Research Journal of Biological Sciences 2 (1): 75-80, 2007

Insecticidal Efficiency of Essential Oils of 5 Aromatic Plants Tested Both Alone and in Combination Towards *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae)

[1]T.S.L. Ngamo, [1]I. Ngatanko, [1]M.B. Ngassou, [2]P.M. Mapongmestsem and [2]T. Hance
[1]University of Ngaoundéré BP 454, Cameroon
[2]Laboratory of Ecology and Biogeography, Research Center on the Biodiversty, 4-5 Place Croix du Sud, 1348, Louvain-la-Neuve, Belgium

Abstract: To prevent attacks of stored grains by insects pest, rural farmers in the past times, regularly introduce aromatic plants in their granaries. These plants were used alone or associated in groups of 2 or 3 plants. Active compounds of these aromatic plants, mostly present in their essential oils have insecticidal properties. In the present work, crude essential oils of some Lamiaceae, as *Hyptis spicigera* (Lam.) *Ocimum canum* (Sims) and *Plectranthus glandulosus* (Hook); of Rutaceae *Vepris heterophylla* (Letouzey) and finally of Astareceae *Echinops giganteus* (Adams) were applied both alone and in balanced combinations on the adults of stored grain pest *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) to evaluate their toxicity. These experiments show that all tested essential oils are insecticide except that of *E. giganteus*. Applied alone, their LD50 vary from 42.91 ppm for the oil of *O. canum* to 349.8 ppm for oil of *V. heterophylla*. Applied in balanced combination *O. canum* and *H. spicigera* expresses no difference ($p<0.05$) between observed (75.83 ppm) and expected (77.46 ppm) LD50. *E. giganteus* which was without any insecticidal efficiency synergise the essential oil already active. Moreover, of 20% *O. canum* in 80% of *E.giganteus* leads to 100% of mortality. While combined in this same proportion *O. canum* and *P. glandulosus* oils also express synergy. *V. heterophylla*, relatively rich in oxygenated sesquiterpenes increases values of the observed LD50 higher and significantly different ($p<0.05$) from the expected values.

Key words: Antagonism, crude essential oils, stored product, *Sitophilus oryzae*, synergy

INTRODUCTION

Cereals and legumes are the major staple crops of the northern Cameroon (Seignobos, 2002). They are generally harvested once a year at fixed period. The storage makes these crops available on markets during the whole year (Ngamo and Leonard, 2004). One of the most important post harvest activities is the storage. Many small holders stored more than 80% of their crops. During the storage losses occurred, they are due to Insects spoliation (Bell, 1994; Bell *et al*., 1998; Buchbauer, *et al*., 2000). Stored grain insect pests are numerous and highly diversify (Danho *et al*., 2000). The Insect bores in the grain and feed on the germ essentially (Dal Bello *et al*., 2002). These noxious activities generally lead to loss of agricultural, nutritional and commercial value of the grain.

To avoid loss of stored products, farmers usually have to relish for traditional tools or on synthetic insecticides. The use of chemicals to protect stored products is an increasing method. In Cameroon, the market of pesticides is open and many insecticides could be bough without restriction. Traditional tools are those coming from the know-how learned from parents and perpetuated for generations. The traditional approach is based essentially on the use of aromatic plants that serve to keep away pests attract by the crop or to kill those already in the granary (Boeke, 2001; Mohan and Fields, 2002; Boeke *et al*., 2004). Peasant also used from time to other inert substances. The introduction of aromatic plants into granaries during the storage is frequent in many localities of the northern Cameroon (Ladang, 2004). Some small holders are able to introduce up to 6 different aromatic plants in their granary during the storage most for the protection of cowpea (Szafranski, 1991; Wink, 1993; Ngatanko, 2004). The most frequent combinations of aromatic plants to protect involves *Ocimum canum, Hyptis spicigera, V. heterophylla* and *Capsicum frutescens* (Lamiri *et al*., 2001).

