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Full Length Research Paper

Bioefficacity of the powder of Melia azedarach seeds and leaves against Callosobruchus maculatus, on cowpea seeds (Vigna unguiculata) in storage

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Cowpea is a legume that participated in the food security in Cameroon. Despite its importance, more than 30 % of its production is lost between the harvest and consumption. The main insect pest of stored cowpea is *Callosobruchus maculatus*. The control strategy is based essentially on the use of chemicals products which are toxic, polluting and expensive. In the search for alternative to chemical control, this study was undertaken to evaluate the insecticide potential of seeds and leaves powder of *Melia azedarach*. Toxicity tests and insecticidal efficacy of *M. azedarach* were performed on 20 (seed weevils), using 6 doses of the powder of seeds and leaves applied in the jars containing 100 g of cowpeas. The control jars did not receive treatments. Mortality of *C. macullatus* weevils and the impact of powders *M. azedarach* were recorded after 96 h and 120 days of infestation. The germination capacity of the treated seeds was evaluated at the beginning and the end of the study. Doses of 2 g and 8 respectively of seed powder and *M. azedarach* sheets applied on 100 g of cowpea led a 100% of weevil's mortality after 96 h. The results on the efficiency test show that at these same doses, treated seeds were not attacked during the four months of storage. Germination rate of cowpea seeds treated with 2 and 8 g of bio-insecticide powders were 99.75 and 99.5%, respectively. Seed and leaves powder of *M. azedarach* may be considered as a powerful bio-insecticide against insect pest of stored cowpea.

Key words: Cowpea; *Callosobruchus maculatus*; *Melia azedarach*; mortality, Bioefficacity.

Abbreviations: Mc_ is the corrected mortality rate; M0_ is the number of dead insects in jars treated; Mt_ is the number of dead insects naturally in the control jars; Ns_is the number of healthy seeds; Na_is the number of attacked seeds; G_is the percentage of germination; NGG_is the number of germinated seeds; NGE_ is the number of seeds sown.

INTRODUCTION

The niebe or cowpea (*Vigna unguiculata* (L) Walp.) is a legume that participates in the food security of many African countries such as Cameroon (Njouenkeu et al., 2010). In Cameroon, its production ranges from 1.2 to 2 t / ha in research stations and 300-500 kg / ha in farmers' fields. In general, most of the production is carried out in the region of the Far North of the country. In this part of the country, cowpea is among the legumes, the second most cultivated after groundnut. Cowpea remains an asset for the people of Cameroon and the Far north in particular in terms of its nutritional importance. Its seeds represent a precious source of vegetable protein, vitamins and income for the man, as well as fodder for animals. The juvenile leaves and the immature pods are consumed as a vegetable (Ouédraogo, 2003; Dudje et al., 2009).

According to FAO, between 60 to 80% of the production of cowpea seeds are stored by farmers in the

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tropics. During the period of storage, there is an important loss of the production (Tapondjou et al., 2002; Ngamo et al., 2007a). These losses are due on the one hand to conditions and storage structures and on the other hand to storage insect pests (Ngamo and Hance, 2007; Ngamo et al., 2007a). In Cameroon, more than 30% of the production is lost between the harvest and the consumption. This proportion is higher in the northern part of Cameroon because of the long period of storage (Ngamo and Hance, 2007). Thus, a third of what is produced never arrives to consumers.

The main insect pest of stored cowpea is *Callosobrucus maculatus*. It causes significant losses of up to 80% (Tapondjou et al., 2002). The infestation of cowpeas by this insect begins at the field on the immature pods being; the larva enters the pod and the seed where it feeds. The adult emerges either in cultures or in attics after harvest. Weevils perforate the cowpea seeds leaving several galleries, which causes not only a reduction in dry weight but also a decrease in seed quality and seed viabilities compromising the organoleptic and nutritional properties of the food (Ngamo et al., 2007a; Ngamo et al., 2007b). Damage caused by insects can cause its total loss of stored seeds in a few months (Leinard and Seck, 1994).

