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Use of Essential Oils of Aromatic Plants as Protectant of Grains During Storage

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Abstract: Post harvest losses of crops during storage are mainly due to insects. To prevent their stored products from insects attacks, farmers often used in the past some traditional tools which are nowadays disappearing without sufficient valorisation. The present research aims to make an inventory of stored grains pests present and of the botanicals used in the protection of stored grains. From these investigations, it clearly shows that, Insects associated to stored grains in Northern Cameroon are primary or secondary pests, detritivores, predators or parasitoids feeding on other insects mainly pests. The major pests of stored grains are the weevils *Sitophilus zeamais* and *S. oryzae* (Coleoptera: Curculionidae) for cereals and *Callosobruchus maculatus* (Coleoptera: Bruchidae) for legumes. To protect their stored grains from losses, 29 plants are introduced in the granaries during their filling with crops, 12 of these are aromatic plants producing essential oil by hydrodistaillation. Nine of these essential oil have insecticidal properties towards *S. zeamais*, *S. oryzae* and *Tribolium castaneum* (Coleoptera: Curculionidae) which are the most important pests of cereal in grains and in flour. In order to designate an essential oil for the pest control by farmers, the criteria define was the relation between the insecticidal activity and the rate of extraction of essential oil from plant material. With this consideration, essential oil of *Lippia rugosa* (Verbenaceae) is among the 4 most used the suitable tool to prevent attacks of *S. zeamais* and *S. oryzae*. In the other hand to control *T. castaneum*, essential oils of *Xylopia aethiopica* (Annonaceae) *Vepris heterophylla* (Rutaceae) and *L. rugosa* are adviced.

Key words: Cameroon, stored grains, aromatic plants, insect pests, essential oils

INTRODUCTION

In African tropical zones, cereals and legumes are kept as grains or flour in storage structures and constitute important source of income for farmers. Efforts are made to protect the stocks because they insure food security for people living in these areas of deficiency (Kéita, 1999) where agriculture is not highly productive and depends on the length of the dry season. Protecting stored product in this case is an emergency. In Northern Cameroon, almost all the harvested grains are stored in familia granaries (Seignobos, 1994; Seignobos *et al*., 1996; Ngamo *et al*., 2004). This aims to prevent hunger by reducing the length of the period during which granaries are empty.

The storage will be successful if at its end, the product stored does not significantly differ in quantity and quality from what was put initially. Unfortunately, this success is never observed. Losses are currently observed in stocks (Bell, 1994; Ntoukam and Kitch, 1998). There are linked to the storage facilities or to the attacks of pests which may be Insects, Rodents or micro organisms. These spoliators are capable of destroying a whole stock if no prevention method is taken. During storage, the most important factor causing losses of grains is insect attacks (Delobel and Tran, 1989; Kreiter, 1989) They feed on break or entire grains, consuming mostly the germ (Dal Bello *et al*., 2001). An attack grain, losses its agronomic, nutritional and commercial value, since it could not be sell or saw. Finally, insect dejection inside the attack grains or inside flour creates contamination of the whole stock characterized by a very bad smell. To avoid these losses, use of industrial chemical is often made by farmers, the use of this tool still in progress (Ngamo *et al*., 2004) while the use of traditional methods is disappearing (Boeke, 2002). Use of chemical is the popular method of crop protection in developing countries, with the development of the phytophar-maceutical industries and

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the liberalisation of the market of insecticides in these countries, it is possible to see in the years to come high occurrence of adverse effects of the use of industrial pesticides (Kumar, 1991). It is therefore useful to develop and popularise efficient pest control method which are user-friendly methods respecting the consumer and the environment.

