisaldehyde, Clay, Contact toxicity, Bruchids

1. Introduction

Cowpea (*Vigna unguiculata* (L.) Walpers) and beans (*Phaseolus vulagris* (L.) (Fabacaea) are important crops for many subsistence farmers in the tropics, especially in Africa, because they contain a high level of protein (20 to 25% and 23 to 30% respectively), and are used as human food (Broughton et al., 2003). In tropical and subtropical countries, dry and ripe seeds of these legumes are currently destroyed by *Callosobruchus maculatus* (F.) and *Acanthoscelides obtectus* (Say) (Coleoptera: Bruchidae), respectively (Delobel and Tran, 1993). Physical, biological and chemical methods have been developed in order to control stored-product insects. In addition, plants extracts (essential and vegetable oils, organic and aqueous extracts) have insecticidal, fungicidal and bactericidal properties (Boeke et al., 2001, Kuiate, 1993). As an insecticide, plant extracts can acts as a contact insecticide, fumigant, and antifeedant or as a repellent (Boeke et al., 2001).

Some constituents of essential oils are insecticidal (Boeke et al., 2001; Hinman, 1954; Burditt et al., 1963; Hammond et al., 2000) and may inhibit growth of insects (Huang et al., 2002). On the other hand, some clays are recognised by some traditional societies for their ability to control insects (Ramaswamy et al., 1995). The use of such powders, aromatised with essential oils or insecticidal pure compounds could have a combined effect of mechanism action and insecticidal action (Ramaswamy et al., 1995; Ndomo et al., 2008).

The family of Rutaceae contains plants with very strong aroma, and their essential oils contain constituents with which the insecticidal activity has already been studied. The study of the essential oil of *Clausena anisum-olens* (Blanco) Merrill (Rutaceae) shows that it contains anethole and methyl chavicol as major components and anisaldehyde as a minor component, (Molino, 2000). Anethole shows insecticidal properties against *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) by inhibiting its reproductive activity (Bazzoni *et al*., 1997) and against *C. maculatus* (Tapondjou et al., 2002). Generally, the biological activity of essential oils is due to the synergy of its major and minor components (Kuiate, 1993). However, constituents of many extracts are now tested individually in order to find out the active component in an extract (Prates et al., 1998, Huang et al., 2002; Tapondjou et al., 2002). In view of the toxicity of many pure compounds, the present study investigates the insecticidal effect of anisaldehyde, a pure compound found in essential oil of *C. anisum-olens* on *A. obtectus* and *C. maculatus*; also to use a clay as a support of this chemical in order to increase the persistence of its insecticidal activity over time.

2. Materials and methods

2.1. Insects

The legume pests *A. obtectus* and *C. maculatus* were obtained from our stock cultures maintained in 5-L glass jars held in a controlled temperature chamber at 27 ± 2°C, 75 ± 5% r.h. and photoperiod of LD 12:12 (hours light:dark) on beans and cowpea seeds as culture medium, respectively.

2.2. Chemicals

Anisaldehyde (anisic aldehyde, 4-methoxybenzaldehyde, $C_8H_8O_2$, MW=136.15; 99.5% purity, Sigma-Aldrich Chemicals GmbH Company, Taufkirchen, Germany) discovered in essential oil of *C. anisum-olens* (Mollino, 2000) was diluted with acetone to prepare a series of concentrations. Quantities of 0, 0.5, 1, 2 and 4 µL of anisaldehyde were diluted in 1 mL of acetone and applied to 40 g of either beans or cowpea corresponding to doses of 0, 0.008, 0.016, 0.033 and 0.066 µL/ g of grain.

2.3. Preparation of aromatized clay powder (ACP)

The mineral material used was fine white clay of smectitic nature and montmorillionitte type already investigated by Tonle (2004) and present in our laboratory in the form of powder with particles less than 106 µm diameter. Preliminary tests were carried out in order to choose the non toxic quantity of clay to insects, and which must be able to remain as a powder not a paste after admixture with tested volumes of anisaldehyde. Four different samples of ACP were prepared by mixing separately 0.5, 1, 2 and 4 µL of anisaldehyde with 0.05 g of clay. The mixtures were manually stirred for 5 min to obtain a homogenous mixture called aromatised clay powder (ACP). The control consisted only of 0.05 g of clay powder without anisaldehyde.