Difficulties related to storage have led farmers to use chemical synthetic insecticides (Dudje et al., 2009). For effective protection of food products and seeds, commonly used pesticides are organochlorines, organophosphates, carbamates and pyrethroids (Gwinner et al., 1996). The interest which the peasant carries to the use of the pesticides nearly explains by its easiness of employment, its speed of action and the almost total elimination of the insects including those useful. However, synthetic chemical insecticides constitute a great danger to the environment and human health, and causes of cases of resistance development (Aubertot et al., 2005; Tabula et al., 2005; Javed et al., 2008; Park et al., 2003; Nerio et al., 2009). They also reduce biodiversity and destroy much of the beneficial organisms, such as decomposers involved in the construction of the humus and biogeochemical cycles (Provost et al., 2003; Glitho et al., 2008).

Much research has been conducted on the use of natural products, especially on plant extracts having insecticidal properties and whose undesirable effects have not been reported in humans (Isman, 2000). Thus the essential oils extracted from plants have been widely used in the fight against pests of grain stocks (Regnault and Hamaoui, 1997; Prates et al., 1998; Tapondjou et al., 2002; Tapondjou et al., 2003; Ngamo et al., 2007a; Nerio et al., 2010). Their insecticidal properties, nematicide, larvicide, ovicidal, sterilizing, repulsive and feeding effect have made several studies (Ngamo et al., 2007a; Kouninki, 2007; Ndomo et al., 2009; Kosma et al., 2011). These botanical origin bio-insecticides appear as a better alternative to fight against specific insects (Ambang et al., 2005).

The *Melia azedarach* is a tree of the family of *Méliacées*, it is originating in India (Nardo et al., 1997). This plant is very widespread and is naturalized in the tropical and subtropical areas. It is largely used in pharmacology traditional and known for its properties insecticide, fungi and medicinal (Kataria, 1994; Huang et al., 1995; Valladares et al., 1999; Carpinella et al., 2002). The methanolic extracts of the sheets and seeds of *M. azedarach* were tested against *Anopheles stephensi* (Nathan et al., 2006). On the African continent more particularly in Algeria, the plant is used as tonic and antipyretic. In South Africa the plant was used for the treatment of leprosy and the relief of the attacks of asthma crises (Oelichs et al., 1983). However, the effect of the powder of seeds and sheets of *M. azedarach* opposite *C. maculatus*, remains unknown. The principal objective of this study is to evaluate the insecticidal potential of seeds and leaves powder of *M. azedarach* against *C. maculatus*, the main pest of cowpea in stock in Cameroon.

MATERIALS AND METHODS

Cowpea variety

This study was carried out with a batch of seeds of a cowpea variety (Br 24 125), considered very sensitive to the attacks of weevil insects. These seeds were sorted, cleaned of impurities and damaged seeds. Before their use in the various stages of the test, the seeds have been sorted in the refrigerator for 24-48 h at 4 °C to remove traces of eggs or larvae may be at the surface.

Cowpea weevil

Weevils used in this study were collected from a lot of cowpea seeds bought in a store IRAD Maroua, using a mouth aspirator. Insects were placed at 25 couples in one-liter jars containing 1kg of cowpea brought at the Meskine market, and not having been treated with insecticides. The openings of these jars were carefully covered with muslin cloth to allow ventilation and prevent the exit of insects and other external contamination. This phase lasted for 30 days to allow the emergence of new adults. After this operation, the cowpea seeds contained in the different jars were screened; adult weevils were removed and breeding continued with cowpeas infested with eggs. This second phase lasted 23 days and got bugs older than 3 days were used to infest cowpea of different treatments. All of these breeding operations was conducted in the laboratory at a 32.40 ± 2.85 °C temperature and 50.93 ± 6.74% of relative humidity.

Obtaining seed powder and leaves of *Melia azedarach*

The fresh leaves and ripe fruits of *M. azedarach* were collected in February 2012 Makabai (Maroua / Cameroon), then dried in the shade for 6 days at 32.40 ± 2.85 ° C temperature and 50.93 ± 6.74% relative humidity. Thereafter, fruits were peeled manually to extract the seeds. The seeds obtained were dried in the laboratory for 10 days at 31 ° C and then ground with a porcelain mortar. As for the sheets, they are made as a powder by grinding in a mortar and sieved using a 0.5 mm mesh sieve. After this operation, seeds and leaves powders were stored respectively in two different dark jars at the laboratory, to avoid any risk of oxidation.

