

## Research Article

### Evaluation of Insecticide Properties of Ethanolic Extract from *Balanites aegyptiaca*, *Melia azedarach* and *Ocimum gratissimum* leaves on *Callosobruchus maculatus* (Coleoptera: Bruchidae)

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**Abstract:** In order to protect crops and food stocks against the grain depreciators while preserving the environment, ethanolic extracts of some plant leaves (*Balanites aegyptiaca*, *Melia azedarach* and *Ocimum gratissimum*) were tested for their insecticide activities on *Callosobruchus maculatus*. In jars and petri dishes and at doses 10, 15, 25 and 50%, respectively biological tests were realized with the extracts obtained by maceration in ethanol (95%) of leaves powders of these plants. The rearing of *C. maculatus* and anti-insect test were conducted in laboratory conditions at a temperature of 29.1°C and a relative humidity of 74%. The results were compared with those of the negative (only ethanol) and positive (Star grain) control. At the end of the first day of exposure, the lowest dose (10%) of *B. aegyptiaca* resulted in a high mortality (85%) of *C. maculatus* and 50% dose of *M. azedarach*, 68.33% of mortality. Seventy two hours after treatment, the highest mortality (100%) was obtained with 50% dose of *O. gratissimum*. All these extracts showed repellent effects proportional to the dose. *O. gratissimum* extract had the highest repellency (Class IV) and also proved highly persistence (63.33% mortality after 24 h of exposure). This Insecticide activity could be correlated to the presence of secondary metabolites such as saponins, flavonoids, alkaloids, phenolics and terpenes. These results suggest that these plant extracts could be used as an alternative in the fight against *C. maculatus* in the areas of culture and seed storage areas.

**Keywords:** *Balanites aegyptiaca*, biopesticide, *Callosobruchus maculatus*, *Melia azedarach*, *Ocimum gratissimum*, *Vigna unguiculata*

## INTRODUCTION

Cowpea is the most widely grown legume in sub-Saharan Africa where it is very important to the community (Ndiaye, 2007). The seeds and leaves are very rich in protein (24-33%) and are used in the preparation of various dishes for food and feed (Bressani, 1985). Cowpea is able to fill gaps dietary protein populations in developing countries that make up three-quarters of the world's population, but produces only a quarter of the global production of meat. This is why it was called "meat of the poor" (Delobel and Tran, 1993). Also, it contributes greatly to soil fertility through symbiotic fixation of atmospheric nitrogen (Pasquet and Baldwin, 1997). Nigeria (2 million tons), Niger (1.773 million tons) and Burkina Faso (350 000 tons) are the three main world producers of cowpea (Soulé and Gansari, 2010; RECA (Réseau National des Chambres d'Agriculture du Niger), 2011). In Cameroon, cowpea production is currently estimated

at 200,000 tons/year. This foodstuff is grown mainly in the northern part of the country but also in the west, east, south-west and north-west (Parh, 1997; Singh *et al.*, 1997).

Despite its various nutritional and economic importances, cowpea fails to cover the qualitative and quantitative needs of populations. Decreases in yield and/or production recorded during crop are due to several factors, including the sensitivity of *V. unguiculata* to diseases and pests (Parh, 1999; Ngakou *et al.*, 2008). The attack by pests whose main storage insect is *C. maculatus* starts in the field and spreads in the storage systems. If no action is taken, this causes a drop in production or total post-harvest of the crop stock (Ngamo and Hance, 2007). Severity of post-harvest losses due to insects led (Labeyrie, 1992) to say that in Africa, the peasant works for insects.

Faced to this situation, several control methods have been proposed among which physicochemical methods such as irradiation, spraying equipment,

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synthetic or natural chemical insecticides (Kitch and Ntougam, 1994; Tapondjou *et al.*, 2002; Kouninki, 2007), the use of resistant varieties to storage insects (Arnason *et al.*, 1992) and biological control (Ngakou *et al.*, 2008). However, the use of chemical insecticides considered to be effective by its rapid effects is a major drawback. These chemicals are responsible of the appearance of resistant insects, destruction of the auxiliary fauna, food poisoning, environmental pollution and ecological disorders (Isman, 2000; Regnault-Roger, 2002). To this must be added the high cost, the unavailability and the insufficient quantity of pesticides (Lienard and Seck, 1994). An alternative to the abuse of synthetic insecticides is the development of new production and protection technics of cowpea including bio-fertilizers and bio-pesticides from botanical extracts (Ngakou *et al.*, 2008; Ndomo *et al.*, 2008). The insecticide effect of plant extracts has been demonstrated by several studies. Rajapakse and Ratnasekera (2008) showed that the ethanolic extract of *Ocimum sanctum* induces a 100% mortality of *C. maculatus* and *C. chinensis* after 3 days of exposure. Adeniyi *et al.* (2010) observed that the ethanolic extract of *O. gratissimum* at the dose of 4% causes 47.33% mortality on *Acanthoscelides obtectus* after only 1.5 h of exposure.

