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Full Length Research Paper

Persistence of insecticidal activities of crude essential oils of three aromatic plants towards four major stored product insect pests

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Essential oils of aromatic plants with insecticidal properties are more and more considered as alternative insecticides to protect stored products. Many banned insecticides have high persistence which allow them to occur at several levels of trophic chains. The aim of the present work is to analyse the persistence of insecticidal activity of crude essential oil of three most used local aromatic plants : *Annona senegalensis* Pers. (Annonaceae), *Hyptis spicigera* L. (Lamiaceae) and *Lippia rugosa* L. (Verbenaceae) towards the four majors stored product insect pests: *Sitophilus zeamais* Motsch., *Sitophilus oryzae* L., *Callosobruchus maculatus* Fab. and *Tribolium castaneum* Herbst. This research revealed that *H. spicigera* essential oil was the most active towards *S. oryzae* with a LD50 = 20.18ppm. *T. castaneum* was the less sensitive insect to the three essential oils tested. During a period of 24 hours *L. rugosa* essential oil was the most persistent, showing mortalities for *S. zeamais* of 80%, *S. oryzae* more than 60%, *C. maculatus* 100% and *T. castaneum* 50%. The two other oils tested were not as persistent as *L. rugosa*. This important persistence of the essential oil of *L. rugosa* could be explained by its high content of oxygenated compounds compared to that of the other oils. This most interesting essential oil is therefore a suitable one for popularisation in strategies of pest management in storage.

Key words: essential oils, aromatic plants, stored products, insect pests, persistence.

INTRODUCTION

One of the major problems with agriculture nowadays is to produce more and more in order to provide food for the population which number is in permanent augmentation. One of the most important constraints of having every day sufficient food is the post harvest preservation of its quality and quantity. During storage, foods are currently destroyed by insects and other pests. In Cameroon, these insects on stored grains are mostly *Sitophilus zeamais* and *S. oryzae* (Coloptera: Curculionidae), *Callosobruchus maculatus* (Coleoptera: Bruchidae) and *Tribolium castaneum* (Coleoptera: Tenebrionidae) (Ngamo et al., 2001). Losses due to these pests are very important according to the system considered. Small holders could loss 80% of their stock because of insects after 6 to 8 months of storage (Ntoukam & Kitch, 1998, Nukenine et al., 2002).

To prevent the loss of crops during storage and on fields, producers usually have a relish on chemical insec-ticides. These tools used frequently and abusively cause consequently pollution of environment and intoxication of consumers. Their residues on treated crops have adver-se effects on human being. With all these defaults, it becomes an emergency to build up storage tools that are user-friendly methods with low adverse effect on environ-ment and on consumers. One of the most important insufficiency of industrial pesticide is their persistence in the environment and their stability throughout trophic chains (Rappe, 1976; Derach, 1986). Essential oils of aromatic plant that have insecticidal properties could be considered as alternative insecticides (Dal Bello et al., 1996; Regnault-Roger et al., 2002). There are volatiles with high insecticidal efficiency and very low persistence. Most of the active compounds of essential oils are specific to particular insect groups

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Table 1. Yield of extraction of essential oil from three aromatic plants of Northern Cameroon.

Aromatic plant	Part collected	Yield (%)
Annona senegalensis	leaves	0.19 ± 0.06 b
Lippia rugosa	flowers	0.51 ± 0.19 a
Hyptis spicigera	flowers	0.46 ± 0.35 a
Chi square		9.32 ** (df = 2)

The yields followed by the same letter do not differed significantly (p<0.01)

Table 2. Expression of the insecticidal efficiency of the three essential oils towards the four major insect pests by the estimation of their LD_{50}.