Experimental design

A total of 56 jars were used to evaluate the insecticidal activity of the powdered seeds and leaves of *M. azedarach*. Jars were placed in 2 lots of 28 jars each. The first batch was designed to study the effect of insecticide powders of *M. azedarach* and the second to the damage assessment caused by *C. maculatus* after 120 days of storage. Thus, in each batch, the jars were divided into 7 groups of 4 jars per treatment. These jars were kept on the bench under the conditions of temperature 32.40 ± 2.85 ° C and humidity of 50.93 ± 6.74% recorded daily using a thermohygrometer placed close jars.

Cowpea infestation

One hundred grams of packaged seeds were introduced into the jars previously marked T0 (control), G1, G2, G3 (for seeds) and F1, F2, F3 (for leaves). Thus, powders were weighed using an Item balance (No. TS400W) sensitive to a thousandth; of1, 1.5 and 2 g for seeds, and 1, 4 and 8 g for leaves; each was introduced in jars containing 100 g of cowpea. Control jars (T0) have not received powders. After weighing, twenty couples of 3 days age weevils were collected using a vacuum cleaner and introduced into each of the jars. These jars were then covered with muslin and sealed with elastic rings in order to avoid any external contamination. All jars were infested on the same day.

Germination tests

Two germination tests were performed. The first was carried out at the beginning of the experiment. Whereas the second test was performed on seeds that have been in constant contact with the bio-insecticide powders after 120 days. the second test was performed on seeds that have been in constant contact with the bio-insecticide powders after 120 days.

Data collection

After 96 hours, the jars contents were observed to determine the toxicity of the seed of leaves powder of *M. azedarach* according to the used doses. For that, jars were sieved to extract adult weevils and leave only the infested seeds. At the same time, the dead individuals were counted. Was considered as death, an individual which didn't move any more after several touched of legs and antennae with a syringe.

Mathematically data were measured as follows:

- 96 hours after their introduction into different jars, the toxicity of different bio-insecticide powders against *C. maculatus* was calculated using the formula of Abbott (1925):

$$Mc = \frac{M_0 - Mt}{100 - Mt} \times 100$$

Where Mc is the corrected mortality rate, M0 is the number of dead insects in jars treated and Mt is the number of dead insects naturally in the control jars.
The doses that caused a higher mortality to 50% of control in a very short time were considered more efficient.
Subsequently, the impact of the bio-insecticide powders on the damages caused by *C. maculatus* has been measured after 120 days.
- The estimated damage was calculated from a batch of 100 seeds (N) which is separated into healthy and attacked seeds.
The percentage of attack (A%) will be:

$$A\ (\%) = \frac{Na}{Ns + Na} \times 100$$

Where: Ns is the number of healthy seeds and Na is the number of attacked seeds. Finally, in terms of germination rate, it was evaluated before and at the end of the experiment.
- The rate of germination was evaluated by counting germinated compared to seeds sown according to the formula:

$$G\ (\%) = \frac{NGG}{NGE} \times 100$$

Where G is the percentage of germination, NGG is the number of germinated seeds and NGE is the number of seeds sown

Table 1 : Evaluation of the toxicity of the *M. azedarach* seeds and leaves powder on *C. maculatus*. In the same column, means followed by the same letter are not significantly different according to the Newman-Keuls test at 5% level. G: treatment with *M. azedarach* seed powder; F: treatment with *M. azedarach* leave powder and T0: control.

