

**POTENTIAL USE OF ESSENTIAL OILS FROM LOCAL CAMEROONIAN
PLANTS FOR THE CONTROL OF RED FLOUR WEEVIL *TRIBOLIUM
CASTANEUM* (HERBST.) (COLEOPTERA : TENEBRIONIDAE)**

By

H Kouninki *¹, L.S.T Ngamo ², T Hance ¹ and M.B Ngassoum ³



Kouninki Habiba

* Corresponding author- Email: kouninki@yahoo.fr

¹Catholic University of Louvain (UCL), Unit of Ecology and Biogeography, Place Croix du Sud 4-5, 1348 Louvain-La-Neuve Belgium,

²Faculty of Sciences, Department of Biological Sciences, University of Ngaoundere, Po Box 454 Ngaoundere Cameroon

³National Advanced School of Agro- Industrial Sciences, University of Ngaoundere Po Box 455 Ngaoundere Cameroon

ABSTRACT

Animal feed is food constituted for breeding stock animals because it possesses vital nutrients for animal growth. Animal feed or their ingredients as constituted by cereal flour and are stored after formulation for ulterior breeds. These provender or cereal grains used are commonly attacked by storage insects principally of the genus *Tribolium*. Firstly, contact and ingestion test by two essential oils of aromatics plants *Ocimum gratissimum* L (Lamiaceae) and *Xylopia aethiopica* Dunal A. Rich (Annonaceae) were done firstly on adults and aged larvae of *Tribolium castaneum*. Secondly, the contact and inhalation test by three essential oils of the aromatic plants *Annona senegalensis* L. (Annonaceae), *Lippia rugosa* L. (Lamiaceae) and *Hyptis spicigera* Lam. (Verbenaceae) were done for the control larvae, young and aged adults of the red flour weevil *Tribolium castaneum* Herbst (Coleoptera : Tenebrionidae) very resistant pest to chemical pesticides. Essential oils of *Ocimum gratissimum* and *Xylopia aethiopica* have no contact and ingestion effect on adults of *Tribolium castaneum*; their insecticidal activity is characterized mostly by their inhibition of the nymphosis of aged larvae of the same species. On the other hand contact and inhalation tests with crude essential oils of *Lippia rugosa* and *Hyptis spicigera* are the most promising because of their efficacy on the other life stages. They are more efficient, with 100% mortality, on larvae at early stages and young adults. On resistant aged larvae and adults, the insecticidal efficacy decreases but remains significant. Since this insect, *Tribolium castaneum* is the major pest of stored flours and provender, the incorporation of these promising essential oils in flours or in storage formulations of these foods for animals could contribute to their better cereal food conservation. The important and indispensable element of cereals for storage, growth and reproduction animal feed could be preserved from insects attack by use of natural product and may contribute to diminish toxicity of feeder, environmental pollution and the resistance phenomenon of insects.

Key words: Essential oils, *Tribolium castaneum*, Pesticides.

French

Potentialités d'utilisation des huiles essentielles des plantes locales du Cameroun pour le contrôle du vers rouge de la farine *Tribolium castaneum* (Herbst.) (Coleoptera : Tenebrionidae)

Les aliments pour animaux sont des aliments composés de plusieurs ingrédients pour élever des animaux parce qu'ils possèdent les éléments nutritifs vitaux pour l'animal. Les provendes ou leurs ingrédients constitués principalement par la farine des céréales; sont entreposés après formulation pour des utilisations ultérieures. Les provendes ou les grains de céréale utilisés sont attaqués au cours du stockage principalement par les insectes du genre *Tribolium*. Premièrement, la toxicité par contact et ou par ingestion de deux huiles essentielles de plantes aromatiques *Ocimum gratissimum* L (Lamiaceae) et *Xylopiya aethiopica* Dunal A. Rich (Annonaceae) a été effectuée sur les larves âgées et les adultes de *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae) ravageur principal de farine ou des provendes. Deuxièmement, la toxicité par contact et ou par inhalation de trois huiles essentielles des plantes *Annona senegalensis* L. (Annonaceae), *Lippia rugosa* L (Lamiaceae) et *Hyptis spicigera* Lam. (Verbenaceae) a été effectuée pour le contrôle des différents stades larvaires, des jeunes et vieux adultes de *Tribolium castaneum* insecte résistant aux pesticides chimiques. Le test par contact et ou par ingestion effectué avec les huiles essentielles d'*Ocimum gratissimum* et *Xylopiya aethiopica* n'ont montré aucun effet sur adultes de *Tribolium castaneum*; leur activité insecticide est caractérisée principalement par l'inhibition de la nymphose des larves âgées. Le test par contact et ou par inhalation montre que les huiles essentielles brutes de *Lippia rugosa* et *Hyptis spicigera* sont les plus prometteuses à cause de leur efficacité sur les autres stades de développement ; elles sont plus efficaces sur larves les plus jeunes et les jeunes adultes avec 100% mortalité, pour les larves âgées résistants et les adultes, l'efficacité de l'effet insecticide diminue mais reste considérable. Vu que cet insecte; *Tribolium castaneum* est le ravageur principal de farines entreposées et des provendes, l'incorporation de ces huiles essentielles prometteuses dans les farines ou lors de la formulation de nourritures pour animaux pourrait contribuer à une meilleure conservation de ces dernières. Les éléments importants indispensables pour l'entretien, la croissance et la reproduction pour l'animal pourraient être conservés contre les attaques des insectes par les produits naturels et contribuer à réduire la toxicité du consommateur, la pollution environnementale et les phénomènes de résistance chez les insectes.

