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Full Length Research Paper

Persistent effect of a preparation of essential oil from *Xylopia aethiopica* against *Callosobruchus maculatus* (Coleoptera, Bruchidae)

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Cowpeas, *Vigna unguiculata* (Walp), are commonly attacked by *Callosobruchus maculatus* (Coleoptera, Bruchidae) during storage. Current methods to prevent losses during storage involve synthetic insecticides. Although they have insecticidal efficiency, these products have adverse effects on consumers and the environment. Plant products such as those of *Xylopia aethiopica* (Annonaceae) are potential sources of natural insecticides. This study assessed the efficiency of a preparation of the essential oil of *X. aethiopica* mixed with its powdered fruits in order to increase the persistence of its effect against *C. maculatus*. The persistence relates to toxicity against adults, the prevention of F1 emergence, oviposition and the development of eggs laid by *C. maculatus*. Four treatments were compared: 1) control, consisting of 200 g of grain, 2) 200 g of grain mixed with 2 g of powder from *X. aethiopica*, 3) 200 g of grain mixed with 0.4 ml of essential oil, and finally 4) 200 g of grain mixed with 2 g of powder plus 0.4 ml of essential oil. The results showed that, with powder alone, crude essential oil, and powder imbibed with essential oil, the toxicity lasted respectively for 3, 9 and 12 days ($p<0.05$). The persistence of the effect of treatment with *X. aethiopica* powder plus essential oil on the mortality and oviposition of *C. maculatus* was better than that of the treatment with the crude essential oil ($p<0.05$ until day 18). Moreover, the *X. aethiopica* powder enriched with essential oil provoked, after 52 days, 100% of development inhibition. The hatching of eggs of *C. maculatus* was also inhibited by all the formulations except the control. The authors results indicate that protection of cowpeas against *C. maculatus* may be possible using a formulation based on the dry fruits of *X. aethiopica*; the powder may increase the persistence of the essential oil.

Key words: *C. maculatus*, *Xylopia aethiopica*, persistence, toxicity, essential oil.

INTRODUCTION

Callosobruchus maculatus Fabricius (Photo 1) infest cowpeas in the field and during storage (Delobel and Tran, 1993; Stoll, 2000). This pest of stored grain is the major cause of loss during cowpea storage and contributes to the deterioration of the quality and quantity of stored grains (De Luca, 1979; Delobel and Tran, 1993; Fleurat-Lessard, 1994). Synthetic insecticides are used currently to control this pest species. These synthetic insecticides, in spite of their efficacy, have well known negative effects on the environment and on non-target species, and represent a potential risk for consumers.

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Photo 1. *C. maculatus* adult used for the experiment.

Photo 2. Dried fruits of *X. aethiocica* used for the experiment.

The use of locally available plant material to protect stored products against insect damage may be an alternative to synthetic pesticides. In Africa in general, and in Cameroon in particular, farmers currently use various local plants to protect their crops during storage (Keita et al., 2000; Boeke et al., 2004; Ngamo et al., 2007a). The essential oils from these common local plants are an important source of bioactive chemicals for the control of insect grain pests (Shaaya et al., 1997; Prates et al., 1998; Bekele and Hassanali, 2001; Park et al., 2003).