In order to contribute to the development of bio-pesticides, this study aims to determine the insecticide and repellent effects of the ethanolic extracts of leaves from *Balanites aegyptiaca* of *Melia azedarach* and *Ocimum gratissimum* on storage insect.

## MATERIALS AND METHODS

**Biological material and pretreatment:** Dry seeds of *Vya* cultivar cowpea, sensitive to *C. maculatus*, were bought from farmers (Far north, Cameroon). *Balanites aegyptiaca*, *Melia azedarach* and *Ocimum gratissimum* leaves were also collected from the same area. In the laboratory, cowpea has been sorted and cleaned of debris and infested grains before storing in the freezer (-20°C) for 48 h. To ensure the healthy state of the seeds, cowpeas were dried in the sun for 2 h. Hundred grams of cowpea were introduced into glass jars initially labeled. For every test, this was done in triplicate.

None sexed beans weevil (*Callosobruchus maculatus*) of 2 to 3 days old were used. In order to obtain a homogeneous population of these animals for biological tests, a mass rearing was carried out with pairs of beans weevil from previously infested bunkers of cowpea section (Agriculture Research for Development Institute, Maroua). According to the method used by Tapondjou *et al.* (2002), this breeding was done on cowpea grain (*Vya* variety) at a temperature of 29.1°C and a relative humidity of 74%. The obtained young animals (2 days old) were thus used for insecticide, insect repellent and persistence tests.

**Extracts preparation:** Fresh *Balanites aegyptiaca*, *Melia azedarach* and *Ocimum gratissimum* leaves were dried in the laboratory at an average temperature (26.8°C) and relative humidity (74%) for 7 days. The dried leaves were ground using a blender to obtain a powder. The powder was then passed through a sieve to obtain uniform size (0.25 mm) particles.

Fifteen gram (Mi) of leaves powder of each plant species were macerated under stirring at room temperature in 100 mL of ethanol (95%) for 48 h. The mixture was filtered through Whatman filter paper (No. 4). Suspensions were dried and weighed (Mr). Extraction yield (R) was calculated by:

$$R(\%) = \frac{Mi - Mr}{Mi} \times 100$$

The resulting filtrate was concentrated (60°C) in a rotary evaporator (Heidolph type). The obtained extracts were stored in a refrigerator (4°C) until used.

**Qualitative phytochemical screening:** Qualitative chemical tests were realized on the crude extracts of *Balanites aegyptiaca*, *Melia azedarach* and *Ocimum gratissimum* leaves. These tests were performed as described by Harborne (1973), Sofowora (1996) and Trease and Evans (1989) to showcase the different classes of secondary metabolites such as saponins, flavonoids, terpenes, tannins, phlobatannins, alkaloids, phenolics and anthraquinones.

**Bioassays:** The bioassays were performed in glass jars (500 cm<sup>3</sup>) and Petri dishes (Ø = 9 cm) containing filter paper (Whatman No. 4). The assembly was randomly disposed on the study bench and repeated 3 times. Each jar was containing 100 g of cowpea grain treated with ethanolic extracts of the leaves.

**Insecticide test by contact with cowpea seeds coated:** Four different doses of ethanolic extracts (10, 15, 25 and 50% w/v, respectively) were previously prepared. Then, 100 g of seeds were uniformly coated with 5 mL of the extract in glass jars. Seeds from negative controls jars were treated only with 95% ethanol, whereas those of positive controls were treated (2 g/kg of seed) with Deltamethrin powder. Jars were then left open in an enclosure air for 45 min to evaporate the dilution solvent (Adebayo and Gbolade, 1994). A beans weevil aspirator was used to infest each jar with 10 pairs of weevils of two days old after emergence in grain. In order to avoid any external contamination, the jars were then covered with canvas gauze and sealed with elastic slings. A dead insect counting was performed on different treatments (42 jars each) every 24 h whitening a period of 4 days. According to the formula presented by Abbott (1925), recorded mortalities (Mo) were expressed as corrected mortality (Mc), taking into

account natural mortality observed in control boxes (Mt):

$$Mc = \frac{(Mo - Mt)}{100 - Mt} \times 100$$

Finney (1971) method based on the probit regression of mortality in function of logarithms of extract doses was used to determine the doses decimating 50% (LC<sub>50</sub>) and individual effective doses for each product.