The combination of these aromatic plants made by peasant is to provide a synergistic effect in the protection of stored grains from the spoliation of insects. This could be understood through the analysis of the reactivity of

Corresponding Author: Léonard S.T. Ngamo, Université de Ngaoundéré, Faculté des Sciences, BP 607, Ngaoundéré

the compounds of the different active plants (Lamiri *et al.*, 2001). Many study up to now concern the insecticidal activity of the crude essential oil of aromatic plat towards stored grain insects (Essam, 2001; Lee, 2000). Moreover recent studies were carry on the combination of majors active compound of essential oils with insecticidal properties (Prates *et al.*, 1998; Kouninki, 2005). Other study on the combination of essential oils of *Ocimum gratissimum* vand *Xylopia aethiopica* (Annonaceae) in one hand, of *Lippia rugosa* (Verbenaceae) and *X. aethiopica* in other hand leaded to their antagonistic effect on the maize weevil *Sitophilus zeamais* (Coleoptera: Curculionidae) (Aoudou, 2006). A synergistic activity was recorded while considering their antifungic activity (Aoudou, 2006). The present study therefore aims to evaluate the traditional know-how through which many aromatic plants are combined in granaries to protect stored grains from attacks of insect pests.

MATERIALS AND METHODS

Insect used for the bioassays: The rice weevil, *Sitophilus oryzae* (L.) (Coleoptera : Curculionidae) used for the tests belonged to the strain collected in November 2003 in the granary of a peasant of Beka hosséré (Ngaoundéré, Cameroon). This strain referenced 24Z/LN/01 is reared in the *In vivo* collection at the Storeprotect laboratory at the University of Ngaoundéré in Cameroon.

Aromatic plants used and their sampling sites: Essential oils used for the tests were extracted from plants collected in different localities of northern Cameroon. Leaves of *V. heterophylla* were collected at Méri (Far North province) at point referenced 14°15,225'E; 10°41,039N; altitude: 496,93 m above sea level. Leaves and flowers of *O. canum* were collected at Maroua (Far North province) at the site geographically referenced 14°24,145'E; 10°39, 214 N; altitude: 375, 1m. Finally, flowers of *H. spicigera*, *P. glandulosus* and *E. giganteus* were collected at Dang (Ngaoundéré, Adamawa province) at the site geographically referenced: 13°33,549'E; 07°25,609N; 1100,5 m. These data were recorded with a GPS Garmin Geko 301.

The collection of all fresh plants materials was made in December 2005. There after the hydrodistillation was carry out through a Clevenger type apparatus. The essential oils obtained were kept alone or in combinations were obtained by mixing fixed volume of targeted oils. Each preparation was stored in a refrigerator at 4°C until use.

Bioassays: Tests were carry out to access the evaluation of the insecticidal activity of crude essential oils obtained both alone and in combination towards *S. oryzae*. Two series of combinations were made, the balanced combinations where each oil contributes for half of the volume tested (50%). The second set of combination of oils was the dilution of one in another. The participation of the essential oil to be dilute was 1; 5; 10; 20; 30 and 40% of the mixture.

In preliminary tests, 8 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 establish the LD50 of each oil.

With a micropipette (Rainin Magnetic-assist) the precise volume of essential oil was pumped and diluted into 0.5 mL of acetone. The solution aspired was uniformly flowed on a disk of filter paper (Whatman N°1) of 9 cm de diameter put into the Petri dish. An amount of 20 insects were introduced in the dish 4 min after. These insects were less than one-month old. The dish was finally covered and sealed, a control with acetone alone, without essential oil was made. For each preparation 5 replications were made. The amount of death insects were counted 24 h after the application.

RESULTS AND DISCUSSION

Toxicity of the essential oils alone: All the essential oils used expressed insecticidal activity toward the rice weevil except that of *E. giganteus* which has no toxicity. *O. canum* is the most active with a LD_{50} at 42.9ppm and *V. heterophylla* is the less active, LD_{50} is 349.8 ppm, *H. spicigera* has its LD_{50} at 112 ppm (Table 1). With the essential oil of *P. glandulosus*, the LD50 is 77 ppm.