The first step in this procedure is to precise the biodiversity of the insects present and to establish their status. To our knowledge, such information is not yet available for the entomofauna of stored grains in Cameroon. Secondly it is clear that in the past, people use plant products to protect their crops from insect spoliation and nowadays, this knowledge is unused (De Groot, 1997). This traditional know-how unvalorised is to be improved. Biopesticides of plant origin are efficient as grain protectants (Jirovetz *et al*., 2005; Ngassoum *et al*., 2004; Ngamo *et al*,. 2004; Tapondjou *et al*., 2002; Boeke *et al*., 2004; Boeke, 2002). The persons continuing to use them are convinced by their efficacy. The aim of the valorisation of these botanicals active on pests is to reduce the intensive use of industrial pesticides by farmer and to help the local producer to put in market clean and safe grains. The present work aims to investigate on the plant used to protect stored grain in northern Cameroon and to come to the designation of the most active plant on major pests.

MATERIALS AND METHODS

Investigation on grains storage in northern cameroon: Prospections were made during 3 years in storage campaign each year from November to February (2002/2003, 2003/2004 and 2004/2005) to establish how peasants stock harvested grains, and to make an inventory of the tools used to prevent insect attacks. Most of the plants cited were founded, identified and collected. The period of collection of the plant was noted and the manner a plant is used in the granary was also noted.

Diversity of the insects associate to the stored grains: During the prospections in farmer compounds, a sample of grain was collected from peasant's stock and take back to the laboratory where it was observed for 7 months, all the insects emerging were taken and identified. The identification was done with the help of the identification guide made by Delobel and Tran, (1993) and Kreiter, (1989). The Centre of Research on the Biodiversity of the Catholic University of Louvain-la-Neuve in Belgium was also contacted for this purpose. All the important insects obtained were also reared.

Hydrodistillation of the plant material collected: Plant material introduces in granaries by farmers were for each sample dried without sun light cut in pieces and distilled in a Clevenger-type apparatus for 4 hours period. The mass of the material distilled was weighed and after the distillation the volume of the essential oil produced was recorded. Hence the rate of essential oil extraction was estimated. The essential oil obtained for each plant was preserved in a flask, sealed and kept in a refregirator at 4°C.

Evaluation of the insecticidal efficiency of the essential oils: Insecticidal activity of each oil was accessed through a contact and inhalation test. For each essential oil, precise volumes ranking from 0.5 to 5 mL were tested. The oil was diluted in 10 mL of acetone. For each trial, 0.5mL of the preparation was deposited on a 9 cm diameter filter disk (Whatman n°1) put in a petri dish of 80 mL, 5 min after application, evaporation of the acetone was completed. Once acetone was evaporated, 20, one month aged adults were introduced in the petri dish, each one covered and sealed with parafilm. For each preparation, 5 replications were made. The control was the same volume of acetone without addition of essential oil. The mortality of insects was recorded 24 h after the treatment.

Some preliminary tests aimed to screen the doses from that without insecticidal activity to that killing 100% of the experimental population. Dose-response curves were analysed by the log-probit method to estimate de DL50 which is the dose of essential oil killing 50% of the experimental population.

To come to the designation of the most active oil on the major pests, the criteria used was the ratio of the DL50 by the rate of extraction of essential oil from each plant considered.

RESULTS AND DISCUSSION

Diversity of insects associated to stored grains in Northern Cameroon: Majors noxious insects to grains during storage are beetles, Coleoptereans and buterflies, Lepidoptereans. In relationship with their damages to grains, they are classified in 4 groups.

- Primary pests, able to attack clean grains. Six insect Families are involved. In Coleoptera Family, they are Curculionidae, Bostrychidae et Bruchidae and in the Lepidopera Family they are mostly Pyralidae (Table 1). One specie was observed: *Ephestia kuehniella.*
- Secondary pests attacking grains already infested by primary pests or associated with break grains or with flours. They are unable to feed on clean and safe grain. They are Coleoptereans from 2 Families, Tene-brionidae with 2 sp. *Tribolium castaneum* and *T. confusum*.The second Family is that of Sylvanidae with one specie *Oryzaephilus surinamensis.*

