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Toxicity of some terpenoids of essential oils of *Xylopia aethiopica* from Cameroon against *Sitophilus zeamais* Motschulsky

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Abstract:

The acute toxicity of essential oils from the whole fruit (EF) or from the fibres of the fruit (FF) of the local aromatic plants *Xylopiα αethiopicα* Dunal (Annonaceae) collected in north Cameroon was evaluated on *Sitophilus zeαmαis* adults. A concentration of 1 ml of essential oil per 100 g of maize seeds was tested to determine weevil mortality after 24 h of exposure. Under these conditions, the essential oil derived from both EF and FF of *X. αethiopicα* led to 100% mortality. In a second step, proportions of active compounds present in the oil of both EF and FF of *X. αethiopicα* were quantified. The toxicity of the four main compounds was tested against *S. zeαmαis*: *α*-pinene, *β*-pinene, *Δ*-3-carene and terpinen-4-ol according to their proportion in the essential oil of the concerned plant part. *β*-pinene and terpinen-4-ol were responsible for 50% of the mortality at the proportion found in EF and FF essential oils respectively. When mixed, a synergic effect of the compounds was observed that restored the mortality percentage observed for the crude oil. It appears that *X. αethiopicα* essential oil could be a potential source of natural and low-cost insecticide to control storage pests.

Keywords : *Sitophilus zeαmαis* ; *Xylopiα αethiopicα* ; essential oil ; monoterpenes ; stored grain protection

1. INTRODUCTION

Insects are the major cause of grain losses during storage (Scotti 1978; Delobel and Tran 1993). In the northern provinces of Cameroon, where cereals play an essential role as human food, the maize weevil *Sitophilus zeαmαis* Motschulsky (Col., Curculionidae) constitutes the main pest in granaries (Seignobos 2000; Ngamo 2004). This post-harvest insect contributes to the deterioration of the quality and the quantity of stored grains (De Luca 1979; Delobel and Tran 1993; Fleurat-Lessard 1994). Synthetic chemical pesticides are usually applied by the farmers to reduce losses during storage. Indeed, the use of chemical insecticides is currently the most widely adopted method for grain protection against stored-product pests. However, the extensive use of these substances has led to the development of resistance in several species (Champ and Dyte 1976; Zettler and Cuperus 1990). This fact, combined with the consumer's demand for residue-free food, prompted researchers to evaluate other alternative reduced-risk control methods for stored-grain protection. These methods include, among others, the application of entomopathogenic fungi (Kavallieratos et al. 2006; Michalaki et al. 2006; Vassilakos et al. 2006), diatomaceous earth (Athanassiou et al. 2003, 2005a,b; Kavallieratos et al. 2005) and plant derivatives (Prakash and Rao 1997; Weaver and Subraman-yam 2000; Athanassiou et al. 2005).

Traditionally, farmers of north Cameroon mix plant material of diverse origin in their granaries to reduce grain pest impacts (Seignobos 2000; Ngamo 2004). Studies of essential oils extracted from these active plants have already shown their potential for pest control (Stoll 1988; Hamraoui and Regnault-Roger 1997; Ngamo et al. 2001; Boeke et al. 2004; Jirovetz et al. 2005) and may contribute to alleviate losses recorded during grain storage. Previous investigations have already shown the potential activity of the essential oil of *Xylopiα αethiopicα* (Dunal) A. Rich. (Annonaceae) that can lead to more than 90% mortality by direct application on adults of *Sitophilus zeαmαis* Motschulsky (Jirovetz et al. 2005; Kouninki et al. 2005). *X. αethiopicα* is a tall aromatic tree, with fairly smooth grey-brown bark (Vedcourt 1971), commonly found in forest gallery along rivers in the southern part of Cameroon. Fruit is aggregated in clusters of minimum five, but more usually of 16-

24 drupeous monocarps. Fruit is dried and used as spice in northern Cameroon.