2.4. Biological tests

2.4.1. Contact toxicity of anisaldehyde

In order to determine the contact toxicity of anisadehyde towards insects, the method used by Tapondjou et al. (2003) was followed. *Acanthoscelides obtectus* and *C. maculatus* assays were conducted on beans and in cowpea seed, respectively. Forty gram samples of grain contained in 270 cm^3 glass jars were mixed with each of the previous test solutions by tumbling for 5 min to ensure even spread of the material over the surface of the grain. In the control jars, grain was treated only with acetone (1 mL) and all jars were manually stirred for 5 min and kept open for 15 min to allow complete evaporation of solvent. The grain was then infested with 1-day-old unsexed adult insects (25 per jar) and each jar was covered with fine porous cloth held with rubber bands; jars were placed in a chamber conditioned at 27 ± 2°C and 75 ± 5% r.h. and a photoperiod of LD 12:12 (hours light:dark). Each treatment was replicated three times. Mortality counts were made daily up for four days.

2.4.2. Contact toxicity of ACP

The contact toxicity of ACP was carried out as previously described with anisaldehyde and in the same experimental condition, however, instead of anisaldehyde, every jar containing 40 g of grain was treated with one sample of ACP previously prepared and manually stirred so that all grain were uniformly coated. Thus lead to the following doses: 0.008, 0.016, 0.033 and 0.066 µL/g of grain (volume of

anisaldehyde per quantity of grains). However, in control jars, the grain was treated with non ACP (0.05 g of clay powder without anisaldehyde).

2.4.3. Effect of anisaldehyde and ACP on F_1 progeny production

After counting mortalities in the above contact toxicity tests on the fourth day, the remaining living adult insects were removed and the glass jars kept under the same experimental conditions until the emergency of F_1 progeny adults. Based on the life cycle of untreated insects (Delobel and Tran 1993), the counting period of F_1 progeny (38 d after treatment) was established so as to avoid an overlap of generations. Percentage of reduction in adult emergence or inhibition rate (% IR) was calculated with the formula used by Tapondjou et al. (2003) as:

$$\%IR = \frac{Cn - Tn}{Cn} \times 100$$

Where *Cn* is the number of newly emerged insects in the untreated (control) jar and *Tn* the number of insects in the treated jar.

2.5. Data analysis

Data obtained from each dose-response bioassay were subjected to probit analysis in which probit-transformed mortality was regressed against log-transformed dose (Finney, 1971); LD_{50} and LD_{95} values were calculated after each day of exposure using the software Poloplus version 2.0. One-way analysis of variance was performed to compare the effect of dose tested for each exposure period. Means were separated using a subsequent Waller-Duncan at 5% significance level with the Software SPSS, 2000 (Steel and Torrie, 1980).

3. Results

3.1. Adults of A. obtectus

The mortality of adult *A. obtectus* increased with increased doses and time (Table 1). There was a significant difference (P<0.05) between the mortalities induced by the highest doses (0.033 and 0.066 µL/g of beans) and the lowest (0.008 and 0.016 µL/g of beans) after 4 d exposure. For anisaldehyde, the LD_{50} and LD_{95} were 0.052 (0.041-0.075) µL/g and 0.196 (0.118-0.563) µL/g of beans respectively, after 2 d exposure.

Table 1 Effect of different doses of anisaldehyde and ACP (aromatized clay powder) on mortality of *Acanthoscelides obtectus* adults