Table 1: Diversity of stored grain insects sampled in northern Cameroon

Insects sampled	Familly	Status*	Hosts or preys
Sitophilus zeamais	Curculionidae	Pst I	Cereal grains
S. oryzae	Curculionidae	Pst I	Cereal grains
Callosobruchus maculatus.	Bruchidae	Pst I	Legumes grains
Tribolium castaneum.	Tenebrionidae	Pst II	Cereal flour/break
T. confusum	Tenebrionidae	Pst II	Cereal flour/break
Oryzaephilus surinamensis	Sylvanidae	Pst II	Cereal flour break
Rhyzopertha dominica	Bostrychidae	Pst I	Cereal grains
Cryptolestes ferrugineus	Tenebrionidae	Det	Cereal grains
Ephestia kuehniella	Pyralidae	Pst I	Cerealgrains/flours
Plodia sp.	Pyralidae	Pst I	Legume and cereal
Sitotroga cerealella	Gechiidae	Pst I	Cereal grains
Acanthoscelides obstectus	Bruchidae	Pst I	Legume grains
Gibbium sp.	Ptinidae	Det	Cereal infested
Oligota sp.	Ptinidae	Det	Cereal infested
Gnatocerus sp.	Tenebrionidae	Det	Cereal infested
Anisopteromalus calandrae	Pteromalidae	Para	*Sitophilus zeamais*
Eupelmus sp.	Ichnemonidae	Para	*Sitophilus sp.*
Cerocephala sp.	Pteromalidae	Para	*Sitophilus sp.*
Dinarmus basalis	Pteromalidae	Para	*Callosobruchus maculatus*
Lyctocoris sp.	Pentatomidae	Pred	*Tribolium sp, R. dominica*
Xylocoris sp.	Pentatomidae	Pred	*Tribolium sp, R. dominica*

* Status : Pst I = Primary pest; Pst II = secondary pest; Det = detritivore; Para = parasitoid; Pred = predator

- Detritivores insects feeding on dust and other decaying material. They are considered as granary cleaners. Four insects were observed, 2 of the Family of Ptinidae, *Gibbium* sp. and *Oligota* sp. and 2 others from the family of Tenebrionidae: *Gnathocerus* sp. and *Cryptolestes ferrugineus*.
- Beneficial insects which are natural enemies of some of the major pests observed. They are in 2 groups: Predators and parasitoids of pests. Four species of parasitoids were observed emerging from grains already attacked by *S. zeamais* and *S. oryzae*. One of them is an Ichneumonidae, *Eupelmus* sp. having *S. zeamais* and *S. oryzae* as hosts. Moreover 3 Pteromalidae: *Dinarmus basalis* emerging from *C. maculatus*, *Cerocephala* sp. having *S. oryzae* and *S. zeamais* as hosts and a last one identified as *Anisopteromalus calandre* emerging from *S. zeamais*.

Two buds, *Xylocoris* sp. and *Lyctocoris* sp. were sampled in products containing *R. dominica*, *T. castaneum* and *T. confusum*. These predators are from the Family of Pentatomidae. They feed on free insect larvae they find in the granary.

Diversity of plants used as grains protectant in northern Cameroon: It comes from the 3 years prospection campaign that 29 plants could be used by farmer to protect grains during storage. They are all introduced in the granary with grain at fill period to repel all insect coming to infest grain and to kill all other insects already present in the granary. These plants belongs to 17 Families, the most important ones are Poaceae with 6 species, Labiateae with 5 species, Asteraceae with 4 species, Annonaceae, Rutaceae and Fabaceae having all 2 species (Table 2).

No evident linkage was observed between the plants and a type of grain to protectthem; All the plants are collected at the period of harvesting. In the far North division with sahelian climate, the knowledge of botanical is strong and the amount of plant known by farmer as crop protectant is high. All efficient plant available is used to protect the stored crops.

Many of the plants used are annual plant or herbs, the entire plant or their leave are collected and dry. They are used intact or matched and transformed in powder. On perrenial plants, leaves or fruits are used, even if the plant is present the whole year, it is only during the period of preparation of the filling of granary the targeted parts of the plant are collected.