Our first aim was to evaluate the insecticidal activity of the essential oil extracted of two parts of *X. aethiopica*: the whole fruit (EF) or the fibre of the fruit (FF). Secondly, we relate the toxicity observed to the variation in the terpenic composition by testing the main terpenes present in the oils at their observed concentrations.

2. MATERIALS AND METHODS

2.1 Insect material

Sitophilus zeamais were collected from farmer's granaries in northern Cameroon in 2003 and were reared in the Laboratory of Ecology and Biogeography (Louvain-la-Neuve, Belgium) on maize in a climate room under constant conditions [28°C temperature, 65% relative humidity (RH)].

2.2 The essential oil sources

In order to select the best plant material to use, we compared the activity of essential oils extracted from the whole fruit (EF) with that extracted from the fibre of the fruit (FF) of *X. aethiopica.* The essential oils extracted from these plants were obtained by a 4-h distillation using a Clevenger apparatus (Noudjou 2004).

Analyses of *X. aethiopica* EF and FF essential oils have been undertaken using a gas chromatography/mass spectrometry (GC-MS) (Agilent HP-6890 gas chromatograph (Agilent Technologies, Waldborn, Germany) coupled to an Agilent 5973 mass spectrometer) on a high performance/-mass spectrometry (HP-5MS) column [30 m × 0.25 mm internal diameter (i.d.); 0.25 μm film thickness]. Helium (1 ml/min) was used as the carrier gas and temperature was programmed from 40 to 210°C at increments of 5°C/min, and from 210 to 280°C at 30°C/min, with a final hold of 5 min at 280°C. The injections were performed in the splitless mode at 250°C. Mass spectra recording conditions were: EI mode at 70 eV, source temperature of 230°C and scanned mass range at 50-350 amu. Identification of the essential oil constituents was made on the basis of their retention data (Kovats index), by comparing their fragmentation patterns with those of the Wiley 275.L computed database, and whenever possible by co-injection of authentic references. The percentage of each compound was measured with a gas chromatography/flame ionisation detector (GC-FID) on an Agilent HP-6890 chromatograph (FID at 250°C) in the same chromatographic conditions as for GC-MS.

2.3 The pure essential oil compounds

The pure essential oil compounds used for toxicity tests were purchased from Sigma-Aldrich (Bornem, Belgium).

2.4 Insecticidal effect of essential oils

Toxicity tests were performed in glass vials (diameter 2.5 cm, height 9.5 cm, volume 40 ml) containing 20 g of maize grains. To prepare these batches of grains, 100 g of maize was mixed with 3 ml of acetone containing 1 ml of essential oil at a final concentration of 1 % (v/w) of essential oil/maize ratio (Bekele and Hassanali 2001; Kim et al. 2003). The acetone was then let to evaporate for 15 min under an evaporator. From these preparations, batches of 20 g were sampled and placed in the glass vials. The control consisted of grains treated with the acetone alone and then let to evaporate for 15 min. Ten adults of *S. zeamais* were introduced per vial with the grains. The vials containing insects were closed with cellulose cover and kept under constant conditions (28°C, 65% RH) at continuous darkness. Each treatment was replicated five times. Individual survival was recorded 24 h later. Insects were considered dead in the absence of movement and when they did not react even to probing.

2.5 Mortality of *S. zeamais* after exposure to the main terpenic compounds present in the *X. aethiopica* (EF and FF) essential oils

Essential oils from *X. aethiopica* EF (extracted from the whole fruit) and from FF (extracted from the fibre part of the drupaceous monocarps) were tested against *S. zeamais.* These two extracts were analysed by GC/MS (Noudjou 2004). The toxicity of each identified compound was individually or synergistically tested using the concentration actually found in the primary extract.