Insect pests	Aromatic plants	LD_{50} (ppm)	r (n=5)
	Annona senegalensis	220.71	0.913***
S. zeamais	*Hyptis spicigera*	234.31	0.917***
	Lippia rugosa	202.32	0.968***
	Annona senegalensis	159.07	0.982***
S. oryzae	*Hyptis spicigera*	20.18	0.887***
	Lippia rugosa	169.23	0.978***
	Annona senegalensis	206.0	0.973***
C. maculatus	*Hyptis spicigera*	100.85	0.952***
	Lippia rugosa	72.12	0.950***
	Annona senegalensis	433.89	0.990***
T. castaneum	*Hyptis spicigera*	346.85	0.967***
	Lippia rugosa	348.47	0.876***

Where ‘r’ is the coefficient. of correlation

(Huang et al., 1997) and not to Mammals (Isman, 2000), many of them are not dan-gerous to humans. They should be considered in pest management strategies. One of the most important step in the use of these aromatic plants is to precise the persistence of their insecticidal activity. The present work focuses on the study of the insecticidal activity of crude essential oils of the three most used local plants for the protection of grain in northern Cameroon: *Annona sene-galensis* (Annonaceae), *Hyptis spicigera* (Lamiaceae) and *Lippia rugosa* (Verbenaceae (Ngamo et al., 2004). In a second phase, the persistence of the insecticidal acti-vity of each essential oil was evaluated.

MATERIAL AND METHODS

Aromatic plants used and their sampling site

Leaves of *A. senegalensis*, flowers of *L. rugosa* and of *H. spicigera* were collected near the campus of the University of Ngaoundéré at the area referenced: longitude: 13°33.549’E, latitude: 07°25.609N and altitude: 1100.5 m. These data were recorded with a GPS Garmin Geko 301. The collection of all plant materials was made in December 2005.

After the collection the plant materials were dried in laboratory condition without sun light during 24 h, cut in pieces, weighed and finally introduced in the Clevenger-type apparatus for its distillation. 4 h after the boiling, the essential oils were collected and its amount measured. Essential oils obtained were kept at 4°C till their use for the bioassays.

Insect pests used

The four insect species used for the test were reared in the Store-protect laboratory at the University of Ngaoundéré in Came-roon. The maize weevil *S. zeamais* used was collected at Ngaoun-déré and has been reared in laboratory conditions since 2003. The rice weevil, *S. oryzae* used for the tests belonged to the strain collected in November 2003 in the granary of a peasant at Beka hosséré (Ngaoundéré, Cameroon) . The cowpea weevil *C. macu-latus* came from Maroua since 2004, finally, the red flour weevil, *T. castaneum* was collected in 2004 at Mokolo in the Far North Province (Cameroon).

Insecticidal efficiency of the three essential oils

Tests were carried out to access the evaluation of the insecticidal activity of crude essential oils towards all the insects. In preliminary tests, several doses were chosen between the doses having no killing effect on the experimental population to the minimal one killing 100% of this population. These experiments aimed to esta-blish the LD_{50} of each essential oil.

With a micropipette (Rainin Magnetic-assist) the complete volume of essential oil obtained for each plant sample was pumped and diluted into 5 ml of acetone. A volume of 0.5 ml of each solution sample was aspired and uniformly flowed on a disk of filter paper (Whatman N°1) of 9 cm diameter put it into separated Petri dishes. An amount of 20 young insects were introduced in each dish 4 min after. These insects were less than one- month old except for *C. maculatus* of three days old. The dish was finally covered, a control with acetone alone, without essential oil was run. For each prepara-tion five replications were carried out. The amount of death insects was counted 24 h after the application.

Persistence of the insecticidal efficiency of the three essential oils

The three essential oils were prepared at their LD_{100} for each insect, diluted in acetone and flowed in Petri dishes at the same time. From the beginning and every 2 h, 20 insects were introduced in each Petri dish for a period of 24 h. For each essential oil, five replications were carried out. 24 h after, the number of death insects was counted.

RESULT AND DISCUSSION

Yield of essential oils extraction

The volume of essential oil produced by every one of the three plants was not important. The yield observed (Table 1) was in all cases less that 1%. The *L. rugosa* and *H. spicigera* flowers produced more essential oil than the *A. senegalensis* leaves.