The insecticidal properties of active compounds of various local aromatic plants have been studied for insect grain pests (Hamraoui and Regnault-Roger, 1997; Stoll, 2000, Paranagama et al., 2003; Kouninki et al., 2005; Shaaya and Kostyukovysky, 2006; Kouninki et al., 2007a). Essential oils from these aromatic plants are generally naturally volatile complex mixtures of organic compounds that give the specific odour and flavour of a plant. The essential oils from these aromatic plants may not present important risks for the environment because of their specificity for insects, their high volatility and finally their low persistence (Isman, 2002; Shaaya and Kostyukovysky, 2006). The insecticidal properties of *Xylopia aethiopica* Dunal (Photo 2) from Cameroon have been investigated against Sitophilus zeamais Motsch., Tribolium castaneum Herbst and *C. maculatus* (Ngamo et al., 2001; Jirovetz et al., 2005; Kouninki et al., 2005 Kouninki et al., 2007a). However, until now, to our knowledge, no preparations have been investigated that improve the persistence of these essential oils (Kouninki et al., 2005). *X. aethiopica* is a tall aromatic tree, with fairly smooth grey–brown bark (Verdcourt, 1971), which is commonly found in the forest gallery along rivers in several localities of the Adamawa province of Cameroon. The fruits are aggregated, and composed of a minimum of 5, but more usually from 16 to 24, drupeous monocarps. The dried fruits are currently used as a spice in Northern Cameroon (Kouninki et al., 2007a). Because of its high volatility, the practical use of the essential oil of *X. aethiopica* as a natural insecticide is limited owing to its short-term effect. To improve its persistence and utility, the preparations need to be easily accessible and inexpensive for farmers. In this context, the aim of this study was first to analyse the insecticidal activity of the powder from the fruits of *X. aethiopica* mixed with its essential oil against adults of *C. maculates*. This was an attempt to increase the persistence of the essential oil to give long-term protection of cowpea grains. The second aim was to evaluate the impact of preparations of *X. aethiopica* on the emergence of the F1 generation of *C. maculatus.* The third was to record the impact of these preparations with respect to the time of oviposition and the development of eggs laid by *C. maculatus*. The effect of an insecticidal formulation may vary with the stage of insect development.

MATERIALS AND METHODS

Plant material and extraction of essential oil

Dried fruits of *X. aethiopica* were purchased in various local markets of Pangar and Ngaoundéré in the Adamawa province of Cameroon. The fruits of *X. aethiopica,* after crushing, were submitted to hydrodistillation for four hours using a Clevenger apparatus. The essential oil obtained was dried over anhydrous sodium sulphate and conserved at 4°C in a refrigerator.

The insects

The strain of *C. maculatus* used was obtained from infested cowpeas collected from farm granaries in Cameroon in 2003. These insects were first identified at the University of Ngaoundéré (Cameroon) and confirmed by a specialist at the Catholic University of Louvain in Belgium. The pest was reared in cowpea seed, *Vigna unguiculata*, at 28°C in the laboratory of the Unit of Ecology and

Table 1. Experimental protocol and days on which parameters were recorded.

Experiment	Days						
Preparation of treatments	**0**						
Insect introduction	0	3	6	9	12	15	18
Mortality observation Oviposition (observation of egg laying)	3	6	9	12	15	18	21
Emergence (F1) observation	43	46	49	52	55	58	61

Biogeography, Research Center on Biodiversity at the Catholic University of Louvain, Belgium. Newly emerged adults were used for the tests.

Powder and essential oil mixture and bioassays involving *C. maculatus*

A powder was obtained from dry fruits of *X. aethiopica* by crushing the fruits in an electric machine (Brand name: Industex, SL. Model Wonder max; Rating 230 BAC/50 Hz, 3000 w) and sifting with a mesh sieve with pore size less than or equal to 1 mm. A preliminary test was done in order to determine the optimum quantity of oil and powder to use for our experimentation.

Four treatments were prepared:

1) The control, which consisted of 200 g of grain without *X. aethiopica* powder.
2) The treatment of 200 g of grain with 2 g of *X. aethiopica* powder (1% of powder, weight of powder per weight of grain).
3) The treatment of 200 g of grain with 400 µl of *X. aethiopica* essential oil diluted in 6 ml of acetone. The evaporation of acetone lasted for 5 min.
4) The treatment of 200 g of grain with 2 g of the powder imbibed with 400 µl of the essential oil of *X. aethiopica.*

Given that, under natural conditions, granaries are not hermetically sealed, small holes were made in the flask covers and the samples were maintained at 28°C and 65% relative humidity.

