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Insecticidal Activity of Spicy Plant Oils Against *Sitophilus zeamais* in Stored Maize in Cameroon

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Abstract: *Ocimum gratissimum* L. (Lamiaceae), *Piper nigrum* L. (Piperaceae) and *Xylopia aethiopica* Dunal A. Rich. (Annonaceae) are common spicy plant species in many recipes in Cameroon. A laboratory experiment was conducted to investigate the insecticidal activities of essential oil extracts from their fresh leaves or dried fruits against *Sitophilus zeamais* Motsch. (Coleoptera, Circulionidae), the principal weevil of stored maize and grain products in the tropics. The assays were carried out using the ingestion and contact techniques. After the extraction of essential oils by hydrodistillation using a Clevenger apparatus, formulations as dustable powder were prepared at 5 and 10%. Probit analysis was employed in evaluating the LD_{50} response. Insect mortality was recorded after 24, 48, 72 and 96 h. Results showed that, significant insect mortality was obtained with all the essential oils used and all the treatments had effective insecticidal activity by ingestion and contact after 96 h compared to untreated control and pirimiphos-methyl (actellic Super 2% dust) formulations. For all the formulations tested, a significant difference ($p<0.05$) was observed between contact and ingestion assays, the contact test being the most active. The mortality rate of *S. zeamais* increased with increase in the concentration of essential oil of the three plants and the duration of exposure of the weevils on the treated substrates. By contact, the essential oil extract from *P. nigrum* fruits was the most effective insecticide (97.2±4.6%) whereas *X. aethiopica's* (97.3±3.7%) was the most effective by ingestion. Results are discussed with regard to the use of the essential oils from the fruits of these plant species by the small-scale farmers as sustainable alternatives and maize grain preservatives against *S. zeamais* during storage to synthetic insecticides. The potential of these non toxic products to protect stored maize against attacks of *S. zeamais* is hereby appraised.

Key words: Maize, *Sitophilus zeamais*, essential oils, insecticidal formulations

INTRODUCTION

Insects are major pests of cereals in storage; they are particularly susceptible to cause deterioration of a whole stock of maize in 8 or 10 months (Bell, 1994a). Insects such as weevils and more especially the genus *Sitophilus zeamais* (Motsch) are currently observed in the maize stocks. The maize weevil, *S. zeamais* (Motsch) is a serious pest of stored maize, causing considerable losses (Prempen, 1971; Okoro *et al.*, 1992; Hidalgo *et al.*, 1998; Obeng-Ofori and Amiteye). The popular manner to avoid post harvest losses due to this pest is, the use of synthetic insecticides. The associated detrimental effects on the environment and health, development of genetically resistant strains, erratic supply and prohibitive costs have become a major concern and thus given impetus to the search for alternative methods of pest control to reduce the use of synthetic insecticides (Schmutterer and Ascher). An alternative was pointed out with the use of natural products as pesticides to control pests (Szafransici *et al.*, 1991). In fact, plant products have played an important role in the traditional methods of protection against crop pests and disease vectors in Africa (Stollg 1988, Poswal and Akpa, 1991). Moreover, some of these natural products protect grain without any observed effects on their germination organoleptic properties (Bell, 1994a, 1994b, Kethar, 1986). The use of locally available vegetable oils as grain protectant is an old practice (Pereira, 1987). In recent years, research on the efficacy of the use of vegetable oils as stored-grain protectants against insects has been intensified (Don-Pedro, 1987, 1989; Kumar and Okonron kwo, 1991;

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Jackai, 1993; Pacheco *et al.*, 1995; Obeng-ofori, 1995; Obeng-ofori and Rechmuth, 1999). In addition, most of the essential oils used in crop protection are extracts from plants formerly known to have insecticidal activity. A popular figure is that of neem seeds (*Azadirachta indica*) and cotton seeds-*Gossypium arboreum* (Schmutterer and Ascher, 1986). Essential oils of aromatic plants and spices are tested in this respect, thus to have an edible protectant for human beings or animals.

The present research was aimed at investigating the insecticidal effects of essential oils extracted from *Ocimum gratissimum*, *Piper nigrum* and *Xylopia aethiopica*, spicy plants of Cameroon. The possibility of using essential oils from these plants as main tools for the protection of maize grains was discussed.