Insecticidal efficiency of the three essential oils

The values of LD_{50} obtained for all pests are presented in the Table 2. The most active essential oil was that having

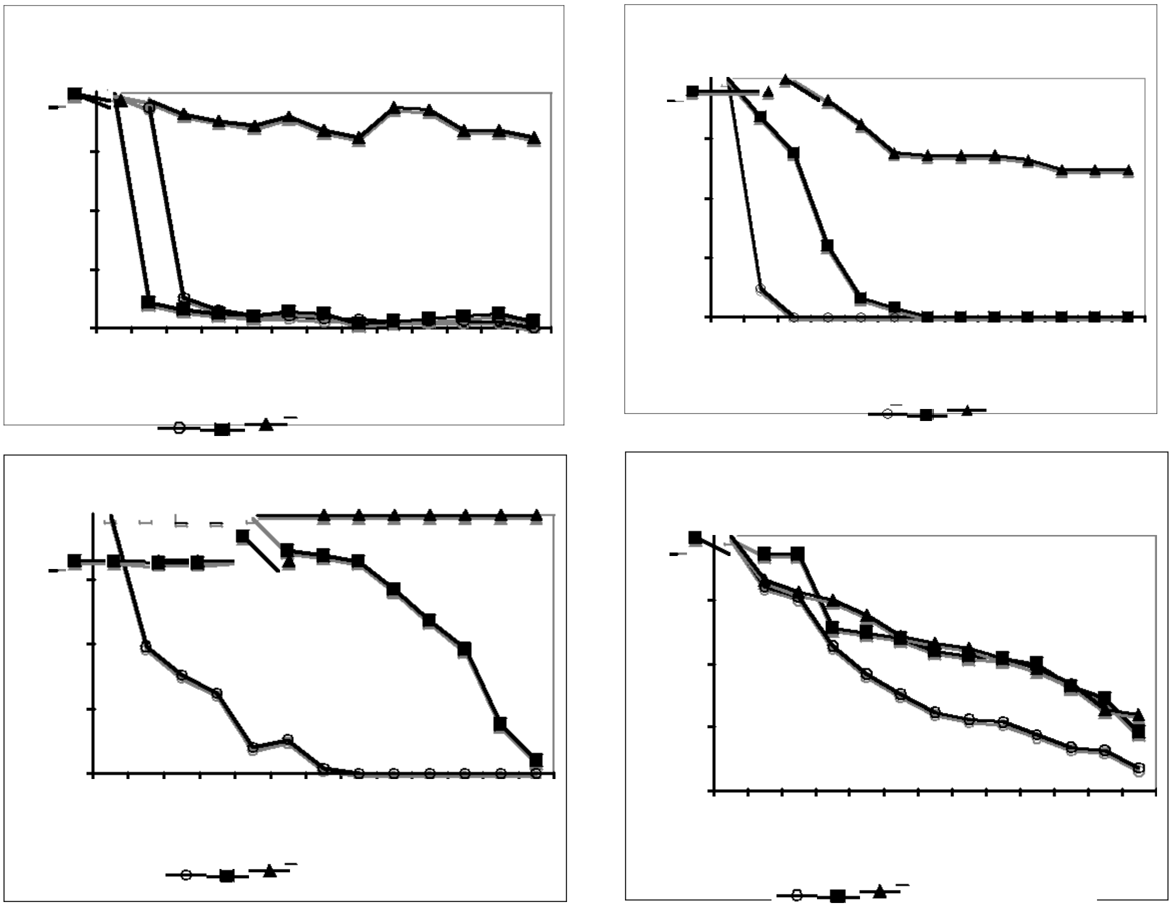

Figure 1. Observation of the persistence of insecticidal activity of three essential oils for a delay of 24 hours starting with maximum efficiency (100% mortality) towards four major pests.

the lowest LD_{50} value. The essential oils did not have the same efficiency on all the pests considered. In the same way, insects had different sensitivity according to the oils. The most active essential oil on *S. zeamais* and *C. maculatus* was that of *L. rugosa*, on *S*. *oryzae* was that of *H. spicigera*. and on *T. castaneum* were both of *H. spicigera* and *L. rugosa* (Figure 1).

All the essential oils tested were insecticides. *T. castaneum* was the less sensitive insect to all these oils. Its lowest LD_{50} is 346.85 with essential oil of *H. spicigera*. The maize weevil was not very sensitive compared to others. The most sensitive insects were *S. oryzae* exposed to *H. spicigera*, which LD_{50} was 20.18 and *C. maculatus* treated with *L. rugosa*, which LD_{50} was 72.12. As shown by Tapondjou et al. (2002), the toxicity of an essential oil depends on the plant species and on the treated insect.