Persistence of insecticidal efficiency of *X. aethiopica* against *C. maculatus*

Flasks containing 200 g of cowpea seeds were treated according to the three protocols described below. For each treatment (including the control) 56 flasks were used. On day 0, after treatment, 20 pairs of 2-day-old adults of *C. maculatus* were introduced to eight flasks for each treatment. In addition, eight flasks for each treatment were infested by 20 *C. maculatus* pairs 3, 6, 9, 12, 15 or 18 days after treatment (Table 1). In each flask, the mortality of *C. maculatus* was recorded 3 days after its introduction. All individuals that did not react after several touches with clamps were considered to be dead.

Effect of *X. aethiopica* formulations on the oviposition and emergence of *C. maculatus*

After the evaluation of mortality in each treatment group, batches of 20 g of grain were weighed for each replication on the same day and removed from the flask. In these batches, each grain was observed under a binocular microscope to determine the weight of seeds on which eggs of *C. maculatus* were present. The percentage of grain showing eggs of *C. maculatus* was evaluated as:

% of grains with eggs = (We / Wi) X 100.

Where

We= Weight of grain with eggs
and
Wi = Weight of the grain used initially in each test (20 g).

The number of insects that emerged for the first generation (F1) was counted 40 days after recording of the F0 mortality.

Effect of *X. aethiopica* essential oil formulated on development of *C. maculatus*

Twenty pairs of *C. maculatus* were introduced into flasks containing 200 g of cowpeas. Two days later, *C. maculatus* pairs were removed. The numbers of emerging adult of *C. maculatus* were counted after 40 days. This delay of 40 days is needed to allow the complete development of *C. maculatus* from eggs to adults. The effect of the four treatments on the development of *C. maculatus* was calculated as follows:

T= (N / Nt) x100

Where

T: Percentage of adults emerging from a treatment
Nt: Total number of adults emerging from all the treatments
N: Number of adults emerging from each treatment.

DATA ANALYSES

The PROC GLM MODE (Generalized Linear Models) in SAS was used to compare the data obtained, after logarithmic transformation. This procedure was used to select the best model for the treatment of our data. The best model is the one which has the lowest AIC (Akaike Information Criterion). In our case, the best model included data on the treatments and the delay in introduction of the insects. The data obtained on the treatments and the delay of insect introduction was compared using two by two tables; a significant test indicates the heterogeneity of our treatments. Treatment means were compared and separated using the GLM procedure at p = 0.05. Data on the percentage of adult mortality (F0), the mean number of individuals that emerged in the first generation (F1), and the proportion of grain with traces of eggs laid

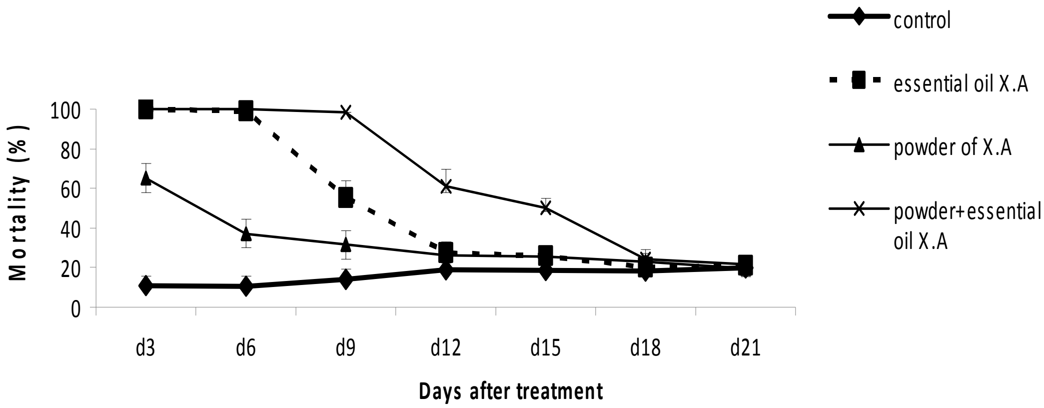

Figure 1. Mortality of *C. maculatus* in relation with the day of its introduction after treatment of grain with *X. aethiopica* formulated.