MATERIALS AND METHODS

Insect rearing: *S. zeamais* was used in this study. This insect was cultured in the laboratory and maintained at 26±2°C and 65-70% R.H in the dark. One hundred adults of mixed sex of this insect were obtained from a laboratory stock culture and reared on 500 g of maize in glass jars; the food media used were the whole maize grains. After 3 weeks of oviposition, the parent adults of *S. zeamais* were removed by sieving the grains with a siever (mesh size 2.0 mm) in order to obtain a homogeneous insect population. The beetles that subsequently emerged were transferred to another jar for further rearing. Seven days after the transfer, the jars were sorted again to obtain fully grown adult insects of, at most seven days old. Then they were used for different bioassays.

Extraction of essential oils: Plants used were previously washed and drained (leaves) or sorted and finely ground to powder (fruits). There were *Ocimum gratissimum* L. (Lamiaceae), *Piper nigrum* L. (Piperaceae) and *Xylopia aethiopica* fruits (Annonaceae). The essential oils of these plants were extracted by steam distillation in a Clevenger type apparatus for 4 h. The oil extracts were dehydrated with anhydrous sodium sulphate and then stored at 4°C in a refrigerator until used. The concentration process yielded 0.44% (v/wt) for *O. gratissimum* L., 3.61% (v/wt) for *P. nigrum* L. and 1.35% (v/wt) for *X. aethiopica.*

Preparation of insecticide formulations: The insecticidal formulations were prepared according to (Keita and Schmitt). It was a powder coming from a mixture of essential oil with kaolin in the ratio of 10^{-4} g of essential oil and a gram of kaolin dust. For the different tests, two doses of the oils (5 and 10%) were formulated as powder. These concentrations were calculated as the ratio between the relative amount of the quantity of the mixture and the quantity of maize to treat.

Treatment procedure: An amount (20 g) of clean maize grain was put in a plastic flask of 25 mL on this maize stock, adults of *S. zeamais* were studied. Two types of test were made: ingestion test or consumption and contact test or direct application.

Evaluation of the insecticidal activity of the oils by ingestion: Toxicity by consumption was assessed by putting each insecticide in the maize container, after the homogenized insects were added to the container. This rearing stayed for 7 days and initial observation was made to assess the mortality rate after 24, 48, 72 and 96 h of exposure to the treated grains before carrying out a cumulative mortality. The same actelic was used as reference insecticide.

Evaluation of the insecticidal activity of the oils by contact: Toxicity by contact was assessed by direct application or the knock down effect of the oils. A mixture was put in a plastic flask and the insects were allowed to be in direct contact with the product for five minutes. After this period, insects were removed and transferred on cleaned maize in flask. Insect mortality rate was noted after 48 and 96 h of exposure to the treated grains. A control was made with actelic, an organophosphorous synthetic insecticide commonly used in Cameroon to control *S. zeamais* in stored maize.

In each of the two experiments, the groups of 20 adults' weevils were introduced in the plastic flask which was covered with a piece of cloth and held with a rubber band. Five replicates were set up for each oil and control. Each treatment had five replicates and was made for all the essential oils and actelic. Insect mortality rate (in %) was calculated for each dish. Mortality was corrected for the natural mortality using the formula proposed by Abbot (1925).

Data analysis: Mortality rate (in %) during experience was transformed into Probit analysis for the determination of LD_{50} (Finney, 1971). Mortality data obtained in the different treatments were analysed using Analysis of Variance (ANOVA) by the statistical programme SAS, to determine if there were statistical differences between treatments in both experiments.

RESULTS AND DISCUSSION

Mean mortality rate was used for comparism. The cumulative mean mortality rate (in %) were adjusted using

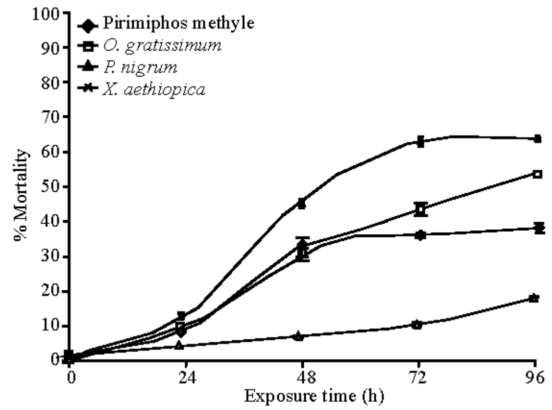

Fig 1: Effect of each substance on the mortality of *S. zeamais* adults in maize treated with 5% of concentration (ingestion test)