Treatments	Doses (g/100 g of cowpea seeds)	Average rate of mortality (%) (standard deviation)
F1	1	70[a] (0,81)
F2	4	80[a] (0,81)
F3	8	100[a] (0,81)
T0	0	2.5[b] (0,8)
G1	1	80[a] (0,81)
G2	1.5	85[a] (0,8)
G3	2	100[a]

Table 2 : Impact of the *M. azedarach* seeds and leaves powder on the damage caused by *C. maculatus.*

Treatments	Doses (g/100 g of cowpea seeds)	damage Seeds (%)	Seeds have more than 2 holes
F1	1	67 (18,56)	35,7 (11,52)
F2	4	24.25 (4,64)	5,75 (2,98)
F3	8	1.5 (1,29)	0
T0	0	96.75 (2,78)	76 (18,95)
G1	1	26 (10,9)	9,25 (11,52)
G2	1.5	7.5 (4,04)	1 (11,52)
G3	2	0	0

Data analysis

The data obtained were submitted to an analysis of variance using the software R. The rates (percentages) of mortality of *C. maculatus* were transformed by calculating the arcsinus of their square roots before the statistical analysis. The averages presenting meaningful differences were classified by the method of Newman and Keuls.

RESULTS

Assessment of the toxicity of the powder of seeds and leaves of *Melia azedarach*

During the assessment of the insecticidal effects of *M. azédarach* seeds and leaves powder, all employed doses resulted in the mortality of *C. maculatus*. The different rates of mortality observed were function of the dose administrated. Thus, mortality rates of 80±0.81; of 85±0.8 and of 100% have been observed after 96 h of exhibition of *C. maculatus* to the *M. azedarach* seed powder at 1; 1.5 and 2 g doses on 100 g of cowpea, respectively (Table 1). For the leaf powder, although the results obtained showed no significant difference (P 0.05 >) with those recorded with seed, at a slight decrease in mortality was observed. In general, depending on the dose and the number of dead individuals, the seed appeared more toxic to *C. maculatus* than the leaf. A significant difference ($P < 0.05$) was observed between the mortality obtained in the treated jars with the *M. azedarach* seeds and leaves powder and control jars (Table 1).

Larvicidal effect of *Melia azedarach* seeds and leaves powder on cowpea

The figure 1 showed the results of the influence of *M. azedarach* seeds and leaves powder on the number of larvae of *C. maculatus* on 100 cowpea seeds. The results showed that the number of larvae in all treated jars was less than the number of larvae recorded in the control jars. The larvicidal effect of *M. azedarach* seeds and leaves powder varied according to the applied dose. Increasing doses for each type of treatment caused the reduction of the number of larvae (Figure 1a and b).

For the seed powder treatment the number of larvae was 3.75 ± 3.09; 2.75 ± 0.95; 0.25 ± 0.5 respectively at a dose of 0.5; 1 and 2 g whereas in the control jars, it is 17.25 ± 3.05 (Figure 1a). For treatment of the leaf powder, 7.5±1.7 larvae have been recorded in the treated jars at 1 g dose. However to the dose of 4 and 8 g on 100 g of cowpea, only 5.25±0.95 and 4.75±0.5 respectively larvae were noted (Figure 1b). Depending on the applied dose, the larvicidal effect of the seed powder is greater than the leaves powder (figure 1 a and b).

Impact of the seed and leaf powder on cowpea seeds damaged and holes number by *C. maculatus* Taking 100 cowpea seeds at random from each jar, we found that damaged seeds vary with applied doses of *M. azedarach* seeds and leaves powder (Table 2). Thus at the dose of 1; 1.5 and 2 g of *M. azedarach* seed powder per 100 g of cowpea, there was 26 ± 10.9; 7.5 ± 4.04 and 0% damaged cowpea seeds ,respectively. For treatments with leaf powder, damaged grain were 67 ± 18.56; 24.25