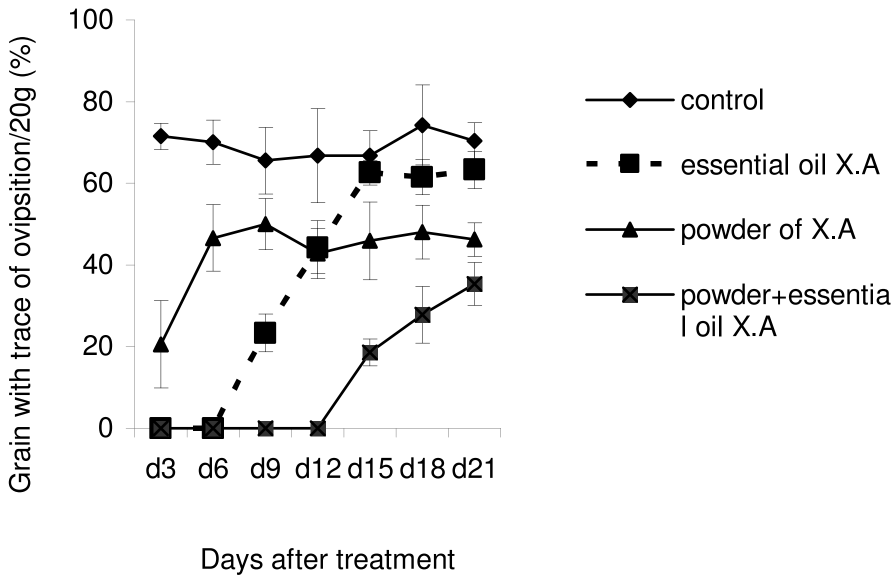

Figure 2. Impact of *X. aethiopica* (X.A) formulated on the oviposition of *C. maculatus* in relation with the day of its introduction.

(oviposition) were used to produce all the figures in the following results.

RESULTS

Persistence of the insecticidal activity of *X. aethiopica* formulations against *C. maculatus*

The percentage of F_0 mortality according to the delay in the introduction of *C. maculatus* after application of the treatments is given in Figure 1. The percentage mortality in the control remained low, although it increased slightly with the delay, probably owing to uncontrolled changes in the conditions of insect rearing. The essential oil and essential oil plus powder led to 100% mortality until 6 and 9 days after treatment. Then mortality decreased rapidly to near control values after 12 days for the oil alone but only after 18 days for oil mixed with powder. Treatment with powder only was less efficient; only 66% mortality was recorded 3 days after introduction of the insects and this reached the control value only on day 12 . There was a significant difference among the treatments ($p<0.0001$). The difference observed for the powder enriched with the essential oil of *X. aethiopica* was significant until day 15, when compared with the other treatments ($p<0.05$).

Consequences for eggs laid by *C. maculatus*

The percentage of grain with eggs of *C. maculatus* for each treatment is presented in Figure 2. With no treatment,