Mots clés: Huiles essentielles, *Tribolium castaneum*, Pesticides.

INTRODUCTION

Insects are the major pests of cereals grain or flour during storage. Damages due to insects affect the quality, the quantity, the commercial and agronomic value of the product. Many pests of stored products are Coleopterans and the most destructive tropical species for cereals belong to the genus *Sitophilus* and *Tribolium* [1]. The control of pests in stored products, principally cereal grains, by use of the chemicals, a common strategy for post harvest loss avoidance, leads to the apparition of many problems like the pollution of environment, toxicity to human being, emergence of resistant pests strains and many others damages [2]. These synthetic pesticides are expensive for the users and may cause potential risk due to the lack of technical knowledge related to their safe use. Among these unexpected effects, the development of resistance by some pests occurred and the lethal effect on non-target species are possibilities. It is the case of *Tribolium castaneum* (Herbst) (Coleoptera, Tenebrionidae) which is nowadays one of the most resistant insects to industrial pesticides. Moreover, *T. castaneum* is a major pest of cereal flour depreciating the quantity and quality of the food [3]. *T. castaneum* in grain storage appears after the implementation of major grain pest as *Sitophilus* species. Any treatment to cure attacks of this pest potentially leads to direct poisoning of consumers because the flour or main components of animal food treated by chemicals pesticides are directly consumed or used for the formulation of provender. Chemical insecticides cannot be mixed with flour or provender because of their adverse effect on food quality [4], very little is reported on the use of chemical natural or synthetic to *T. castaneum* in flour or provender destined to the alimentation. It becomes, therefore, useful to build up alternative methods of controlling pest by methods that are user-friendly as the use of agents with high efficacy on the pest and low persistence in the food. There are needs to develop and popularise such control techniques that are clean and user-friendly as the used of natural essential oils. These natural products as essential oils are often highly specific and biodegradable of low persistence. In the early seventies, an alternative was the use of natural products as pesticides to control pests during storage; some of these natural products protect grain without any observed effects on their germination, their smell and their taste [5-8]. Ethno botany has therefore, played a very important role in the protection of crops against pests in Africa and Asia [9-15]; plants were used at the time in granaries by the farmers naturally to protect their produce. Most of the essential oils or vegetable oils used in crop protection are extracted from plants formerly known to have insecticidal activity by the population. A popular figure is that of neem seeds (*Azadirachta indica*) and cotton seeds (*Gossypium hirsutum*) [2]. Recent research [16, 17] on this topic focused on the oils which are environment and consumer-friendly.

Essential oils are secondary metabolites abundant in aromatic plants families such as Lamiaceae and Annonaceae, and contain a large number of compounds such as monoterpenes and sesquiterpenes. Essential oils of aromatic plants and spices are tested for their potential as protective agents for human and/or livestock feeds. In this respect thus to have a protectant edible for human beings or animals, many researches

pointed out the use of essential oils from aromatic plants as the best way to control pests without leading to human and animal toxicity [16, 18]. Many aromatic plants are known to possess insecticidal activity, to repel ovipositing insects and reduce the progeny. Essential oils are known to exhibit low toxicity to mammals, and the most terpenoids and phenols found in plant essential oils have minimal toxicity and have even been approved as flavouring agents in food [16, 18].