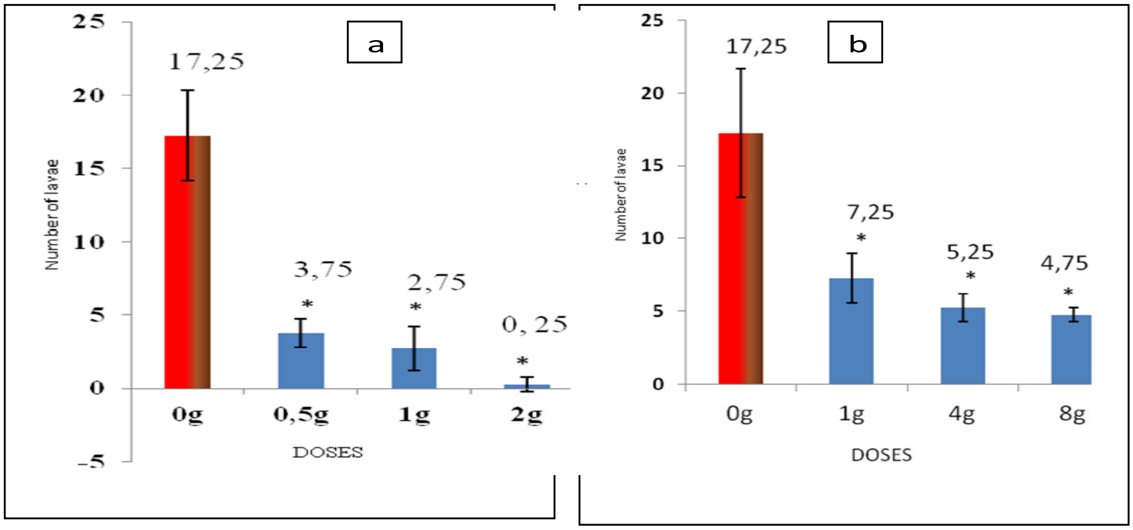

Figure 1 : Larvicidal effect of the *M. azedarach* seed powder (a) and leaves powder (b) on cowpea Seeds

Table 3 : Evaluation of germination rate of cowpea seeds before and at the end of the test.

Germination rate (%) Begining of test	Treatments	Doses (g/100 g of cowpea seeds)	Germination rate (%) End of test
	F1	1	6.5
	F2	4	63
	F3	8	99.5
	T0	0	1.75
99.75	G1	1	21,25
	G2	1.5	96.5
	G3	2	99.75

± 4.64 and 1.5 ± 1.29% at a dose of 1; 4 and 8 g per 100 g of cowpea ,respectively. However, in the control jars, 96.75 ± 2.78% of the damaged cowpea seeds were recorded after 120 days.

After the test, the results showed that in the control jars 76 ± 18.95 cowpea seeds had the holes number ≥ 2 (Table 2). However, in the jars treated with seed powder of *Melia azedarach*, the holes number was 11.52 ± 9.25; 2.98 ± 1 and 0 at a dose of 1; 1.5 and 2 g per 100 g of cowpea ,respectively. For treatments with leaves powdered of *M. azedarach*, it was 35.7 ± 11.52; 0 and 5.75 ± 2.98 at a dose of 1; 4 and 8 g per 100 g of cowpea each respectively. It is found that compared to the values obtained in the treated jars with *M. azedarach* seed and leaves powder, the damage caused by *C. maculatus* were more concentrated in the control jars.

Impact of *Melia azedarach* seeds and leaves powder on germination

The germination test made at the beginning of the test gave a germination rate of 99.75%. When cowpea seeds were in direct contact with the seed and leaves powder of *M. azedarach*, the germination rate varied depending on the administered dose. Thus in the treated jars with seed powder at 1; 1.5; and 2 g doses per 100 g of cowpea each, germination of cowpea seeds were 21.25; 96.5; 99.75%, respectively. In the jars with the treated leaf powder at 1; 4 and 8 g doses per 100 g of cowpea each, the germination rate were 6.5; 63; and 99.5%, respectively. In contrast, the control jars (T0), in which insects are in direct contact with the cowpea seeds, germination rate reached 1.75% (Table 3).