These plants interfered negatively with the biology of insect pests (Boeke, 2002; Boeke *et al*., 2004). They can kill the pest, reduce its development or repel it. The important number of plants cite is an expression of the difficulty peasant face during the storage of their crops.

Production of essential oils: Only 12 of the 29 plants sampled produce essential oil (Table 3). They are all aromatic plants. The treatment done by peasant with the part of the plant used showed that they are mixed with crops or put in several layers so that the aroma coming from the plant is in direct contact with the grains. This process leads to maximum efficiency of the treatment.

The essential oil extraction rate is established for each plant. *X. aethiopica* is the best essential oil producer with a rate of 7.6% followed by *L. rugosa* at 4.7%.

Aromatic plants producing essential oils are mostly Labiateae with 6 plants; Annonaceae, Asteraceae and Rutaceae having each 2 plants. More over, only 4 of these

Table 2: Aromatic plants cited with their site of utilisation, the part of the plant use and the targeted grains protected

Aromatic plant	Family	Division	Part sampled	Grains
Annona senegalensis	Annonaceae	Ad, En, No	Leaves	Ma-MS
Xylopia aethiopica	Annonaceae	Ad, En	Fruits	Ma M
Hyptis spicigera	Labiae	Ad, En, No	Entire plant	MS-C-B-V
Hyptis suavolens	Labiae	En	Entire plant	MS-C-B-V
Plecthrantus globulosus	Labiae	Ad	Leaves	MS-C-B-V
Occimum canum	Labiae	En	Entire plant	Ma-MS-C
Occimum gratissimum	Labiae	En	Entire plant	Ma-MS-C
Thelopogon elegans	Poaceae	En, No	Entire plant	Ma
Eleusine indica	Poaceae	En, No	Entire plant	Ma
Andropogon gayanus	Poaceae	En	Entire plant	Ma-MS-C
Eragrostis tiemula	Poaceae	En	Entire plant	Ma-MS
ciliaris	Poaceae	En	Entire plant	Ma-MS
Sorghum arundinaceum	Poaceae	En	Entire plant	MS
Laggera pterodonta	Asteraceae	Ad	Leaves	Ma-M
Blumea aurita	Astéraceae	En	Entire plant	Ma-MS-C
Boswellia dalzielli	Burseraceae	En	Leaves	Ma-MS-C
hirsuta	Fabaceae	En	Leaves	Ma-MS-C
Eriosena glomeratum	Fabaceae	En	Leaves	MS
Combretum glutinosum	Combretaceae	En	Leaves	MS-C
Vepris heterophylla	Rutaceae	En	Leaves	MS-C
Clausena anisata	Rutaceae	En	Leaves	Ma-M-C-S
Ipomea fistulosa	Convolvulaceae	En	Leaves	Ma-MS
Ficus Thonningi	Moraceceae	En	Leaves	Ma-MS-C
Strychnos spinosa	Loganiaceae	En, No	Leaves	MS- C
Nelsonia canescens	Acanthaceae	En	Leaves	MS
Lippia rugosa	Verbenaceae	Ad	Entire plant	Ma
Mitracarpus villosus	Rubiaceae	En, No	Leaves	MS-V
charntia	Cucurbitaceae	En	Leaves	Ma-M
Vernonia sp.	Asteraceae	Ad	Leaves	MS

Division of the country where the use is made: Ad = Adamaoua; En = Extreme North; No = North. Grains stored: Ma=maize; M=millet, S=sorghum; b = beans; C= cowpea; V = voandzeia

Table 3: Extraction rate of essential oils produced by the aromatic plants with precision on their uses in storage