The experimental set up which was used for the crude essential oil test was also used in this case; 100 g of maize was mixed separately with each compound diluted in 3 ml of acetone. The concentrations used correspond to

those found in the raw essential oil. After the grain coating, acetone was let to evaporate under an evaporator for 15 min. The control included grain treated with acetone alone; 20 g of maize grain was introduced in glass vials (diameter 2.5 cm, height 9.5 cm, volume 40 ml). The vials containing insects were kept under the aforementioned conditions used for testing the whole essential oils. Survival was recorded in five replicates, 24 h after treatment.

2.6 Data analyses

The percentages of mortality were transformed in arcsine square-root and were analysed by one-way analysis of variance (ANOVA). Untransformed mean values were compared and ranked by a Duncan's test at P = 0.05.

3 RESULTS

3.1 Yields of essential oil extraction

Yields (v/m) of the essential oil extractions reached 5% for EF and 2.6% for FF parts.

3.2 Chromatographic analyses of *X. aethiopica* essential oils EF and FF

The essential oil derived from EF and FF differed regarding the main terpenic constituents, FF being richer in terpinene-4-ol and poorer in *α*- and *β*-pinene (table 1). Sixty-eight compounds in total were detected, among which 51 and 57 were identified from FF and EF, respectively. Four monoterpenes represented the main part of the oil: *α*-pinene, *β*-pinene, *Δ*-3-carene and terpinen-4-ol. These four molecules represented a total of 55.73% of the EF essential oil with a *β*-pinene dominating (38.17%), and 41.21% of the FF essential oil but with more than the half the *β*-pinene obtained from the EF essential oil (16.79%). The chemical structures of the main terpenic compounds of the essential oils of *X. aethiopica* are shown in fig. 1.

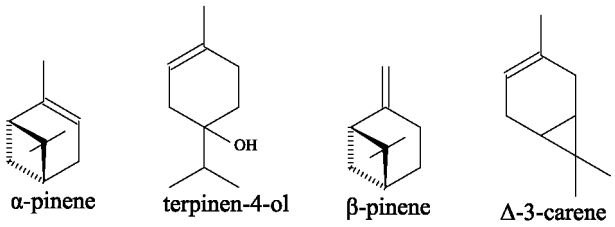
Table 1. *Essential oil composition of* Xylopia aethio-pica *extracted from the whole-fruit extract (EF) and from the fibre (FF)*

Compounds	*X. aethiopica* essential oil proportion (%)	
	Fruit fibre (FF)	Whole fruit (EF)
α-Thujene	0.98	1.48
α-Pinene	4.80	10.20
α-Fenchene	0.43	ND
Sabinene	0.19	ND
β-Pinene	16.79	38.17
α-Phellandrene	0.35	0.50
α-Terpinene	1.25	1.57
p-Cymene	2.46	1.06
β-Phellandrene + 1.8-cineole	9.39	8.77
Z-β-Ocimene	0.76	1.13
δ-3-Carene	2.92	2.81
γ-Terpinene	0.35	0.92
α-Terpinolene	0.81	0.79
Allo-ocimene	0.27	0.67
Total monoterpene hydrocarbons	41.76	68.07
trans-Sabinene hydrate	0.51	0.64
p-Menth-2-en- 1-ol	0.61	0.31
trans-Pinocarveol	1.88	1.48
Myroxyde E	0.27	0.62
Isopulegol	0.27	0.46
β-Pinene oxide	0.31	0.17
cis-p-Mentha-1,5-dien-8-ol	0.37	0.17
Terpinene-4-ol	16.70	4.55
Cryptone	0.56	0.35
α-Terpineol	2.78	0.91
Myrtenal	1.73	1.69
Verbenone	ND	0.61