**Ethanolic extracts repellency:** The repellent effect of plant extracts against weevils was assessed using the method of preferential zone on filter paper as described by McDonald *et al.* (1970). Discs of filter paper (Ø = 9 cm) were cut into two equal parts and 0.5 mL of each extract at different doses (10, 15, 25 and 50%, respectively) was uniformly deposited on one half of the disc giving a final dose of 0.32, 0.47, 0.79 and 1.57% /cm<sup>2</sup>, respectively. The other half part of the disc received only 0.5 mL of ethanol. After 30 min of the complete solvent evaporation, the two halves discs were rebound by means of an adhesive tape at their underside. The reconstituted filter paper disc was then placed in a Petri dish (bottom in contact with the bottom of the box) and a set of 20 non-sexed adult insects, aged up to two days (after leaving the grains) was placed in the center of each disc. After 2 h, the number of insects present on the filter paper treated with the extract (Nt) and the number of those present on the non treated filter paper (Nc) were identified. For each dose, this was performed in triplicate. The Repellency Percentage (PR) was calculated using the formula proposed by Alzouma (1992):

$$PR = \frac{Nc - Nt}{Nc + Nt} \times 100$$

The average percentage of repulsion for the different extracts was calculated and ranked (McDonald *et al.*, 1970) in one of the different classes from 0 to repulsive V: class 0 (PR<0.1%), class I (PR = 0.1 - 20%), class II (PR = 20.1 - 40%), class III (PR = 40.1 - 60%), class IV (PR = 60.1 - 80%) and class V (PR = 80.1 - 100%).

**Determination of the extract persistence:** This was evaluated by observing the persistence of the extracts used and the extent of insect mortality over time. This test was conducted with effective doses of three extracts (10% of *B. aegyptiaca*, 50% of *M. azedarach* and 50% *O. gratissimum*). To perform this test, two lots of Petri dishes (2 times 105 boxes) were used. In the first batch, 0.5 mL of each leaves extract was placed on filter paper (Whatman No. 4) discs covering the bottom of the Petri dishes (Ø = 9 cm). The 105 treated boxes (21 boxes/extract) were immediately closed and kept in the

Table 1: Extraction yield (g/100 g of dried leaves) and qualitative composition of ethanolic extracts obtained from *Balanites aegyptiaca*, *Melia azedarach* and *Ocimum gratissimum*

Characteristics	Source of ethanolic extract		
	<i>B. aegyptiaca</i>	<i>M. azedarach</i>	<i>O. gratissimum</i>
Extraction yield (%) <sup>1</sup>	30.86±0.32 <sup>a</sup>	24.57±0.21 <sup>b</sup>	23.43±0.13 <sup>c</sup>
Saponins <sup>2</sup>	++	+	+
Flavonoids	++	+	+
Tanins	-	+	++
Alcaloids	+	++	+
Anthraquinons	-	-	-
Phenolic compounds	++	+	+
Terpens	+	+	++
Phlobatanins	-	+	+

<sup>1</sup>: Mean values from triplicate measurements±standard deviation, values in the same line followed by different letters are significantly different (p<0.05); <sup>2</sup>: Intensity of compound detection: + = low, ++ = high, - = absent

laboratory for the following periods: 0, 4, 8, 12, 16, 20 and 24 h, respectively. Negative control boxes were treated with ethanol and the positive control with Deltamethrin. At time 0 h, treated papers from the Petri dish of the first batch were transferred in a block of 15 empty Petri dishes of the second batch. Then 20 young non sexed weevils have been introduced and the boxes were closed and sealed. For each time zone and box, this was performed in triplicate. Insect mortality was observed 24 h after their introduction. Recorded mortalities in the Petri dishes were expressed against the control according to the formula proposed by Abbott (1925).

**Statistical analysis:** The obtained data in triplicate were subjected to the analysis of variance (one-way ANOVA) to compare means. The Waller-Duncan test was used to determine differences between these means. Data on the mortality of weevils were corrected by Abbott's formula (1925). All results were considered significant at p<0.05. SPSS (Statistical Package for Social Sciences) version 10.0 was used for these analyses.