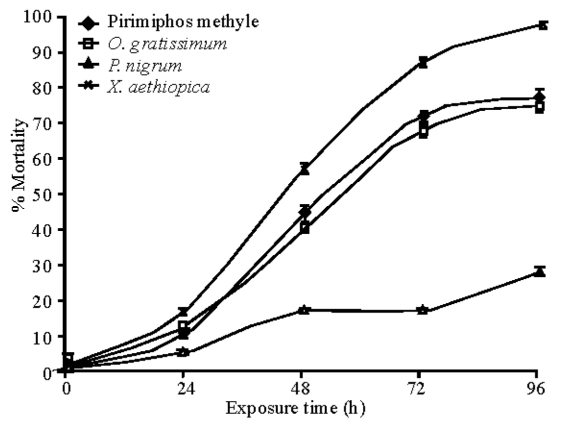

Fig 2: Effect of each substance on the mortality of *S. zeamais* adults in maize treated with 10% of concentration (ingestion test)

Abbot's correction. Figure 1 and 2 represented the mortality rates after consumption of *S. zeamais* with respect to the concentrations (5 and 10 %) of the essential oils and pirimiphos-methyl. The mortality rate of *S. zeamais* increased with the exposure time and increase in the concentration of the essential oils from the three plants and methyl- pirimiphos.

X. aethiopica and *O. gratissimum* had higher mortality rates (63 and 53 %) recorded respectively) than the control (37%) with 5% of essential oil; this was not the case of essential oil from *P. nigrum (*16 %) (Fig. 1). The essential oil from *X. aethiopica* exhibited the best insecticide activity by consumption test, with a mortality rate of 97.3 % recorded after 96 h of exposure at 10 % of concentration (Fig. 2). The mortality rate of *S. zeamais* by contact also increased with the exposure time and the concentrations of the essential oils (Table 1). Unlike in the

Table 1: Insecticidal effects by contact of essential oils from *O. gratissimum, P. nigrum* and *X. aethiopica* on *S. zeamais* at various doses (5 and 10 %)

	Exposure time (h)			
	48		96	
	Concentrations (%)			
Essential oils	5	10	5	10
O. gratissimum	66.1±5.8*	76.9±5.0*	85.9±6.9*	85.7±5.3*
P. nigrum	88.5±6.8*	95.6±5.0*	92.7±5.8*	97.2±4.6*
X. aethiopica	71.4±6.4*	86.8±5.9*	85.0±4.7*	90.1±6.7*
Control (actelic)	59.7±3.1*	69.8±2.9*	75.5±2,2*	83.1±1.9*

*: percentage of mortality

Table 2: LD_{50} calculated for mortality after 96 h of exposure of *S. zeamais* Motsch. While experiments with each essential oil

Essential oil	Ingestion	Contact
O. gratissimum	75.15	48.55
X. aethiopica	83.19	45.59
P. nigrum	16.95	22.89

LD_{50} is the lethal concentration of essential oil which kills 50 % of the number of insects used for each essay

case of consumption, the essential oil from *P. nigrum* showed the highest insecticidal activity with a maximum mortality rate of 97.2 % recorded after 96 h of exposure at 10 % of concentration. Furthermore, all the essential oils tested exhibited a higher insecticidal activity by contact than the control (actelic). For all the plant materials tested, the results of probits analysis (Table 2) which determined LD_{50} showed that the mortality rates obtained by consumption were higher than those obtained by direct application. This means that we needed a smaller concentration of essential oil by contact test than by ingestion test. The probits analysis confirmed the results obtained with both. So the contact test used, on *S. zeamais* Motsch., was more efficient than the ingestion test. Moreover, statistical analysis showed that there was a significant difference ($p<0.05$) between the two types of test.