Cuminal	ND	0.22
Piperitone	ND	0.15
Phellandral	ND	0.25
Bornyl acetate	ND	0.15
p-Cymen-7-ol	ND	0.21
2*E*,4*Z*-Decadienal	ND	0.19
Total oxygenated monoterpenes	26.00	13.12
Monoterpens NI	0.94	1.24
δ-Elemene	1.70	1.59
α-Cubebene	0.45	0.38
Longycyclene	0.27	0.21
α-Copaene	1.38	0.58
β-Cubebene	0.61	ND
Z-Caryophyllene	1.22	1.05
γ-Elemene	1.10	ND
α-Guaiene	0.10	ND
cis-Phenyl limonene	0.31	ND
α-Amorphene	1.67	ND
Epi-bicyclosesquiphellandrene	ND	0.56
β-Duprezianene	ND	0.68
cis-Phenyl limonene	ND	0.31
Dehydro-aromadendrene	ND	0.50
Germacrene D	4.11	3.61
β-Selinene	ND	0.09
10-Epi-zonarene	ND	0.33
α-Muurolene	ND	0.68
cis-Cadina-1,4-diene	ND	0.15
cis-Eudesma-6,11-diene	0.56	ND
Bicyclogermacrene	0.14	ND
Z-*γ*-Bisabolene	0.77	0.37
δ-cadinene	2.18	0.80
E-*γ*-Bisabolene	0.17	0.13
α-Cadinene	0.19	0.11
α-Calacorene	0.12	ND
Selina-3,7(11)-diene	ND	0.18
Total sesquiterpene hydrocarbons	17.32	12.31
Iso-ascaridole	0.22	ND
Methyl perillate	ND	0.48
Cabreuva oxyde A	0.29	0.26
Spathulenol	1.03	0.35
Caryophyllene oxyde	0.38	0.17
Thujopsan-2-a-ol	0.21	0.13
Geranyl-2-methyl butanoate	0.40	0.20
Epoxy-allo alloaromadendrene	1.82	0.43
Total oxygenated sesquiterpenes	4.36	2.02
Sesquiterpene NI	6.41	2.12
13-Epi-manoyl oxyde	0.85	0.60
Other NI diterpenes	0.51	0.13

Results in area %.
NI, unidentified; ND, not detected.

***Fig. 1**. Chemical structures of the four main terpenic compounds of* Xylopia aethiopica *essential oils (EF, FF)*



3.3 Toxicity of the crude essential oils used

Both oil sources caused 100% mortality of *S. zeamais* after 24 h (table 2).

***Table 2.** Toxicity of two sources of essential oils of* Xylopia aethiopica *on* Sitophilus zeamais *after 24 h of exposure (1 ml of oil/100 g of maize seeds)*

Test material	Mortality (%; mean ± SE)
Xylopia aethiopica (FF)	100 ± 0.00 a
Xylopia aethiopica (EF)	100 ± 0.00 a
Acetone	0.2 ± 0.44 b

Mean values followed by the same letter dot not differ significantly at $P < 0.05$ (Duncan's test).

3.4 Toxicity of the main terpenic compounds identified in the *X. aethiopica* essential oils

The toxicity against *S. zeamais* of the four main terpenes applied on grains individually or synergistically at their actual concentration found in the two sources of oils are shown in table 3. It appeared that, at the concentration tested, *α*-pinene and Δ-3 carene did not cause any mortality. For a concentration corresponding to its EF proportion; *β*-pinene provoked more than 50% mortality whereas only 10% when the FF concentration was used. In contrast, terpinene-4-ol was responsible for more than 50% mortality in FF extract. The synergistic effect of the two compounds restored the activity observed for the crude oil.