The Table 1 releases that all the 3 Lamiaceae, *H. spicigera*, *O. canum* and *P. glandulosus* are the most efficient plants. The differences observed in the efficiency of these oils are linked to their chemical composition 3

Table 1: Insecticidal activity of the essential oil alone by contact observed 24 h after application

Preparation	LD50 (ppm)	Equation	r
Echinops giganteus	nd	nd	nd
Hyptis spicigera	112	Y= 3.64x – 2.46	0.905***
Ocimum canum	43	Y= 12.81x – 15.91	0.979***
Plectranthus glandulosus	77	Y= 6.02x – 6.36	0.85*
Vepris heterophylla	350	Y= 4.21x – 5.71	0.944***

nd = not defined

Table 2: Insecticidal activity of balanced combinations of essential oils observed 24 h after application

Balanced combinations (50% + 50%)	LD_{50} Observed	LD_{50} Expected	Khi[2]
Echinops giganteus + Ocimum canum	12.5	50	22.89***
Hyptis spicigera + Ocimum canum	77.46	75.83	0.017 ns
Plectranthus glandulosus + Ocimum canum	70.63	100	0 ns
Plectranthus glandulosus + Echinops giganteus	25	100	100***
Vepris heterophylla + Ocimum canum	103.81	195.97	28.33***
Vepris heterophylla + Hyptis spicigera	182.13	230.9	5.758*

Table 3: Insecticidal activity of mixtures made of various proportion of an essential oil of high insecticidal activity (*Ocimum canum*) dilute in that of an essential oil without insecticidal activity (*Echinops giganteus*)

	Mixture (100%)		Mortality (%)		
Dose (ppm)	*O. Canum* (%)	*E. giganteus* (%)	Observed	Expected	Khi[2]
2.25	1	99	38.75	2.5	31.86***
11.25	5	95	62.5	32.5	9.47***
22.5	10	90	65	20	23.82***
45	20	80	92.5	78.75	1.10 ns
67.5	30	70	90	86.25	0.08 ns
90	40	60	100	97.5	0.03 ns

(Regnault-Roger, *et al.*, 2002). The amount and the diversity of the molecules active on *S. oryzae* are not the same for all the oil tested. Indeed, the 3 Lamiaceae are rich in monoternes. Limonene is the most important compound in essential oil of *O. canum*. Limonene is a compound particularly toxic for *S. oryzae* (Bekele and Hassanali, 2001). This reason can explain the high toxicity of *O. canum* compare to other tested oils. Toxicity of essential oil of *Hyptis spicigera* is attributed to1,8 cineol, to carvacrol, to α-pinene and to β-pinene which are present in this oil. Toxicity of these compounds are demonstrated on Coleopteran (Kouninki, 2005). Sabinene, E-β ocimene, limonene and myrcene are the major compounds of *V. heterophylla* they are reported as active compounds on *S. oryzae* (Park *et al.*, 2003). Moreover, *E. giganteus*, a plant used by producer shows no efficiency. Indeed, essential oil of *E. giganteus* contains exclusively sesquiterpenic compounds (Menut *et al.*, 1996), in the other hand, the most efficient insecticidal effect of essential oil is due to their monoterpenes (Jirovetz *et al.*, 1997; Isman, 2000). From these experiments no insecticidal could be expected from *E. giganteus*. If its use is frequent, there is a reason for that. some important compounds are perhaps inactive by themselves and may need the presence of some other to express their activity.

Toxicity of balanced combinations of essential oils: In balanced combination the insecticidal efficiency observed was in most of the cases different from that expected. The LD_{50} values decreased in all combinations even if the differences observed are not significant. These significant reductions of LD_{50} express potentialised insecticidal efficiency of the combination. The combination *O. canum* with *E. giganteus* is the most active one with LD_{50} at 12.5 ppm. It is followed by the combination of *P. glandulosus* and *E. giganteus* with a LD_{50} at 25 ppm (Table 2). In this 2 situations, the combination of the 2 essential oils leads to an observed LD_{50} which is the quarter of the expected value. In the combination of *P. galdulosus* and *O. canum* which are the more active oils alone, the combination has no synergy. No significant difference is observed between the observed and expected LD_{50}.