## RESULTS AND DISCUSSION

*Callosobruchus* sp., (Coleoptera: Bruchidae), is a major pest of economically important leguminous grains (Talukder and Howse, 1994). In the present study, the insecticide effects of ethanolic extract from *B. aegyptiaca*, *M. azedarach* and *O. gratissimum* leaves on mortality of *C. maculatus* are established.

**Extraction yield and phytochemistry of the extracts:** Table 1 shows that the extraction yields varied significantly (p<0.05) according to the type of leaves used. Highest values are obtained with *B. aegyptiaca* (30.86 g/100 g of dried leaves). Although extraction yield is low with *O. gratissimum* (23.43%), Adeniyi

*et al.* (2010) observed very low value (11.10%) with the same plant leaves. This could be due to climatic and edaphic conditions between the two sites and period of the leaves harvest (Ndomo *et al.*, 2009).

Qualitative phytochemical analysis revealed that all the ethanolic extracts contain saponins, flavonoids, alkaloids, phenolics and terpenes (Table 1). *B. aegyptiaca* leaves extract is rich in saponins, flavonoids and phenols meanwhile that of *M. azedarach* exhibit important quantity of alkaloids. Tannins and terpenes are much more present in the *O. gratissimum*

leaves extract. However, anthraquinones were absent in all the leaves extracts. In agreement with Adeniyi *et al.* (2010) flavonoids, tannins, alkaloids, terpenes and phlobatanins have been also identified in the ethanolic extract of *O. gratissimum* leaves. But, they did not detect the presence of saponins in the extracts. This could be explained by the difference in soil and climatic conditions of crop circles (Ndomo *et al.*, 2009). Similarly, Jorge *et al.* (2009) showed the presence of alkaloids, flavonoids, tannins and saponins in the hydroethanolic extracts of *M. azedarach*. The high

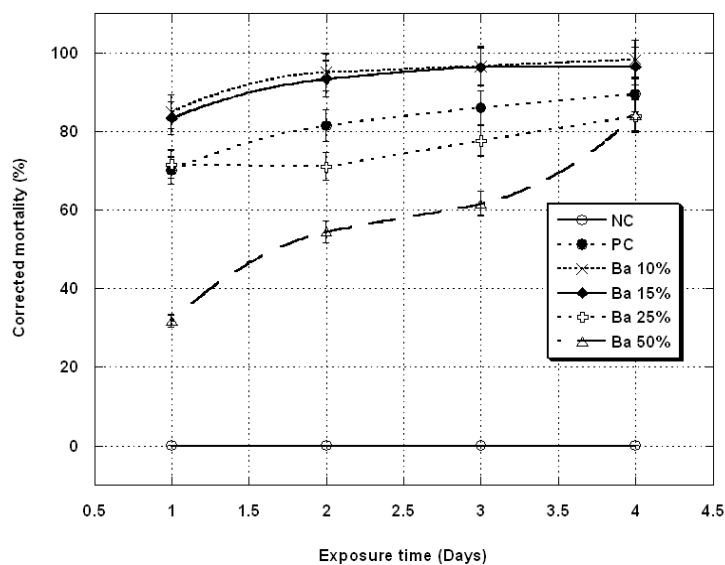


Fig. 1: Variation of cumulative mortalities of *C. maculatus* in function of time (days) at various doses (10, 15, 25 and 50%, respectively) of ethanolic leaves extract from *Balanites aegyptiaca* (Ba); this was compared to deltamethrin and ethanol used respectively as Positive (PC) and Negative (NC) control