***Table 3.** Proportion and toxicity of main terpenic compounds of EF and FF essential oils of* Xylopia aethiopica *on* Sitophilus zeamais *after 24 h of exposure - comparison with whole essential oils*

Treatment	Tested concentrations (ml/100 g)	% Mortality due to EF	Tested concentrations (ml/100 g)	% Mortality due to FF
α-Pinene	0.10	0.0 ± 0.0 c	0.05	2 ± 0.44 d
β-Pinene	0.38	50 ± 1.00 b	0.17	10 ± 0.70 c
Δ-3-Carene	0.03	0.0 ± 0.00 c	0.03	0.0 ± 0.00 d
Terpinene-4-ol	0.05	2 ± 0.44 c	0.17	50 ± 0.70 b
α-pinene + *β*-pinene + *Δ*-3 carene + terpinene-4-ol	0.56	98 ± 0.44 a	0.42	96 ± 0.55 a
Whole essential oil	1.00	100 ± 0.00 a	1.00	98 ± 0.44 a
Control	0.00	0.00 ± 0.00 c	0.00	0.00 ± 0.00 d

Mean values followed by the same letter in the same column do not differ significantly at $P < 0.05$ (Duncan's test).

4 DISCUSSION

Many studies have been undertaken in order to evaluate the potential of local plant resources to control pests during grain storage in Africa (Keita et al. 2000; Bekele and Hassanali 2001; Ngamo et al. 2001; Kouninki et al. 2005). The local plants are a part of ancestral knowledge which exhibit interesting anti-insect activities and are currently available.

The essential oil composition of *X. aethiopica* was first analysed by Jirovetz et al. (2005). However, the proportion and composition differ quite greatly from the values presented herein, most probably because of the difference in geographical origins, extraction protocol, and part of the plant tested (Azevedo et al. 2001; Pascual-Villalobos and Ballesta-Acosta 2003; Shaaya and Kostyukovysky 2006). As we have shown by complementary analyses (Noudjou 2004), composition could vary greatly not between whole fruits and fibres but also according to the origin of *X. aethiopica* monocarps. These differences in composition necessitate the establishment of standardization procedures to allow comparisons, for instance based on rigorous bioassays using a laboratory strain of *S. zeamais.* However, it appears that *X. aethiopica* fruit has a great control capacity on *S. zeamais*, regarding its knockdown effect.

The fruit of *X. aethiopica* is locally used as a spice. However, the status of *X. aethiopica* in Cameroon is not clear and should be investigated for sustainable harvesting. Presently, in their region of growth, trees are cut in order to collect the fruits, and no planting is done. *X. aethiopica* activity seems to be related to *β*-pinene and terpinene-4-ol concentrations. Moreover, a synergistic effect was observed when these two monoterpene hydrocarbons were combined. Farmers usually used the entire fruits of the plants mixed with the grains in the storage. Similarly, in southern and eastern Africa, some farmers usually mix stored products with dry leaves of *Ocimum suave* Wild. (Lamiaceae), *Ocimum kilimandscharicum* Guerke (Lamiaceae) and *Ocimum kenyense* Ayob. Ex A. J. Paton (Lamiaceae) against pest infections (Bekele 1994). According to the present study, the quantity of the essential oils which is needed for the protection of 1000 kg of maize grain is very large. As such, this pesticide alternative seems impractical for large-scale applications because of the large amount of plant materials needed. The cost of this kind of protection must also be taken into account. A solution could be the combination of essential oil use with other means of control: physical (Field et al. 2000), biological (Lucas and Riudavets 2002) or, if no other possibilities are available, with specific insecticides (Don-Pedro 1989). For example, Tembo and Murfitt (1995) showed that the combination of plant oils with pirimiphos-methyl at certain dose was equal in efficiency to pirimiphos-methyl. As a result, these two formulations controlled *Sitophilus granarius* on wheat in 24 h of exposure and remained effective after 90 days. Further experiments are needed to verify the persistence of oil toxicity on eggs and larvae of *S. zeamais* before potential applications or possible formulations.

In conclusion, *X. aethiopica* essential oil could contribute in the protection of maize grain during storage. According to the present results, it will be necessary to use the whole fruit of *X. aethiopica* to extract essential oil in order to gain in quantity of essential oil. However, attention must be paid to the variation in oil composition according to the geographical origins. In that context, the determination of the insecticidal activity of the main compounds offers interesting perspectives to find other and richer sources of these active molecules.

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