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Full Length Research Paper

Chronic toxicity of essential oils of 3 local aromatic plants towards *Sitophilus zeamais* Motsch. (Coleoptera : Curculionidae)

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The maize weevil, *Sitophilus zeamais* is a major stored grain pest currently controlled by chemical pesticides. This commonly used control method to prevent post-harvest losses leads to pollution of the environment and intoxication of consumers. Essential oils of aromatic plants are more considered as good control alternative tools. The amount of active volatile of essential oils present in granaries is almost as infra lethal doses. The present work aimed to analyse the chronic toxicity of low doses of essential oils of *Annona senegalensis* Pers. (Annonaceae), *Hyptis spicigera* L. (Lamiaceae) and *Lippia rugosa* L. (Verbenaceae). These plants are toxic to the pest at high doses. At the dose 2.5 x 10-2 ml/ml, they all reduced the oviposition of *S. zeamais*. Moreover *L. rugosa* and *H. spicigera* were the most active of the biological potential of *S. zeamais* reducing significantly its amount of grains attacked (F = 8.63) and that of the rejected flour (F = 41.04***). This chronic toxicity therefore prevents grains from destruction.**

Key words: Aromatic plants, chemical control, essential oils, feeding deterrence, *Sitophilus zeamais*, oviposition.

INTRODUCTION

The maize weevil *Sitophilus zeamais* (Motsch.) is the most important post harvest insect on maize in Cameroon, Ngamo et al. (2004). The most destructive strains are able to cause losses of up to 90% of the stock after 5 months of storage, (Nukenine et al., 2002; Boura, 2006). To prevent these losses, small holders currently use chemical insecticides which nowadays are the most popular tools to achieve any pest control in the area. This abusive utilisation of chemicals endangers the environment which is polluted and the consumers feeding on treated grains carried great quantity of residues of pesticides, (White and Leesch, 1995; Dal Bello et al., 2001; Regnault-Roger et al., 2002). There is need to develop alternative methods with low adverse effects on consumers and less persistent in the environment.

In the past time, peasants used local aromatic plants that they introduced in their granaries with crops in order to kill insects present or to repel those coming to infest their stored products. Nowadays, with the development of phytopharmaceutical industries and liberalisation of chemical insecticide markets this traditional know-how popular is disappearing (Boeke, 2002). Many studies have documented plants used to protect crops from pest and insect attacks (Stoll, 2000; Boeke, 2002; Regnault-Roger et al., 2002). Many of these botanicals are aromatic plants producing essential oils where main compounds are highly volatiles with low persistence (Papachristos and Stamopoulos, 2002; Park et al., 2003). Essential oils with these properties are good alternatives tools to replace

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Table 1. Toxicity of essential oils of *Annona sengalensis*, *Hyptis spicigera* and *Lippia rugosa* towards *Sitophilus zeamais* 24 h after their application on filter paper in Petri dish.

	5×10^{-2}	7.5×10^{-2}	0.1×10^{-2}	LD_{25}	r
A. senegalensis	50%	80%	99%	2.3×10^{-2}	0.87
H. spicigera	68%	98%	100%	2.2×10^{-2}	0.91
L. rugosa	51%	91%	100%	2.2×10^{-2}	0.93

highly persistent chemical insecticides in the control of stored grain insect pests (Konstantopoulou et al., 1992; Shaaya et al., 1997; Ngamo et al., 2001). The targeted aromatic plants produce low amount of essential oil and their popularisation may lead to abusive exploitation which may endanger them and cause lost of biodiversity. Moreover, within granaries, the volatiles present permanently are not in great amount. From this analysis, another way to protect the biodiversity is the use of low doses of these plants to treat the stored grains. These low doses will perform chronic toxicity towards the pest via reduction of appetite, reduction of the growing rate, of the mating and oviposition (Kumar, 1991; Huang et al., 1997). In the present studies, experiments were carried out to evaluate the impact of application of low doses of essential oils of *Annona senegalensis* (Annonaceae), *Hyptis spicigera* (Lamiaceae) and *Lippia rugosa* (Verbenaceae) on the reproduction, nutrition and attacks of *S. zeamais* on maize.

MATERIAL AND METHODS

Collection of plants and hydrodistillation

Plant materials were collected in the Guinean savannah surrounding the campus of the University of Ngaoundéré in the Adamawa province of Cameroon, near the point referenced latitude 07°25.11N and longitude 13°22.5E and the altitude 1036 m. Leaves of A. *senegalensis*, flowers of *H. spicigera* and of *L. rugosa* were collected, dried without sunlight, cut in pieces and distilled. This extraction of essential oils was carried out in a Clevenger-type apparatus during 4 h.

Insect rearing and evaluation of insecticidal efficiency of essential oils

The maize weevil used was of the strain 01Z/LN/01 of the in vivo collection of insect grain pests of Storeprotect at the University of Ngaoundéré. This strain is in collection since 1999 in an incubator monitor at 27°C temperature. Adult insects used for the tests were of one month age.

A micropipette was used to remove 250, 500, 750 and 1000 µl of each essential oil and diluted it in 10 ml acetone to formulate insecticides. For each preparation, 0.5 ml was pumped and flowed regularly on a disk of filter paper (Wathmann n°1) placed in a Petri dish. After this application 20 insects were introduced in the dish 4 min later and it was closed. Mortality of insect was noted 24 h after the treatment. For each trial, 5 replications were made. From this experiment, the dose killing 25% (LD25) of the experimental population was estimated.