pExposure time (day)		Mortality ± SD (%) Dose (µL/g of grains)					LD_{50} (µL/g of grains)	LD_{95} (µL/g of grains)
	Product	**0.000**	**0.008**	**0.016**	**0.033**	**0.066**		
1	Anisaldehyde	0.0±0.0a	2.7±2.3ab	2.6±2.3ab	5.3±4.6b	32.0±4.0c	0.125 (0.083-0.319)	0.750 (0.302-6.854)
	ACP	0.0±0.0a	5.3±2.3a	5.3±2.3a	37.3±4.6b	72.0±2.3c	0.043 (0.034-0.061)	0.151 (0.094-0.417)
2	Anisaldehyde	0.0±0.0 a	2.7±2.3 a	8.0±4.0ab	25.3±4.6 b	64.0±7.3c	0.052 (0.041-0.075)	0.196 (0.118-0.563)
	ACP	0.0±0.0a	8.0±4.0a	10.7±2.3a	46.7±4.6b	82.7±4.6c	0.034 (0.028-0.045)	0.117 (0.078-0.257)
3	Anisaldehyde	0.0±0.0a	6.7±2.3a	9.3±2.3a	30.7±10.0b	86.7±6.1c	0.038 (0.031-0.047)	0.117 (0.082-0.218)
	ACP	0.0±0.0a	13.3±2.3b	17.3±6.1b	70.7±2.3c	92.0±4.6d	0.025 (0.021-0.030)	0.076 (0.057-0.122)
4	Anisaldehyde	0.0±0.0a	10.7±4.6b	10.7±2.3b	33.3±4.6c	94.7±4.6d	0.033 (0.026-0.044)	0.101 (0.067-0.231)
	ACP	0.0±0.0a	14.7±2.3b	20.0±4.0c	82.7±2.3d	97.3±2.3e	0.021 (0.019-0.024)	0.054 (0.046-0.069)

For a given row means followed by the same letter are not significantly different (p>0.05) at Waller-Duncan test.

The effect of the ACP based on the mixture of clay with different doses of anisaldehyde on beans was dose dependent (Table 1). The highest dose (0.066 µL/g of beans) induced 72.0% mortality after 1 d exposure. This mortality increased to 97% after 4 days whereas the lowest dose (0.008 µL/g of beans)

induced 15% of mortality. No mortality was recorded in the control jars after 4 d. There were significant differences (P<0.05) between mortalities induced by the doses of 0.034 (0.028-0.045) and 0.117 (0.078-0.257) µL/g of beans during the 4 d of assay. The LD_{50} and LD_{95} values of ACP were 0.034 µL/g and 0.057 µL/g of beans, respectively, after 2 d. Additionally, anisaldehyde and ACP were significantly different (P<0.05).

3.2. Adults of C. maculatus

As with *A. obtectus*, there was a dose-dependent evolution in mortality of adults of *C. maculatus* in cowpeas treated with anisaldehyde (Table 2). The mortalities induced by the highest doses 0.033 and 0.066 µL/g were significantly different (P<0.05) during the 4 d of exposure. The LD_{50} and LD_{95} were 0.031 (0.026-0.039) µL/g and 0.132 (0.088-0.263) µL/g of cowpeas after 2 d.

Table 2 Effect of different doses of anisaldehyde and ACP (aromatized clay powder) on mortality of *Callosobruchus maculatus* adults

Exposure time (day)		Mortality ± SD (%) Dose (µL/g of grains)					LD_{50} (µL/g of grains)	LD_{95} (µL/g of grains)
	Product	0.000	0.008	0.016	0.033	0.066		
1	Anisaldehyde	0.0±0.0a	0.0±0.0a	5.3±4.6a	45.3±12.8b	72.0±10.6c	0.041(0.035-0.049)	0.117 (0.088-0.188)
	ACP	0.0±0.0a	4.0±0.0ab	10.0±2.0b	42.7±8.3c	76.0±4.0d	0.039 (0.034-0.045)	0.132 (0.101-0.197)
2	Anisaldehyde	0.0±0.0a	15,3±5.0ab	20.0±6.9b	49.3±16.2c	82.7±2.31d	0.031 (0.026-0.039)	0.132 (0.088-0.263)
	ACP	0.0±0.0a	14.0±3.4b	21.3±2.0b	50.±5.2c	86.7±4.6d	0.030 (0.026-0.034)	0.119 (0.089-0.183)
3	Anisaldehyde	0.0±0.0a	16.0±4.0b	24±13.8b	61.3±15.1c	94.7±2.31d	0.026 (0.021-0.033)	0.095 (0.065-0.188)
	ACP	0.0±0.0a	21.3±2.3b	26.7±3.4b	64.7±6.4c	98.7±2.3d	0.022 (0.018-0.026)	0.072 (0.053-0.120)
4	Anisaldehyde	0.0±0.0a	37.3±2.3b	41.3±15.1b	78.7±6.1c	98.7±2.3d	0.016 (0.013-0.019)	0.062 (0.045-0.108)
	ACP	0.0±0.0a	34.7±2.3b	42.0±3.4b	80.0±8.0c	100.0±0.0d	0.016 (0.013-0.018)	0.055 (0.042-0.086)

For a given row means followed by the same letter are not significantly different (p>0.05) at Waller-Duncan test.