The objective of this study was to determine the sensitivity of the red flour weevil *T. castaneum* adults to the insecticidal properties of essential oils of *Ocimum gratissimum* L. (Lamiaceae) and *Xylopia aethiopica* Dunal A. Rich (Annonaceae) on one hand as contact and ingestion insecticides. On the other hand, the contact and inhalation insecticides of *Hyptis spicigera* Lam (Lamiaceae), *Annona senegalensis* Pers. (Annonaceae) and *Lippia rugosa* (Verbenaceae) essential oils against larvae and adults of *T. castaneum*. All these plants are known to have high insecticidal effect on *T. castaneum* recalcitrant to agricultural insecticides.

MATERIALS AND METHODS

Extraction of essential oils

The five chosen plants were collected in Northern Cameroon in dry or fresh forms. The essential oil extraction was done using a Clevenger apparatus for 4 hours. The hydro distillation process utilized fresh leaves of *A. senegalensis*, *L. rugosa* and *O. gratissimum* and the dried flowers of *H. spicigera* and dry fruits of *X. aethiopica*. The essential oils extracted were dried over anhydrous sodium sulphate and stored at 4°C until analysed and used in experimentation.

Formulation of *Xylopia aethiopica* and *Ocimum gratissimum* essential oils for contact and ingestion toxicity tests on adults and aged larvae of *Tribolium castaneum*

The essential oils obtained from fresh leaves *O. gratissimum* and dried fruits of *X. aethiopica* were formulated as powder using the sorghum flour (*Sorghum bicolor*) according to a preliminary test made to determine flour where *T. castaneum* develops quickly. A concentration of 300ppm was used for the bioassays. Adults and aged larvae (fourth instars) were reared in treated sorghum flour; and mortality was assessed by contact and ingestion. The amounts of dead adults were evaluated after 4 days, the rate of nymphosis was calculated 14 days after the treatment.

Formulation of *Annona senegalensis*, *Hyptis spicigera* and *Lippia rugosa* essential oils for contact inhalation toxicity tests on *Tribolium castaneum* larvae and adults

A precise volume of crude essential oil (250, 500, 750 and 1000 µl) was diluted in 10ml of acetone to constitute 4 essential oils concentrations of *A. senegalensis*, *H. spicigera*. and *L. rugosa*. On a disk of 9cm diameter of filter (Whatman n°1) put in a

petri dish of 80 ml of volume, 10 ml of the preparation was put. After 5 min, the acetone was evaporated after which the tests were conducted. The control was the acetone without any addition of essential oil. All the tests were done under laboratory conditions. After the complete evaporation of acetone, 20 adults of each pest were introduced in the petri dish. The petri dish was covered and sealed with parafilm. The set up was to have the active product act by contact and inhalation. The 4 concentrations and the control were made and the 5 doses tested were 0, 152.43, 297.62, 436.04, 548.18 ppb. The mortality of insects was recorded 24 hours after the treatment. Death or mortality was determined and concluded by failure organism to react after several touches.

Two-days aged and 3-months mature adults of the red flour weevil were used for the tests concerning adult stage; young and aged. All the 5 larval stages were considered; morphological features were used to separate different larval instars.

The red flour weevil considered in this work was reared in the laboratory one year prior to the experiments. The rearing is carried out at laboratory in an incubator at 27.5°C. For each trail 20 adults were used and 5 replications were made. Results were subjected to one way analysis of variance (ANOVA I) and compared to that of control via a Duncan's test.

RESULTS

Contact and ingestion toxicity of the formulation of essential oils of *Xylopi* *aethiopica* and *Ocimum gratissimum* used against adults and aged larvae of *Tribolium castaneum*

Table 1 shows the effect of essential oils used against the adults of *T. castaneum*: No insecticidal effect was noted for the two essential oils used after four days exposure. All adults survived after the treatment; there was no significant difference ($p>0.001$) between the obtained data.

The toxicity by contact or by ingestion on the aged larvae exposed to the essential oils used is recorded in Table 2. No insecticidal effect on the two essential oils was observed on aged larvae after 7 days. It was at the nymphosis stage that a possible effect on the success of the formation of the nymph occurred; and no dead larvae were noted. This reduction in the rate of the nymphosis was only due to *X. aethiopica*, *O. gratissimum* had no significant effect on this reduction ($p>0.001$). *X. aethiopica* essential oil seems to be more effective than *O. gratissimum* essential oil.

Contact inhalation toxicity of essential oils of *Annona senegalensis*, *Hyptis spicigera* and *Lippia rugosa* against different larval and adult stages of *Tribolium castaneum* at different doses

Mortality of *T. castaneum* at different development stages due to various concentrations of the three essential oils of *A. senegalensis*, *H. spicigera* and *L. rugosa* tested are shown in Table 3. With *L. rugosa*, first, second instars larvae and young adults were very sensitive as the mortality observed was greater than 75%. At the dose 152.43 ppb aged adults and aged larvae are resistant, the mortality observed was lower than 25% (Table 3). The augmentation of the dose did not modify the resistance of the fourth and fifth instars larvae; they remained resistant till the dose 548.18 ppb where only the fifth instars remain resistant.