Analysis of the chronic toxicity of essential oils

The pest was treated by the LD25 of each oil on filter paper into a dish for a period of 24 h. Insects surviving after this treatment were removed and immediately used for assays. A control was made with only acetone, for each trial, 5 replications were made.

Evaluation of attacks on grains

A total of 20 treated insects were reared on 100 g of safe maize in a 1200 ml flask during 100 days. After this delay, grains were weighted, the attacked ones counted and the rejected flour weighted.

Evaluation of impact on oviposition

A total of 10 treated adults or 5 pairs, male and female were put on 100 g of safe grains for 10 days and were removed after this period. On the grain marks of egg laying from *S. zeamais* were checked. The colorimetric method performed by Holloway (1985) was used. Maize grains were introduced for 2 min in 50°C water containing 5% fuschin. Fuschin colours insect secretions surrounding the egg in pale-red. These coloured structures are counted under binocular lens.

Evaluation of impact of essential oil on insect appetite

The treated insects were weighted before and after rearing on safe maize. After 48 h observation, they were removed and the grains weighted. The feeding deterrence index was calculated using the Isman et al. (1990) formula,

$$FDI\ (\%) = (C-T)/C) \times 100.$$

Where FDI is the Feeding Deterrence Index, C is the consumption of insect without essential oil and T the consumption of insects treated with essential oils.

RESULTS

Toxicity of essential oils

The tested doses of the 3 essential oils expressed toxicity towards *S. zeamais*, the real mortality ranged from 68 to 100% for *H. spicigera*, significantly different from that of *L. rugosa* and *A. senegalensis* (Table 1). The dose for each oils killing from 25 to 30% of the experimental population is 2.5 x 10-2 ml/ml, being the LD25.

The difference observed among the mortality due to these oils is due to their active volatiles mostly monoter-

Table 2. Influence of low doses of tested essential oils on the damages of *Sitophilus zeamais* on 100 g of maize through the amount of attacked grains and the rejected flour after 100 days of rearing.

	Mean number of attacked grains	Mean weight of rejected flour (mg)	FDI (%)
Acetone	37.4±4.87 a	31.74±3.01 a	
A. senegalensis	21.8±3.03 b	17.91±1.32 b	22.716 a
H. spicigera	17.4±2.51 b	16.28±1.49 bc	9.138 b
L. rugosa	18.2±3.27 b	15.31±2.1 c	6.788 c
	F = 8.63 ** (df=3; 19)	F=41.04*** (df=3; 19)	X^2 = 18,28*** (df=2)

Table 3. Influence of the tested essential oils on the number of eggs laid per female of *Sitophilus zeamais* during a 10 days period.

	Mean number of eggs counted	Range
Acetone	18.8±4.23	a
Anona senegalensis	5.4±1.34	b
Hyptis spicigera	2.2±0.84	b
Lippia rugosa	1.6±1.14	b
		F=21.946*** (df =3 ; 19)

Values followed by the same letter within the column do not differ significantly after one-way ANOVA test followed by a classification by the Duncan's multiple range test

penes which are very active on insects (Liu and Ho, 1999; Huang et al., 2000; Kouninki, 2005).

Impact of low doses of essential oils on *Sitophilus zeamais*

Infra lethal doses of essential oils reduced significantly vital activities of *S. zeamais*. The amount of attacked grains was 38 in control significantly different from the 20 counted in treated grains (Table 2).

The rejected flour due to noxious activities of insects is important in control at 31.74 mg significantly different from the amount produce in presence of essential oils. The reduction due to essential oils is most important with *L. rugosa* where only 15.31mg of flour were rejected, significantly different from the 17.91 mg rejected by *A. senegalensis*.

Values of the grains attacked and weight of flour followed by the same letter within the column do not differ significantly after one-way ANOVA test followed by a classification by the Duncan's multiple range test. The chi2 test was used to compare the FID values.

The reduction of the rejection of flour is a consequence of the interference of the essential oil in the physiology of *S. zeamais*. The impact of the essential oils on the appetite of the maize weevil is real (Table 2). The FDI is lower with *L. rugosa*, intermediary with *H. spicigera* and higher with *A. senegalensis*. All these differences are significant. The reduction of appetite which acts on the physiology of the pest begins by the recognition of the volatiles of the oils by the chemoretpors of the pest (Ducrot, 2002). Other investigations on essential oils of *Evodia rutaecarpa* and of *Elletaria cardamomum* had already made evidence on their anti appetent potentialities at low concentrations on *S. zeamais*, Liu & Ho (1999), Huang et al. (2000).

Impact of low doses of essential oils on the oviposition of *Sitophilus zeamais*

The amount of eggs laid by treated adults is significantly lower than that of adults of the control (Table 3). In the control, in 10 days, 5 females laid 19 eggs but when treated by any of the tested oils, the amount of eggs laid varied from 2 to 5 eggs.

It is clear that low doses of essential oil reduced the amount of egg laid. Previous work focussed on the ovicidal properties of these oils and demonstrated that some essential oils are able to halt the egg incubation, Liu and Ho (1999) or to kill the emerging larvae (Huang, 2000). The present work gives evidence on the negative impact of the application of these essential oils on adult's oviposition.

Conclusion

Lethal concentrations of essential oil need important quantity of material for their extraction. Low doses of essential oils progressively reduce the survival potential of the insect pest: the appetite and oviposition are reduced;

consequently, the damages of pest on grains are alleviated.

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