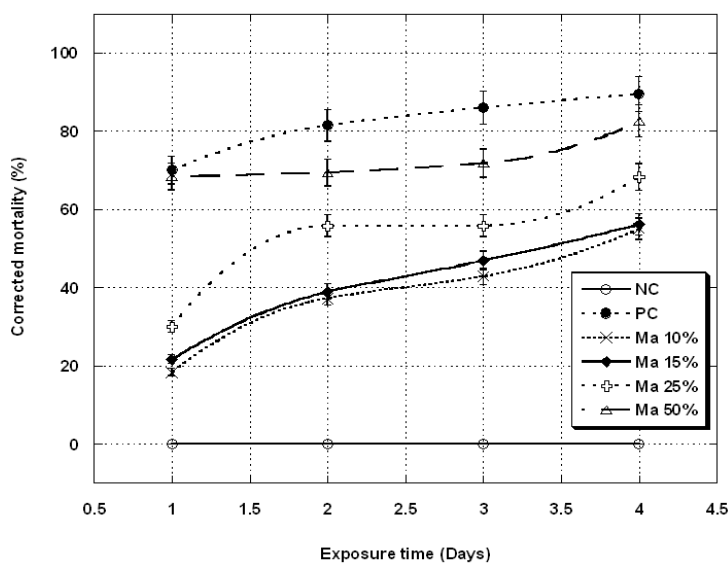


Fig. 2: Variation of cumulative mortalities of *C. maculatus* in function of time (days) at various doses (10, 15, 25 and 50%, respectively) of ethanolic leaves extract from *Melia Azedarach* (Ma). This was compared to deltamethrin and ethanol used respectively as Positive (PC) and Negative (NC) control

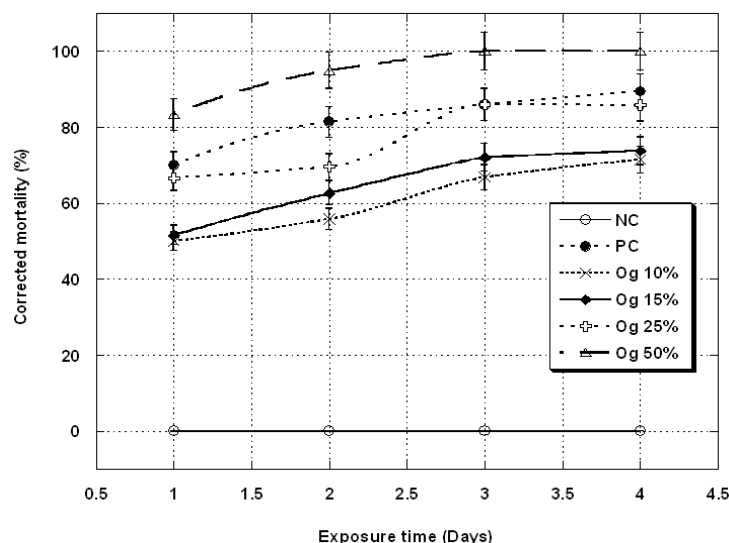


Fig. 3: Variation of cumulative adult mortalities of *C. maculatus* in function of time (days) at various doses (10, 15, 25 and 50%, respectively) of ethanolic leaves extract from *Ocimum gratissimum* (Og). This was compared to deltamethrin and ethanol used respectively as Positive (PC) and Negative (NC) control

content in secondary compounds of these extracts could be correlated to their high insecticide activity (Kabar and Gichia, 2001).

**Leave extracts toxicity:** Figure 1 to 3 present the variation of corrected and cumulative mortality of *Callosobruchus maculatus* in function of time at different doses of ethanolic extracts of *B. aegyptiaca*, *M. azedarach* and *O. gratissimum* leaves respectively. During 4 days of exposure, no mortality was recorded with the negative control (ethanol).

From Fig. 1, it appears that the two lowest (10% and 15%) dose of ethanolic leave extracts causes a high mortality rate (83-85%) at the first day of exposure. This effect remains high throughout the trial period. Highest doses of *B. aegyptiaca* leaves extract exhibit lower mortality rate. This suggests that mortality is conversely proportional to the amount of dose used. However, no significant difference was observed between 10 and 15% doses of the extract. Mortality rate caused by positive control is significantly ( $p < 0.05$ ) lower than that observed with 10 and 15% doses and higher than that recorded with high (25-50%) doses. After 4 days of exposure, 10% dose causes 98.33% of *Callosobruchus maculatus* mortality. With the other two extracts and contrary to *B. aegyptiaca* leaves extract (Fig. 1), we notice that the mortality rate of *C. maculatus* adult increase with the time and dose (Fig. 2 and 3). Whatever the dose used, observed mortalities caused by *M. azedarach* is lower than those recorded with the positive control.