An analysis of the chemical composition of these essential oils elicits the high content of geranial (>30%) in the essential oil of *H. spicigera* and *L. rugosa.* Also, linalool (>15%) was one of the major compounds identified. These compounds, with -phellandrene, terpinolene, (+)-limonene have high toxicity towards *S. oryzae* (Park, 2000). Other compounds contained all the essen-tial oils in very low amount; they were nerol, borneol, cinnaldehyde, eugenol. They have insecticidal properties (Huang et Ho, 1998; Huang et al., 2001; Kim et al., 2002). The insecticidal efficiency observed is both due to major and minor compound of each active oil (Ahn et al., 1998; Cimanga et al., 2002; Park et al., 2002; Regnault-Roger et al*.,* 2002).

Persistence of the toxicity of the three essential oils towards the four insect pests

During the 24 hours period, the toxicity of the tested oils did not remain the same except for that of *L. rugosa* on *C. maculatus*, where the toxicity still at 100%. The essential oil of *H. spicigera* had very short duration of toxicity, after 4 h its toxicity decreased to 20% on *S. zeamais*, while on *S. oryzae*, no significant toxicity was observed after the same delay. On *C. maculatus*, the toxicity of *H. spicigera* decreased to 50% after two hours, to 15% after eight hours, and after 12 hours no observable toxicity was recorded. With *T. castaneum*, the

Table 3. Relative amount (%) of active compounds of the essential oils studied.

Aromatic plants	OMT	HMT	Active compounds
L. rugosa (Ngassoum et al. 2002a)	71.6	19.7	Geraniol, 1-8 cineole, linalool
Hyptis spicigera (Noudjou 2004)	27.29	43.11	Myrcene, 1-8 cineole
A. senegalensis (Jirovetz et al., 2002)	7.42	32.9	Linalool, limonene, phellandrene

OMT= oxygenated monoterpenes ; HMT : hydrogenanted monoterpenes

maximum toxicity of the first hours decreased to 50% after 6 h, and after 24 h it remained significant.

The essential oil of *L. rugosa* was the most persistent. It remained complete on *C. maculatus* , killing 100% of insects above 75% on *S. zeamais*, and at about 70% on *S. oryzae* after 24 h. For the most resistant pest, *T. castaneum*, 30% of the exposed insects were killed after 24 h.

The toxicity of essential oil of *A. senegalensis* decreased rapidly during the first hours of observation. *S. zeamais* was at 15% after two hours, *S. oryzae* at 10% after 8 h, *C. maculatus* at 50% after 20 h and while *T. castaneum* expressed 50% mortality after 10 h and 20% after 24 h.

Persistence of the insecticidal activity of essential oil was in relationship with its chemical composition, the sensitivity of the target pest to the active compounds of the essential oil (Obeng-Ofori et al., 1997).

Active compounds that sustain essential oil insecticidal efficiency are monoterpenes. Essential oil of *L. rugosa* is made of 71.6% oxygenated monoterpenes and 19.7% of hydrocarbonates (Ngassoum et al*.,* 2002) (Table 3). This amount is greater than that of *H. spicigera* which is 27.3% (Noudjou, 2004) and that of *A. senegalensis* 7.4% (Jirovetz et al*.,* 2002) . This difference could explain the variation observed in the persistence of the insecticidal activity of these essential oils. Oil of *L. rugosa*, rich in oxygenated monoterpenes, is less volatile than hydrogenated compounds. They spread slowly.

The activity of essential oils decreased with the time because of their high volatility. The rythm of the reduction of their activity was not the same for all the three oils tested. Oils with high content of hydrogenated compounds loss their activity quicker than those containing mainly oxygenated compounds (Huang & Ho, 1998; Regnault-Roger et al., 2002). The speed of the oxydation of hydrogenanted monoterpenes is greater for compounds as sabinene, 1,8 cineole, -pinene (Kim et al., 2002). This oxydation leads to the reduction of the insecticidal efficiency of the oil.

Conclusion

In pest management strategies, aromatic plants with long lasting insecticidal efficiency should be considered. These considerations must take into account the pest species or the type of stored products. *L. rugosa* was the most active plant mainly on *C. maculatus, S. zeamais* and *S. oryzae*. Its essential oil had the highest amount of oxygenated monoterpenes. The essential oil of *A. senegalensis* was persistent mainly on *C. maculatus* and on *T. castaneum*, The persistence was the same as that of *L. rugosa*.

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