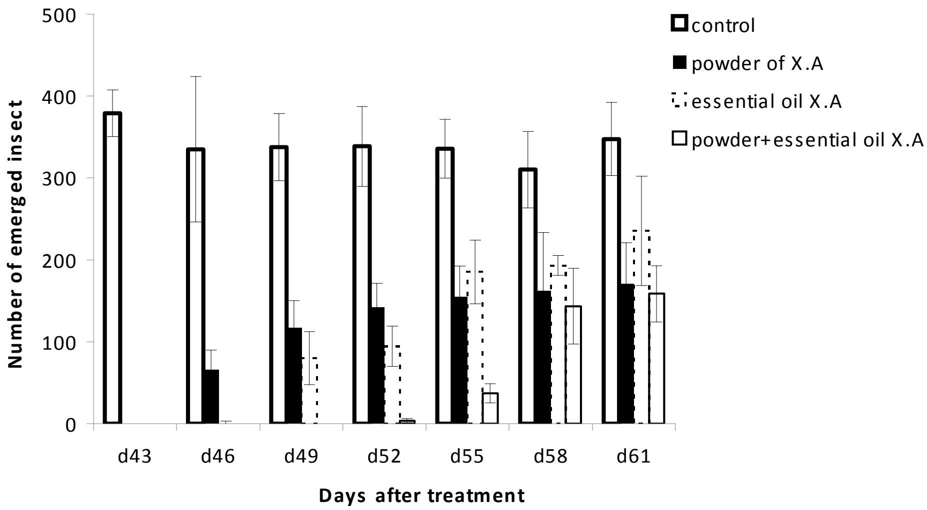

Figure 3. Impact of the efficiency of persistence of a preparation of *X. aethiopica* (X.A) on the emergence of *C. maculatus.*

70% of the grain carried eggs in the control. This value remained constant during the 21 days of observation. Eggs appeared on grains that were treated with the crude essential oil of *X. aethiopica* on day 9 only and reached a level only slightly less than that observed in the control on day 21. When grain was treated with the oil mixed with the powder from *X. aethiopica,* the first eggs were recorded on day 15 and the percentage of grain with eggs reached only half that of the control on day 21. Powder alone also had an impact on egg laying and reduced the percentage of grain with eggs throughout the experiment. The treatments had significant effects on oviposition in *C. maculatus* ($p<0.0001$

Effect on F1 *C. maculatus*

The effect of each treatment on F1 is presented in Figure 3. The number of F1 adults of *C. maculatus* that emerged 40 days after the introduction of the insect was constant for each day and reached an average for the control of 310.1±46.5 to 379.2±28.5 by day 61. With the powder alone, the first adults appeared only after 46 days and the number remained less than for the control on day 63. The first adults in the treatments with essential oil and essential oil plus powder were recorded only after 49 and 52 days, respectively, and the number remained relatively low. There was a significant relationship between the delay in the introduction of *C. maculatus* and its emergence ($p<0.0001$).

Effect of *X. aethiopica* essential oil and powder on the development of *C. maculatus*

The Figure 4 shows the number of adult *C. maculatus* produced when eggs were hatched under the different treatments with *X. aethiopica.* It is clear that in the control, the percentage of F1 that emerge is higher than in samples treated with powder or essential oil; a strong reduction was observed with the essential oil treatment. The powder from *X. aethiopica* and powder plus essential oil did not allow any development, which shows the interesting potential of the powder itself in control of egg development. There was a significant difference among the data obtained for all the treatments ($N = 32$; $F = 74.82$; $p<0.0001$).

DISCUSSION

Past investigations on the essential oils of several aromatic plants have proven their toxicity towards insect grain pests on many occasions (Shaaya et al., 1997; Prates et al., 1998; Bekele and Hassanali, 2001; Huang et al., 2002; Park et al., 2003, Kouninki et al., 2005; Kouninki et al., 2007a). Very few studies exist of the persistence and the possibility of increasing the duration of the beneficial activities of these essential oils (Kouninki et al., 2005; Ngamo et al., 2007b). The results show that the powder from *X. aethiopica* mixed with the essential oil of the same plant has the potential to give longer-term protection of grain against *C. maculatus.* Moreover, the powder itself presents a high inhibitory potential for the development of eggs to adults. Considering the low activity of the powder, this is probably due to the volatility of the essential oil.

The essential oil of *X. aethiopica* is already known for its insecticidal activity (Ngamo et al., 2001; Jirovetz et al., 2005; Kouninki et al., 2005 ; Kouninki et al., 2007b). The effect on mortality, oviposition, emergence and egg hatching is related to the terpenic composition of the