Percentage of toxicity of adult insects from all negative control samples indicated that the ethanol (95%) used for bioassay was not toxic to the insects. Since highest dose extract took much time to

concentrate, the observed loss of effectiveness of *B. aegyptiaca* leaves extract may be related to the time and temperature used to reach the desired concentration. During this concentration time, multiple bonds breaking between molecules and massive evaporation of active components can occur. Schumutterer (1990) showed that the loss of the extract efficiency can be explained by active principles denaturation. Mortalities observed with these extracts may be due to the presence of certain components in the extracts that block the transmission of nerve impulses by inhibiting the hydrolysis of acetylcholine causing paralysis or death of the weevils (Keane and Ryan, 1999). It could also be attributed to the presence of terpenes in their structures (Metcalf and Metcalf, 1992). Monoterpenes mainly target octopamin receptors which are found in insects (Essam, 2001). The highest mortality (100%) caused by *O. gratissimum* extract can be correlated to its high terpenes content (Table 1). Even at lowest concentration (4%) ethanolic extract of *O. gratissimum* causes 47% of beans weevil (*Acanthscelides obtectus*) mortality (Adeniyi *et al.*, 2010). It appears clearly that the mortality of *C. maculatus* varies with the time, the dose and the type of ethanolic extract used (Fig. 2 and 3). Similar observations were also noticed by Kiradoo and Srivastava (2011) on the mortality rate of *C. chinensis* treated with crude, aqueous, ethanolic and diethyl ether plant extract from *O. basilicum*, *O. sanctum* and *Mentha spicata*.

**Repellent effect of ethanolic extracts:** Table 2 presents the percentages of repulsion of *B. aegyptiaca*, *O. gratissimum* and *M. azedarach* leaves extract at different doses (10, 15, 25 and 50%, respectively). We

Table 2: Influence of doses and the source of ethanolic extract on the repellence rate of *Callosobruchus maculatus*. The extracts are obtained from *Balanites aegyptiaca*, *Melia azedarach* and *Ocimum gratissimum* leaves

Doses	Repellence rate (%)			
	Positive control	<i>B. aegyptiaca</i>	<i>M. azedarach</i>	<i>O. gratissimum</i>
10%	/	0.00±0.00 <sup>c</sup>	10.00±1.00 <sup>b</sup>	60.00±5.00 <sup>a</sup>
15%	/	0.00±0.00 <sup>c</sup>	10.00±1.00 <sup>b</sup>	63.33±5.17 <sup>a</sup>
25%	/	6.67±1.77 <sup>c</sup>	16.67±1.78 <sup>b</sup>	90.00±10.00 <sup>a</sup>
50%	/	20.00±2.00 <sup>c</sup>	46.67±2.28 <sup>b</sup>	100.00±7.00 <sup>a</sup>
Mean	50.00±5.00 <sup>b</sup>	6.67±1.43 <sup>d</sup>	20.84±2.51 <sup>c</sup>	78.33±9.72 <sup>a</sup>
Class	III	I	II	IV

Mean values from triplicate measurements±standard deviation; Values in the same line followed by different letters are significantly different ( $p < 0.05$ )

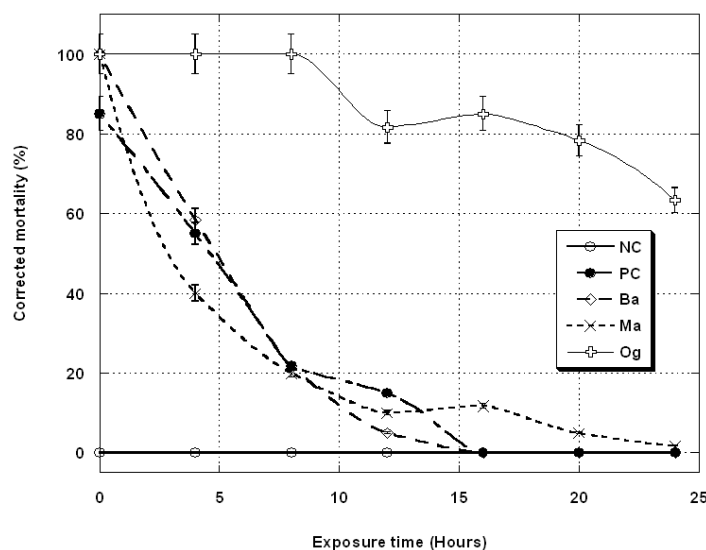


Fig. 4: Effect of ethanolic extracts on the adult mortality (%) of *C. maculatus* in function of time during persistence. This was compared to deltamethrin and ethanol used respectively as Positive (PC) and Negative (NC) control. The extracts are obtained from *Balanites aegyptiaca* (Ba), *Melia azedarach* (Ma) and *Ocimum gratissimum* (Og) leaves