Insecticidal activities of essential oils from various plants have been demonstrated in several previous studies (Irovetz *et al*., 1997, 1998; Bouda *et al*., 2001; Toppndiou *et al*., 2005). Although their mode of action is not clearly understood, it has been suggested in the case of vegetable oils that, insect death is caused due to anoxia or interference in normal respiration resulting in suffocation (Don-Pedro, 1989; Schoonhoven, 1978). Some oils act as grain surface protectants, preventing stored products from any insect attacks (Schmutterer and Ascher, 1986). The toxic effects of phytochemicals on *S. zeamais* depend on several factors among which are the chemical composition of crude oil and insect susceptibility (Tapond *et al*., 2005). The analyses of the chemical composition of these 3 essential oils have been done (Jirovetz *et al*., 1997; 1998; Gopal *et al*., 1993).

The components like monoterpens and terpinenes (1,8-cineol, pinenes and limonene) which have an activity on insects (Jirovetz *et al*., 1998) have been found in those 3 essential oils. However, essential oils have a flaw on preservation. It would be important to maintain them in darkness for avoid oxidation in the light and it would be also important to maintain them in a saturated atmosphere (Valnet, 1998). The fruits of *X. aethiopica* and *P nigrum* and the leaves of *O. gratissimum* are common household spices (easily use during cooking of the foodstuffs) and medicines in many traditional African communities (Bauer *et al*., 1990). To prepare them as pesticide dust by simple drying and grinding is relatively cheap and practical. They can, therefore be easily accepted by rural communities as cheaper and safer alternative to the more expensive and often hazardous conventional grain protectants. They also possess potentials for the manufacture of ecologically sound pesticides for the pollution-conscious industrialized world (Okoro *et al*., 1992). In other words, these oils could be utilized in association in pest management against insects feeding on grains or insects present within the stock.

CONCLUSION

The application of essential oil insecticide mixtures may reduce the use of synthetic insecticides and hence reduce health hazards to applicators and consumers. In this regard, the trials carried out showed that *X. aethiopica* and *P. nigrum* have significant insecticide activity by consumption and contact respectively. Treatment of grains with these essential oil mixtures could have important practical applications in area of the world where insecticides are expensive, in short supply and essential oils are readily available. This has an important practical implication in the search for and use of plants for pest control. Further studies will be carried out to assess the activity of the entire plants dusts.

REFERENCES

Abbot, W.S., 1925. A method of computing the effectiveness of an insecticide. J. Econ. Entomol., 8: 265-267.

Bauer, K., D. Garbe and H. Surburg, 1990. Common Fragrance and Flavour Materials. (2nd Ed.), VCH 30, pp: 297-301.

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

Bell, A., 1994b. Moyens et méthodes traditionnels de protection des stocks. Bulletin d'information, GTZ, Eschborn., pp: 21.

Bouda H., L.A. Tapondjou, D.A. Fontem and M.Y.D. Gumedzo, 2003. EEffect of essential oils from leaves of Ageratum conyzoides, Lantana camara and Chromolaena odorata on the mortality of Sitophilus zeamais (Coleoptera, Curculionidae). J. Stored Prod. Res., 37: 103-109.

Don-Pedro, K.N., 1987. Insecticidal activity of plants oils against stored product pests. Ph.D. Thesis, University of London, pp: 135.

Don-Pedro, K.N., 1989. Mechanisms of action of some vegetable oils against Sitophilus zeamais (Motsch.) (Coleoptera: Curculionidae) on wheat. J. Stored Prod. Res., 25: 217-223.

Finney, D.J., 1971. Statistical Method in biological Assay. 2nd. Griffin, London.

Gopalakrisnan, M., Nirmala-Menon, K.P. Padmakumari, A. Jayalekshmy and C.S. Narayanan, 1993. GC analysis and odor profiles of four new Indian genotypes of *P. nigrum* L. J. Essentia Oil Res., 5: 247-253.

Hidalgo, E., D. Moore and G. Le Patourel, 1998. The effect of different formulations of Beauvera basssiana on Sitophilus zeamais in stored maize. J. Stored Prod. Res., 34: 171-179.

Jackai, L.E.N., 1993. The use of neem in controlling cowpea pests. IITA Research Report, 7: 5-11.

Jirovetz L., G. Buchbauer and M.B. Ngassoum, 1997. GC-MS analysis of essential oils from Cameroonian plants used as spices in local foodstuff. Recent res. Devel. In: Agric. Food Chem., 1: 241-255.