At all doses D3 (436.04 ppb) and D4 (548.18 ppb) young adults exhibited similar sensitivity as young larvae, no significant difference is observed for their mortality. The fourth and fifth larval instars remained highly resistant, no significant mortality was observed. At 548.18 ppb less than 10% of mortality was noted in these groups.

Essential oils of *H. spicigera* expressed high mortality on the first larval instars and on young adults with doses 436.04 and 548.18 ppb. At this last dose, even aged adults are highly sensitive; the mortality observed was above 95%. The fifth larval instars were resistant at all the doses tested, no important mortality was observed. At 548.18 ppb less than 15% of mortality was noted. This observation was the same with *L. rugosa* where the rate of mortality was greater in all cases. *A. senegalensis* causes mortality which is not so important as for the two other essential oils. This means that *A. senegalensis* will be less toxic than the other essential oils used against larvae and adults of *T. castaneum*.

There are indications that early larvae stages and young adults are the most essential oil insecticide vulnerable stages in the development of *T. castaneum* activity. *L. rugosa* expressed the most efficient insecticidal properties; it was very efficient on sensitive stages even with low doses. *A. senegalensis* was efficient but at very high doses on sensitive stages, with a similar situation as *H. spicigera* where the efficiency was noted with high doses. The aged larvae were the resistant stages to these oils. The analysis of the value of the LD₅₀ (Table 4) expressing essential oil efficacy, points out sensitive pest stages, *L. rugosa* pointed out very low indeterminate LD₅₀. *A. senegalensis* expressed the high value of the LD₅₀ with resistant stages, 11 007.58 ppb for the fifth instars larva and 6748.07 ppb with the fourth instars larvae (Table 4). *H. spicigera* had, with resistant stages, low LD₅₀.

DISCUSSION

The use of chemical synthetic pesticides poses problems to human health and their environment because of their persistence and effects on non-target organism and the insect resistance. Nowadays, many studies demonstrate the value of utilization of natural products as the safe alternatives to replace synthetic chemical pesticides to control storage pest [15-23]. The tests made in this study were principally experimental and are focused on important pests of stored flours and provender, the *Tribolium* species. The treated grains can be used as seed by farmer to maintain the health of the grain. There is no work on the preservation of flour or provender against *T. castaneum*, and these aliments are the principal source of nutrients for all pests and their larvae as they in live in flour or broken grain. Chemical synthetic pesticides cannot be used in association with flour or sources of provender because of their direct consumption by humans or animals.

The utilization of aromatic plant essential oils as protectant for stored grains for human consumption is important as an alternative to chemical pesticide. The low essential oil persistence and the fact that sources of plants are also used as spice by the population indicate safety and potential contribution in protection against attack of *Tribolium* species.

The inhibition of the nymphosis observed in this study is due to active compounds found in the essential oils used that varied in the function of the plant. The aged larvae are more sensitive than adults when exposed to the toxicity of vapours, an insect stage dependant phenomenon [19]. The non-effect observed against adults of *T. castaneum* may be due to insufficient essential oil quantity of plants tested.

Essential oil of *X. aethiopica* had an effect on the red flour weevil, acting as an insect growth regulator. The *X. aethiopica* essential oil may be used to kill major pests of flour or provender. The incorporation of these essential oils in the animal food to fight against insects in general and *T. Castaneum* in particular, major pest of flour and feeding stuff is to be considered in integrated pest management in stored products. These results also show that it is possible to treat the major component to build up another formulation of animal food.

Plant materials with insecticidal properties have been used for generation throughout the world. These traditional post-harvest practices are particularly relevant for small-scale subsistence farmers for their storage commodities. Botanicals have many advantages over synthetic pesticides because of their low persistence, their specificity and the fact that they are commonly also used by local people as spice. The insecticidal activity of these spices is due to the presence of monoterpenes in their constituents [15, 17, 21-23]. Aromatic compounds such of the spices used in these studies are generally active on insects at a very low dose and until now no study demonstrates contraindications of these plant essential oils in vertebrates [14].