notice that after 2 h of exposure, *O. gratissimum* extracts exhibit the highest (60.00%) repulsion at the lowest dose (10%). Even at highest doses (50%), the other extracts (*B. aegyptiaca* and *M. azedarach*) cause 20 to 47% of repulsion. No degree of repulsion is observed with *B. aegyptiaca* extract at 10 and 15% doses. Compare to the positive control (50.00%), only *O. gratissimum* exhibit high value of repulsion (78.33%). This suggests that *O. gratissimum* leaves extract is the most repellent extract. In the light of these results, it should be noted that all these extracts have a repellent activity towards these beetles. It also appears that the repugnant effect is deeply correlated to the dose.

During the present study, the three plant extract showed significant ( $p < 0.05$ ) different repellent effect, that of *O. gratissimum* leaves exhibiting the highest. According to the ranking proposed by McDonald *et al.* (1970), the ethanolic extracts of *B. aegyptiaca*, *M. azedarach* and *O. gratissimum* can be ranked in repulsive classes I, II and IV respectively. Tandon and Sirohi (2009) also noticed that repellency significantly changed with dose used. They also studied the repellent

effect of *M. azedarach* ethanolic extract on *Raphidopalpa foveicollis* and they classified this extract in repellent class II. These repulsive effects can be attributed to the major components of the extracts (saponins, flavonoids, phenols, alkaloids) in association or not with terpenes. Some studies showed that the toxicity and repellency of extracts were related to the presence of terpenes in their composition (Metcalf and Metcalf, 1992; Asawalam *et al.*, 2006).

**Persistence of ethanolic extracts:** The toxicity and persistence of the ethanolic extracts (*B. aegyptiaca*, *M. azedarach* and *O. gratissimum*) used were tested on *C. maculatus* on a filter paper. It appears that *C. maculatus* is sensitive to the three extracts used. They exhibit an insecticidal activity by direct contact (Fig. 4). Mortalities are high (100%) for all extracts when insects are put onto the filter paper treated at time 0 h. The insecticidal activity decreases when the time between filter paper treatment and insect exposure increases. Thus we observe a reduction in mortality to 21.67% 8 h after treating filter paper with *B. aegyptiaca* extract. After 12 h, it decreases to 5% before reaching

0% at 16 h after treatment. With *O. gratissimum*, mortality remains relatively high throughout the test (100% at 0 h and 63.33% at 24 h) presenting this extract as the most persistent. No weevil mortality was recorded in Petri dishes treated with only ethanol (negative control).

Since ethanolic extract from *O. gratissimum* leaves showed a different and high persistence compared to the other extracts difference, we can suggest that these differences may be related to the chemical composition of the extracts tested, the latter having more or less affinity with the filter paper. With the time, extracts lose their effects by exposure on the filter paper, but with its polar nature, it could retain some volatile compounds thereby emphasizing the persistence of the product (Obeng-Ofori *et al.*, 1998). Ndomo *et al.* (2008) also observed a highly significant loss of toxicity after 24 and 36 h following treatment of *Acanthoscelides obtectus* with essential oil from *Clausena anisata* leaves. The loss of efficiency by extracts could also be explained by the denaturation of toxic constituents, thus confirming the biodegradability of the plant extracts (Schumutterer, 1990) which is an advantage in limiting the toxicity of biopesticides. It would be convenient to conduct a comprehensive study to identify and elucidate the structure of compounds conferring insecticide effects in plant extracts in order to assess the toxicity of treated seeds.

### CONCLUSION

It is clear from this study that the ethanolic extracts of *Balanites aegyptiaca*, *Melia azedarach* and *Ocimum gratissimum* leaves possess insecticide properties and can help to reduce and prevent the adverse effects of chemical insecticides. Toxicity tests show a high mortality rate of beans weevil (*Callosobruchus maculatus*) during all the 4 days of exposure. With a 10% dose, *B. aegyptiaca* extract causes the highest mortality (85%) on the first day of exposure and with the 50% dose; *O. gratissimum* causes 100% of mortality 72 h after treatment. These extracts were also dose-dependent insect repellent. *O. gratissimum* extract is more repellent (class IV) and persistent than the two other extracts. These ethanolic extracts have potential insecticides against *C. maculatus* and can be used as an alternative to fight against weevils in the areas of culture and seed storage. For judicious use these extracts should be applied consistently to counteract any weevil development.

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