Jirovetz L., G. Buchbauer and M.B. Ngassoum, 1998. Aroma compounds of leaf and flower essential oils of the spices plant *Ocimum gratissimum* L. from Cameroon. Ernährung-Nutiti., 22: 395-397.

Keita, S.M., A. Belanger and J.P. Schmitt, 1999. Use of Ocimum in Cowpea Protection in Storage Against Bruchids in Guinea. 30th Int. Symp. Essential Oils (ISEO), Bell Flavors and Fragrances, Leipzig (Germany), pp: A-14.

Kethar, C.M., 1986. Use of three derivated non-edible oils surface protectants of stored legumes against *Callosobruchus maculates* and *C. chinensis*. Proc. 3rd neem Conf., Nairobi Kenya, pp: 535-542.

Kumar, R. and N.O. Okonronkwo, 1991. Effectiveness of plant oils against some Bostrychidae infesting cereals in storage. Insect Science and its Application, 12: 77-85.

Obeng-Ofori D. and C. Reichmuth, Plant oils as potentiation agents of monoterpenes for protection of stored grains against damage by stored products beetle pests. Int. J. Pest Manag., 45: 155-159.

Obeng-Ofori D. and S. Amiteye, 2005. Efficacity of mixing vegetable oils with Plirimiphos-methyl against the maize weevil, *Sitophilus zeamais* Mostchulsky in stored maize. J. Stored Prod. Res., 41: 57-66.

Obeng-Ofori, D., 1995. Plant oils as grain protectants against infestations of *Cryptolestes pusillus* and *Rhyzopertha dominica* in stored grain. Entomologica Experimentalis et Applicata, 7: 133-139.

Okoro, O.F.N., L.C.N. Madubuike and O.O. Perpetua, 1992. Protection of stored maize *Zea mays* L., against *Sitophilus zeamais* L. with non toxic natural products: Potentials of *Xylopia aethiopica* and *Piper guinense*. Acta Agronomica Hungarita, 41: 131-135.

Pacheco L.A., M.F.P.P.M. De Castro, D.C. De Paula, A.L. Lourencao, S. Bolonhezi and M.K. Barbieri, 1995. Efficacy of soybean and castor oils in the control of *Callosobruchus maculatus* (F.) and *Callosobruchus phaseoli* (Gyllenhal) in stored chick-peas, *Cicer arietinum* (L.). J. Stored Prod. Res., 31: 221-228.

Pereira, J., 1983. The effectiveness of six vegetable oils as protectants of cowpeas and bambara groundnuts against infestation by *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae). J. Stored Prod. Res., 46: 25-30.

Poswal, M.A.T. and A.D. Akpa, 1991. Current trends in the use of traditional and organic methods for the control of crop pests and diseases in Nigeria. Trop. Pest Manag., 37: 329-333.

Prempeh, H.R.B.A., 1971. Maize crop: Harvesting, processing and storage in Ghana. Maize the Wonder crop. Symposium U.S.T., Kumasi, pp: 18-87.

Schmutterer, H. and K.R.S. Ascher, 1986. Natural pesticides from the neem trees (*Azadirachta indica* A Juss) and other tropical plants. Proc. 3rd Neem Conf. Nairobi., pp: 517-523.

Schoonhoven, A.V., 1978. The use of vegetable oils to protect stored beans from bruchid attack. J. Econ. Entomol., 71: 254-256.

Stoll, G., 1988. Natural Crop Protection Based on Local Farm Resources in the Tropics and Subtropics. (3rd Edn.), Josef Margraf, Langen. Gernany, pp: 217.

Szafranski, F., E. Bloszyk and B. Drozdz, 1991. Activité biologique des extraits de quelques plantes des environs de Kisangani (Zaïre). Belg. J. Bot., 124: 60-70.

Tapondjou L.A., C. Adler, D.A. Fontem, H. Bouda and C. Reichmuth, 2005. Bioactivities of cymol and essential oils of *Cupressus sempervirens* and *Eucalyptus saligna* against *Sitophilus zeamais* Motschulsky and *Tribolium confusum* du Val. J. Stored Prod. Res., 41: 91-102.

Valnet, J., 1998. Aromathérapie: Traitement de maladie par les plantes. (10th Édn.), Maloine. A. Paris (France), pp: 639.