The positive effect coming from the combination of these oils could be explained by the synergy in their active compounds which together put together their effect to come to most efficient activity. The interaction observed may be an expression of a quantity of active compound available. If the active compounds are the same or act in the same way the combination could have low positive effect but if the active compound are different and interact in different ways, their association may leads to high positive effect. There is a synergic effect between menthol and terpineol (Belaiche *et al.*, 1996). Moreover a synergic effect between essential oil of *O. gratissimum* and that *X. aethiopica* and between essential oil of *L. rugosa* and that of *X. aethiopica* (Aoudou, 2006). However, mechanism of this phenomena still unknown.

Toxicity of highly insecticidal essential oil diluted in a non insecticidal one: Expected mortality due to the dilution of essential oil of *O. canum* in that of *E. giganteus* is in all case lower than that observed (Table 3). More than 90% of insect mortality was observed with only 20% of *O. canum* in the mixture. Above t his proportion no more effect was observed. *E. giganteus* which alone could not induce any mortality here reacts like a synergist of *O. canum*. Insecticidal efficiency of *O. canum* is highly improved while dilute in *E. giganteus*.

The same situation was observed with *P. glandulosus* which is another very active essential oil

Table 4: Insecticidal activity of mixtures made of various proportion of an essential oil of high insecticidal activity (*Plectranthus*) dilute in that of an essential oil without insecticidal activity (*Echinops giganteus*)

	Mixture (100%)		Mortality (%)		
Dose (ppm)	*P. glandulosus* (%)	*E. giganteus* (%)	Observed	Expected	Khi^2
0.75	1	99	95.8	0	95.86***
3.75	5	95	84.06	2.5	76.85***
7.5	10	90	97.28	21.33	46.63***
15	20	80	98.53	31.40	34.68***
22.5	30	70	100	29.43	38.48***
30	40	60	100	72.98	4.22*

Table 5: Insecticidal activity of mixtures of various proportion of 2 essential oils of high insecticidal activity (*Ocimum canum* and *Plectranthus glandulosus*) dilute one in another

	Mixture (100%)		Mortality (%)		
Dose (ppm)	*P. glandulosus* (%)	*O. Canum* (%)	Observed	Expected	Khi^2
0.75	1	99	15	12.5	0.23ns
1.87	5	95	45	6.25	29.3***
3.75	10	90	48.75	10.5	24.69***
7.5	20	80	71.67	13.75	39.27***
11.25	30	70	95	42.5	20.05***
15	40	60	91.25	98.75	0.3 ns

(Table 4). At the proportion of 1%, while no mortality is expected, 95.6% of mortality was observed in the pest population. Indeed, the composition of essential oil of *E. giganteus* show presence of certains compounds named lignans (Philogene, 1991). These compounds have no important effects, but can increase biological effect of others efficient compounds when they are combined (Tene *et al.*, 2004). Therefore, this phenomenon could explain increasing efficiency of essential oil of *E. giganteus* in combinations. The beneficial effect of combining several plants in granaries in order to have full control of pests of stored products is hereby proved the last combination involved the 2 more active essential oils. *P. glandulosus* was dilute in *O. canum* in the various proportions as describe previously. Observed mortalities were in all cases greater than expected ones. Nevertheless the rate of increase was not as important as it could be imagined. At the proportion of 1% of *P. glandulosus*, the observed mortality was only 15% whereas in the combination with *E. giganteus*, this same proportion leads to 96.8% mortality. Ther is absence of synergic effect with the combination of 2 phenols (Pibiri, 2006). This explain that the combination of 2 substances of same nature or more can not produced a significant synergic effect. However, essential oil of *O. canum* and *P. glandulosus* are both rich in monoternes, that would be the reason why the synergic effect of their combination is low than their combination to essential oil of *E. giganteus*. In the combination, the 2 oils do not perform the same task (Table 5).

It is possible that in these combinations, an essential oil without any insecticidal potential could be source of synergist of active compounds. Some of these natural synergists are known as lignans. For essential oils without lignans but only with active compounds, the insecticidal effect observed is due to the combination of active compounds from both essential oils. The effect observed looks like an additive effect.