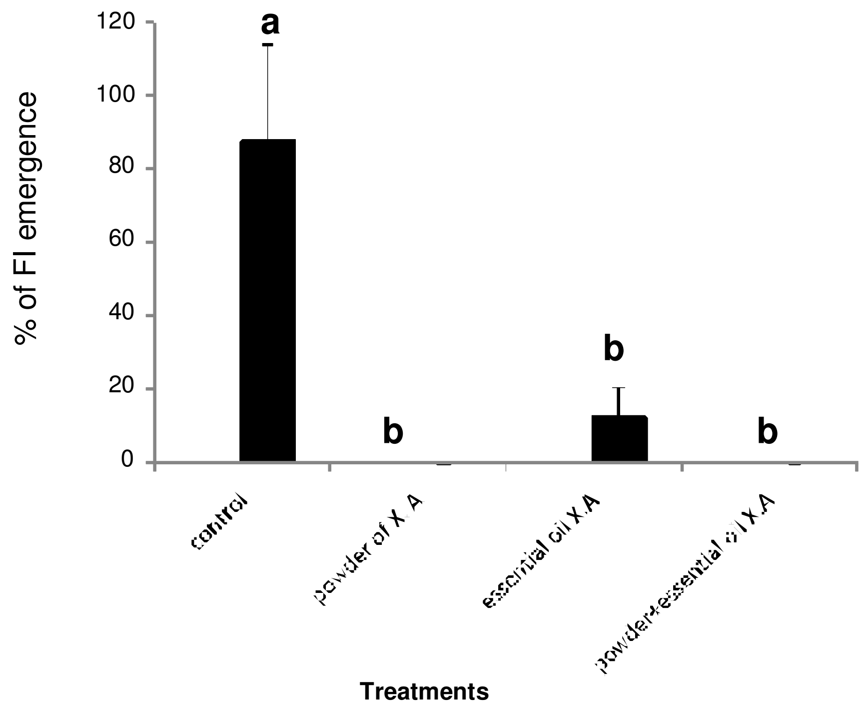

Figure 4. Impact of *X. aethiopica* formulated on the development of eggs laid by *C. maculatus* after 40 days.

applied treatments, as shown by Kouninki et al. (2007a) with *S. zeamais*. In that case, β-pinene and terpinene-4-ol were responsible for 50% of the insect mortality observed. A combination of α-pinene + β-pinene + Δ-3 carene + terpinene-4-ol, four terpenes present in the essential oil of *X. aethiopica,* could lead to 96% of mortality of *S. zeamaïs*. These compounds are particularly volatile and this may explain the decrease in insecticidal activity of the essential oils with time. This possibility has been already stated by several authors (Hamraoui and Regnault-Roger, 1997; Isman, 2002; Shaaya and Kostyukovysky, 2006). The mix of powder and essential oil probably increases the persistence of the insecticidal activity for two reasons. First, because the powder itself contains on average 4% of essential oil, the concentration of the active principle increases when both are mixed. Second, lipids or other components present in the fruit powder may adsorb the terpens, slowing down their release in the flask.

It also seems that these essential oil preparations may influence oviposition and egg survival, because the treatments have consequences on emergence of the next generation of insects. Plant compounds in general, and the essential oils of aromatics plants in particular, are known to reduce oviposition and the development of different life stages in insects (Lambert, 1985; Bekele and Hassanali, 2001; Tapondjou et al., 2002; Boeke et al., 2004; Kouninki et al., 2007a). The authors results show that the powder, or a preparation of the essential oil, of *X. aethiopica* has clear effects on the development of the eggs of *C. maculatus* to adults because no emergence was observed for the treatment with powder alone or in combination with essential oils. This significant reduction in the emergence of F1 progeny suggests an ovicidal toxic effect of our insecticidal preparations of *X. aethiopica.* The preparations could have an effect on oviposition together with an ovicidal action on *C. maculatus*. The combination of essential oil plus powder of *X. aethiopica* opens a new perspective on the management of pest populations because it combines a knock down effect at the beginning and persistence for nearly two weeks, followed by negative consequences on the development of *C. maculatus* that reduces the size of the future generation. However, because grains are stored for 3 to 9 months, several applications will be needed for adequate protection (Sutherland et al., 2002). This corresponds to an important cost, as well as to the requirement for large quantities of *X. aethiopica* extract. Under our conditions, 2 l of essential oil and 10 kg of powder are required per application to 1000 kg of grain. Therefore, for practical use, improvement in the preparation is required.

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