CONCLUSIONS

With the dominance of synthetic pesticides capable of poisoning humans and animals, polluting water and environment, enhancing pest resistance, environmental persistence and effect on non target species, search of alternative methods for the protection of stored products against insects is imperative. The availability of plant essential oils and their long history as flavouring agents in food is indication of their relative safety to human. The aromatic plants are well known by the population and some which exhibit insecticidal activity against pest storage would be welcomed because its use as essential oils in low quantity can help the population to reduce losses during storage and preserve the environment against pollution by synthetic chemicals. The essential oils of *X. aethiopica* and *O. gratissimum* have no effect on adult of *T. castaneum* and act as insect growth regulator by contact and ingestion. The early larvae stages and young adults are very sensitive to *A. senegalensis*, *H. spicigera* and *L. rugosa* essential oils to aged larvae and adults by contact and inhalation. For a better control of these pests, an integrated approach in killing them by essential oil needs to account the preservation of natural enemies of the concerned pests such as parasitoids or predators present in stored food.

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Table 1. Efficacy of essential oils of *Xylopiya aethiopia* and *Ocimum gratissimum* on adults of *Tribolium castaneum* after 4 days (mean of individual which survive after the treatment)

Treatment	<i>X. aethiopia</i>	<i>O. gratissimum</i>	Control
Dose (ppm)	300	300	0
Mean (n=5; p>0.05; ns)	9.6±0.9	10±0.0	10±0.0

Table 2. Effect of essential oils of *Xylopiya aethiopia* and *Ocimum gratissimum* (dose: 300 ppm) on the nymphosis of aged larvae of *Tribolium castaneum* after 14 days

Treatment	<i>X. aethiopia</i>	<i>O. gratissimum</i>	Control
% nymphosis	75±11.7 ^b	87±8.2 ^{ab}	92±5.7 ^a

Values followed by the same letter do not differ significantly, F= 8.78; df=2; 14; p<0.001

Table 3. Insecticidal activities expressed by mortality by contact and inhalation of five doses of tree essential oils *Annona senegalensis*, *Hyptis spicigera* and *Lippia rugosa* on larval and adult stages of *Tribolium castaneum*. (D0=0ppb; D1=152.43ppb; D2=297.62ppb; D3=436.04ppb; D4=548.18ppb)

	Plants	Larvae					Adults	
		1 st	2 nd	3 rd	4 th	5 th	Young	Aged
D0	Control	0	0	0	0	0	0	0
D1	<i>A senegalensis</i>	31 ^a	20 ^{ab}	0 ^c	0 ^c	0 ^c	10 ^b	0 ^c
	<i>H spicigera</i>	34 ^a	19 ^b	9 ^c	6 ^c	0 ^c	16 ^b	6 ^c
	<i>L rugosa</i>	100 ^a	73 ^a	30 ^b	24 ^{bc}	16 ^c	100 ^a	35 ^b
D2	<i>A senegalensis</i>	53 ^a	40 ^a	2 ^b	0 ^b	0 ^b	65 ^a	7 ^b
	<i>H spicigera</i>	66 ^a	41 ^b	30 ^b	12 ^c	3 ^c	56 ^a	27 ^b
	<i>L rugosa</i>	100 ^a	83 ^a	41 ^b	31 ^{bc}	23 ^c	100 ^a	42 ^b
D3	<i>A senegalensis</i>	62 ^a	57 ^b	6 ^c	0 ^c	0 ^c	98 ^a	58 ^b
	<i>H spicigera</i>	80 ^a	58 ^b	56 ^b	20 ^c	5 ^d	79 ^a	57 ^b
	<i>L rugosa</i>	100 ^a	88 ^a	48 ^b	34 ^b	33 ^b	100 ^a	47 ^b
D4	<i>A senegalensis</i>	72 ^b	68 ^b	22 ^c	6 ^d	4 ^d	100 ^a	77 ^{ab}
	<i>H spicigera</i>	97 ^a	72 ^b	62 ^b	31 ^c	13 ^d	100 ^a	91 ^a
	<i>L rugosa</i>	100 ^a	95 ^a	70 ^b	47 ^c	38 ^c	100 ^a	68 ^b

Values followed by the same letter within a line do not differ significantly, p<0.05

Table 4. Values of the LD₅₀ of the essential oils tested on different larvae stages and adults of *Tribolium castaneum*

Plants	Larvae					Adults	
	1 st	2 nd	3 rd	4 th	5 th	Young	Aged
<i>A senegalensis</i>	278.16	359.12	964.96	6748.07	11007.58	233.93	433.89
<i>H spicigera</i>	210.67	349.15	420.32	1168.91	1216.89	244.04	346.85
<i>L rugosa</i>	/	70.67	354.63	720.04	1017.68	/	348.47

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