ACKNOWLEDGEMENTS

This study was carry out with a financial support of the Belgian Cooperation for Development through the convention Storeprotect, PIC 2003, Cameroon.

REFERENCES

Aoudou, Y., 2006. Essai d'optimisation de la protection des graines de maïs contre et *Sitophilus zeamais* Motsch (Coleoptera: Curculionidae) *Aspergillus flavus* Lab$_1$ avec les huiles essentielles de cinq espèces végétales. Mémoire de Diplôme d'Etude Approfondie. Département des Sciences Alimentaires et Nutrition, Ecole Nationale Supérieure des Sciences Agro-Industrielle, Université de Ngaoundéré (Cameroun).

Bell, A., 1994. Emploi des substances végétales comme produits de protection des stocks contre le grand capucin du grain (*Prostephanus truncatus)* et autres ravageurs. GTZ, Eschborn, Allemange, pp: 7.

Belaiche, T., D. Rutledge, C. Ducauze et and A. Tantaoui-Elaraki, 1996. Etude comparative des effets simultanés de trios terpènes sur trios moisissures. Sciences des aliments, pp: 16.

Bell, A., O. M,ck and H. Schneider, 1998. La protection intégrée des denrées stockées est une affaire rentable! GTZ, Eschborn, Germany, pp: 42.

Buchbauer G., L. Jirovetz, M.B. Ngassoum O. Adjoudji and L.S. Ngamo Tinkeu, 2000. Insecticidal activity and chemical composition of essential oils of *Ocimum gratissimum* and *Xylopiaaethiopica* from Cameroon. In Abstract book of the 31st International Sym.

Boeke, S.J., 2001. Traditional africain plant products to protect stored cowpeas against insect damage; the battle against the beetle. P.h. D thesis, Wageningen Uni., pp: 151.

Bekele, J. and A. Hassanali, 2001. Blend effect in the oxicity of the essential oils constituent of *Ocimum Kilimandssharicum* and *Ocimum kenyense* (Labiateae) on two post harvest insect pest. Phytochem., 57: 385-391.

Boeke, S.J., I.R. Baumgart, J.J. Loon van, A. Huis van, M. Dicke and D.K. Kossou, 2004. Toxicity and repellence of African plants traditionally used for the protection of stored cowpea against *Collosobruchus maculatus*. J. Stored Prod. Res., 40: 423-438.

Danho M., E. Haubruge, C. Gaspar and G. Lognay, 2000. Sélection des grains-hôtes par *Prostephanus truncatus* (Coleoptera; Bostrichidae) en présence de grains préalablement infestés par *Sitophilus zeamais* (Coleoptera: Curculionidae). Belg. J. Zool., 130: 30-39.

Dal Bello, G., S. Padin, C. Lopez lastra and M. Fabrizio, 2001. Laboratory evaluation of Chemical-biological control of the rice weevil (*Sitophilus oryzae* L.) in stored grains.

Essam, E., 2001. Insecticidal activity of essential oils: octopaminergic sites of action. Comparative Biochem. Physiol., Part C, 130: 325-337.

Isman, M.B., 2000. Plant essential oils for pest management. Crop Protection, 19: 603-608.

Jirovetz, L., G. Buchbauer and M.B. Ngassoum, 1997. Investigation on the essential oils of dried fruits of *Xylopia aethiopica* (West african peper tree) and *Xylopia parviflora* from Cameroon. Nutrition/ Ernährung 22 (VII-VIII), pp: 324-325.

Kouninki, H., 2005. Etude de la toxicité des huiles essentielles de quelques plantes traditionnellement utilisées au Nord Cameroun contre les Bruchidae et les Curculionidae. Mémoire présenté en vu de l'obtentuion du Diplôme d'Etude Aprofondies en biologie. Université Catholique de Louvain. Faculté des Sciences. Département de Biologie. Unité d'Ecologie et de Biogéographie, centre de recherche sur la biodiversité, pp: 82.

Lamiri, A., S. Lhaloui B. Benjilali and M. Berrada, 2001. Insecticidal effects of essential oils against Hessian fly *Mayetiola destructor* (Say). Field Crpos Res., 71: 9-15.