DISCUSSION

In Cameroon, cowpeas are food products that contribute to food security. However, cow pea weevil, *C. maculatus*, is one of the main constraints to their conservations (Leinard and Seck, 1994; Ngamo and Hance, 2007). This work reveals and highlights the potential insecticide effect of *M. azedarach* seeds and leaves powder in the fight against *C. maculatus*. Indeed, the potential of plants for food preservation stored in Cameroon against insects have been the subject of several studies (Tapondjou et al, 2002. Tapondjou et al, 2003. Ambang et al, 2005. Ngamo and Hance, 2007). Their works are based on the use of essential oils, extracts and powders of plants to fight against pests in storage food (Tapondjou et al, 2002. Tapondjou et al, 2003. Ambang et al. 2005 Ngamo and Hance, 2007). At the heart of this work, it appears that the mortality rate of weevils increased after 96 h with increasing doses of seeds and leaves powder of *M. azedarach*.

It reaches its optimum at a dose of 2 g / g for the seed powder and 8 g / g when the powder of the leaves is used. These results corroborate with those obtained by Ambang et al. (2005) in their work on the insecticidal effects of extracts of the yellow laurel seeds (*Thevetia peruviana* Pers) on a pest of stored *Sitophilus zeamais* Motsch. This mortality is due to the biological activity of triterpenoids and limonoids present in the seed and leaves powder of *M. azedarach* (Carpinella et al., 2002). Indeed, triterpenoids and limonoids have an antinutritional effect, inhibit food intake and cause death and deformities in future generations (Carpinella et al., 2002). In this study, the insecticidal activity of the seeds and leaves powder of *M. azedarach* may be explained by their penetrating and / or direct toxicity of their triterpernoide and limonoid component. This hypothesis was verified by Mulla and Su (1999) who showed that the presence of triterpenoids, steroids and alkaloids have insecticidal activity thereby inhibiting the insect development.

The emergence of the adult forms decreases with increasing dose. This is due to the active components in the powder would have ovicidal and larvicidal effects, reducing hatching and preventing the penetration of larvae in grains and consequently suppresses the emergence of the next generation. Moreover, the absence of the emergence at a dose of 2 g is due to the higher toxicity in seeds. Similar results were observed by Ambang et al. (2005) during the evaluation of extracts of yellow laurel seeds (*Thevetia peruviana* Pers) on *S. zeamais*, a stored pest present in Cameroon. The number of damaged seed also varies depending on applied dose and the type of powder (leaf or seed). In general, the treated jars with *M. azedarach* seed powder contain less damaged cowpea seed than the one treated with leaves powder.

This is due to the fact that the active components of *Melia azedarach* is more concentrated in the seeds than the leaves. Also, we find that increasing doses result in an increase of the active components and at the dose of 2 and 8 g of the *M. azedarach* seeds and leaves powder, no damaged cowpea seed are observed. The more holes is ≥ 2, plus there is a loss of organoleptic quality of food, loss of market value and reduced germination of the seed. Leinard and Seck (1994) showed that *C. maculatus* causes a reduction in the seeds quality, seed viability and a total loss of cowpea if no treatment is applied. More Tchanou (1995) showed that a relationship exists between the number of holes and the germination capacity of cowpea seeds. Concerning the germination, *M. azedarach* seed and leaves powder not affect the germination of cowpea seeds and this corroborates with the results obtained by Stoll (1988) who showed that the germination capacity of wheat treated with lilac of India before being placed in reserve was not affected by at planting.

CONCLUSION

At the end of this study in laboratory IRAD Maroua, cowpea section and whose overall objective was to evaluate the insecticidal activity of the *M. azedarach* seeds and leaves powder in the fight against *C. maculatus*, it appears that these powders have an insecticidal effect. Indeed, the results obtained in this work show that 2 and 8 g / g respectively for the *M. azedarach* seeds and leaves powder, the mortality rate of *C. maculatus* was 100% after 96 h exposure to these bio-insecticides. In addition, at these same doses, the damaged cowpea seeds and the holes number ≥ 2 are relatively 0 after 4 months of storage.

Thus, it is clear that the *M. azedarach* seed and leaves powder prevents the development and also reduces the emergence of adult *C. maculatus*. Germination tests showed that the *M. azedarach* seed and leaves powder applied at 2 and 8 g per 100 g of cowpea, have a positive effect on the germination of cowpea seeds. And *M. azedarach* seed and leaves powder may be considered an excellent bio-insecticide that farmers can use in the fight against insects cowpea storage.

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