The mortality of *C. maculatus* adults increased with the dose of ACP applied (Table 2), and is more pronounced than with anisaldehyde applied alone. Also, there were significant differences (P<0.05) between the lowest doses (0.008 and 0.016 µL/g) and the highest (0.033 and 0.066 µL/g). The LD_{50} and LD_{95} were 0.030 (0.026-0.034) µL/g and 0.119 (0.089-0.183) µL/g of cowpea, respectively, after 2d. However, according to the LD_{50}, LD_{95} and their confidence intervals during the 4 d exposure, there was no significant difference between the effect of anisaldehyde and ACP at tested doses.

3.3. F_1 progeny production

Anisaldehyde and its ACP reduced the production of progeny of *A. obtectus* and *C. maculatus* (Table 3). The percentage of inhibition of these adults insects at F_1 increased with the dose of anisaldehyde or ACP applied. However, at lowest doses the inhibition of F_1 progeny production of these beetles induced by ACP is more pronounced than that caused by anisaldehyde at the same doses.

Table 3 Inhibition rate of anisaldehyde and ACP (aromatized clay powder) on F_1 progeny production of the two bruchids

Dose (µL/g of seeds)	Reduction in F1 progeny production (% of untreated)			
	A. obtectus		*C. maculatus*	
	Anisaldehyde	ACP	Anisaldehyde	ACP
0	0	0	0	0
0.008	59.6	88.3	38.1	45.4
0.016	76.9	90.9	71.4	90.9
0.033	90.4	100.0	90.5	100.0
0.066	100.0	100.0	100.0	100.0

4. Discussion

Anisaldehyde as well as its ACP were toxic against adults of *A. obtectus* and *C. maculatus*. The current results are in agreement with the report of Kuiate (1993) who mentioned that even minor components, alone or in association with other components, could have biological activity. Many pure compounds, especially terpene have been evaluated for their insecticidal activities against stored-product insects (Prates et al., 1998; Huang et al., 2002). The study of some aldehydes has also attracted the attention of some researchers in their use as insecticides (Ferguson and Pirie, 1948; Hinman, 1954; Burditt et al., 1963; Hammond et al., 2000). Our results corroborate with those of Hammond et al., (2000), who found that propanal, (*E*)-2-pentenal, and 2-methyl-(*E*)-2-butenal have excellent potential as post-harvest insect control agents. These chemicals killed 100% of aphids with little or no detectable harm to a majority of the commodities tested (naked and wrapped iceberg lettuce, green and red table grapes, lemon, grapefruit, orange, broccoli, avocado, cabbage, pinto bean and rice). This could explain the toxicity of anisaldehyde tested in this study.

Based on the LD_{50} and LD_{95} level during the 4-d exposure, ACP was more toxic than anisaldehyde towards *A. obtectus* adults. In fact, the adsorbent properties of this particular clay powder have been tested by Ndomo et al. (2008) who have used this clay to maintain the persistence of insecticidal essential oil of *C. anisata* against *A. obtectus* over the course of time; However, *C. maculatus* was more susceptible than *A. obtectus* at tested doses. The differences in response by different insect species could be attributed to the morphological and behavioural differences between the species (Delobel and Tran, 1993)

The use of such clay powders aromatized with essential oils or chemicals has a two-fold advantage due to the combined effects of the mechanism of action by the powder, which blocks the insect movement, filling intergranular spaces at high doses, and insecticidal action itself due the chemical (Ramaswamy et al., 1995). Additionally, Tonle (2004) mentioned that the adsorbent character of a clay powder is inversely proportional to the diameter of the particles. Consequently, the use of clay particles powder of very small diameter could increase its capacity of fixing anisaldehyde and ,thus, increase persistence of the insecticidal activity of this product over time. This could provide a solution for Ferguson and Pirie (1948), Hinman (1954) and Burditt et al. (1963) who concluded in their studies that the low to moderate toxicity of aldehydes with three or more carbons made them too weak for commercial insecticide applications.

In spite of the efficiency of anisaldehyde as insecticide against *A. obtectus* and *C. maculatus* adults in this study, further research is needed using other pest insects, in order to broaden its spectrum of action. Research must also determine the toxicity of both anisaldehyde and ACP on rats in order to assess its potential hazards for workers and consumers.

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