Aromatic plants	Life cycle	Rate (%)	Use in storage
Annona senegalensis	Perennial	1.9	Layers
Xylopia aethiopica	Perennial	7.6	Mixture
Hyptis spicigera	Annual	0.3	Layers or mixture
Hyptis suavolens	Annual	0.18	Layers or mixture
Plecthrantus globulosus	Annual	0.52	powdered
Occimum canum	Annual	0.9	Layers or mixture
Occimum gratissimum	Annual	0.3	Layers or mixture
Laggera pterodonta	Annual	0.07	layers
Blumea aurita	Annual	0.022	Layers or mixture
Clausena anisata	Perennial	0.07	Mixture
Vepris heterophylla	Perennial	0.48	Mixture
Lippia rugosa	Annual	4.7	Layers or mixture

plants are persistant, 8 are annual herbs growing in the rainy season and sawn in the dry season at the period of filling of granaries from November to February.

Insectcidal activity of essential oils on major pests :All the essential oils obtained had not exhibit toxicity towards insects. Bio assays were carried out on the major cereal pests *S. zeamais* and *S. oryzae* with the essential oil of the 4 most common plants of the zone: *A. senegalensis*, *H. spicigera, O. canum* and *L. rugosa* (Ngamo *et al*., 2004). Table 4 precises that considering only the DL50, *O. canum* essential oil is more active on *S. oryzae*. When this dose is put in relationship with the availability of the essential oil through its rate of extraction, *L. rugosa* appears as the suitable plant to use for the control of the major cereal pests.

With the same consideration, all 8 aromatic plants producing insecticidal essential oil were tested on the major flour pest, *T. castaneum* which is also the most resistant pest to synthetic insecticides. Table 5 makes clears that *V. heterophylla* is according to the DL50 the plant producing the most active essential oil. When this is analysed in relationship with the rate of essential oil production, *X. aethiopica, V. heterophylla* and *L. rugosa* are the most active plants to use for the control of *T. castaneum*.

X. aethiopica is a very good plant in the context of crop protection using essential oil. It has the highest rate of essential extraction. Moreover its insecticidal activity on insects is greater than that of others. Another fact is that, locally, *X. aethiopica* is a sp. use by local population and also exported for pharmaceutical purposes.

Table 4: Insecticidal efficiency of essential oil of 4 most used plants of Northern Cameroon in relationship with their rate of production of the essential oil

N° Aromatic plants (R%)	Insect grain pest	DL50	DL50/R
Annona senegalensis (1,9)	*S. zeamais*	240.13	126.38
	S. oryzae	163.63	86.12
Hyptis spicigera (0,3)	*S. zeamais*	184.25	614.17
	S. oryzae	50.38	167.93
Ocimum canum (0,9)	*S. zeamais*	162.38	180.42
	S. oryzae	210.38	233.76
Lippia rugosa (4,7)	*S. zeamais*	119.63	25.45
	S. oryzae	117.00	24.89

Table 5: Insecticidal efficiency of 8 essential oils towards *Tribolium castaneum* in relationship with their rate of extraction

Aromatic plant (R%)	DL50	DL50/R
Annona senegalensis (1,9)	433.88	228.36
Xylopia aethiopica (7,6)	244.75	32.20
Hyptis spicigera (0,3)	346.50	1155.0
Ocimum canum (0,9)	88.6	98.4
Ocimum gratissimum (0,3)	210.13	700.43
Clausena anisata (0,07)	651.0	9300
Vepris heterophylla (1,12)	49.44	44.14
Lippia rugosa (4,7)	348.25	74.10

Essential oils acts on insects through their aroma compounds which are highly volatile and biodegradable. Their potential for bioaccumulation and their persistence are very low (Noudjou, 2004, Jirovetz *et al.*, 2002; Kouninki, 2005; Ngamo *et al.,* 2004). Their are therefore good alternatives products for the use of industrial pesticides with have adverse effect on consumers and on environment because of their high potential of bio accumulation.

CONCLUSION

The use of essential oils as tools for protection of stored products against the attacks of insect pests is a user friendly method. In northern Cameroon, *X. aethiopica V. heterophylla,* and *L. rugosa* are plants to promote developed and popularise for the use of their essential oil for the protection of stored grains from attacks of insect pests.

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