Lee, S.E., 2002. Biochemical mechanisms confering cross-resistance to fumigant toxicites of essential oils in a chloropyrifos-ethyl resistant strain of *Oryzaephilus surinamensis* L. (Coleoptera: Sylvanidae). J. Stored Prod. Res., 38: 157-166.

Ladang Yassi, D., 2004. Contribution à la réussite de stockage de *Penissitum glaucum* et *Sorghum bicolor* par l'analyse phénologique des infestations et l'utilisation des huiles essentielles des plantes aromatiques. Mémoire de maîtrise en Biologie et Physiologie animale. Faculté des Sciences, Département des Sciences Biologiques, Université de Ngaoundéré, (Cameroun) pp: 80.

Menut, C., G. Lamaty, P. Weyerstah, l.H. Marschall I. Seelmann and P.H. Amvam Zollo, 1996. Aromatic plants of tropical Central Africa. Part XXXI. Tricyclic sesquiterpenes from the root essential oil of *Echinops giganteus* var. *lelyi* C.D. Adams, 126: 415- 421.

Mohan S. and P.G. Fields, 2002. A simple technique to assess compounds that are reppellent or attractive to stored-product insects. J. Stored Prod. Res., 38: 23-31.

Ngatanko. I., 2004. Analyse de la gestion post récolte de *Vigna unguiculata* (Walp) (Fabaceae) et évaluation de l'importance insecticide des huiles essentielles de trois plantes aromatiques. Mémoire de Maîtrise en Biologie et Physiologie Animale, Université de Ngaoundéré, Cameroun, pp: 81.

Ngamo Tinkeu and S. Léonard, 2004. A la recherche d'une alternative aux Polluants Organiques Persistants utilisés pour la protection des végétaux. *Bulletin d'informations phytosanitaires*. n°843 Avril-Juin 2004, pp: 23.

Philogene, B.J.R., 1991. L'utilisation des produits naturels dans la lutte contre les insectes: Problème et perspectives. La lutte anti-acridienne. Faculté des Sciences, Université d'Ottawa, Ottawa, Canada KIN6N5. Ed. AUPELF-UREF, John Libbey Eurotext, Paris, pp: 269-278.

Prates, H., T. Santos, J.P. Waqui, J.M. Fabris, J.D. Oliveira, A. Forster and J.E. Embrapa, 1998. Insecticidal activity of monoterpenes against *Rhyzoperhta domonica* (F.) and *Tribolium castaneum* (Herbst). J. Stored Prod. Res., 34: 243-249.

Park, I.K., S.G. Lee and D.H. Choi, 2003. Insecticidal activities of constituents indentified in the essential oil from leaves of *Chamaecyparis obtusa* against *Callosobruchus chinensis* (L.) and *Sitophilus oryzae* (L.). J. Stored Prod. Res.

Pibiri, M., 2006. Assainissement microbiologique de l'air et des systèmes de ventilation au moyen d'huiles essentielles. hèse de doctorat, Architecture environnemental, Institut des infrastructures, des ressources et de l'environnement, section d'architecture, école polytectnique fédérale deLuzanne, ausanne rance), EPFL, 2006.

Regnault-Roger, C., B.J.R. Philogène and C. Vincent, 2002. Biopesticides d'origines végétales. Tec and Doc, (Eds.), Paris, pp: 337.

Szafranski, F., 1991. Activité biologique des extraits de quelques plantes des environs de Kinsangani (Zaïre). Belgian J. Botany, 124: 60-70.

Seignobos., 2002. Stratégies de conservation du grain. Atlas de la province de Extreme-Nord Cameroun, pp: 12.

Tene, M., P. Tane, B.L. Sondengam and J.D. Connolly, 2004. Lignans from the roots of *Echinops giganteus.* elsevier ull-text article, Phytochem., 65: 2101-5.

Wink, M., 1993. Production and Application of Phytochemicals from an Agricutural Perspective. In: Van Beek, T.A., Breteler, H. (Eds), Phytochem. Agric. Oxford, UK, 34: 171-213.