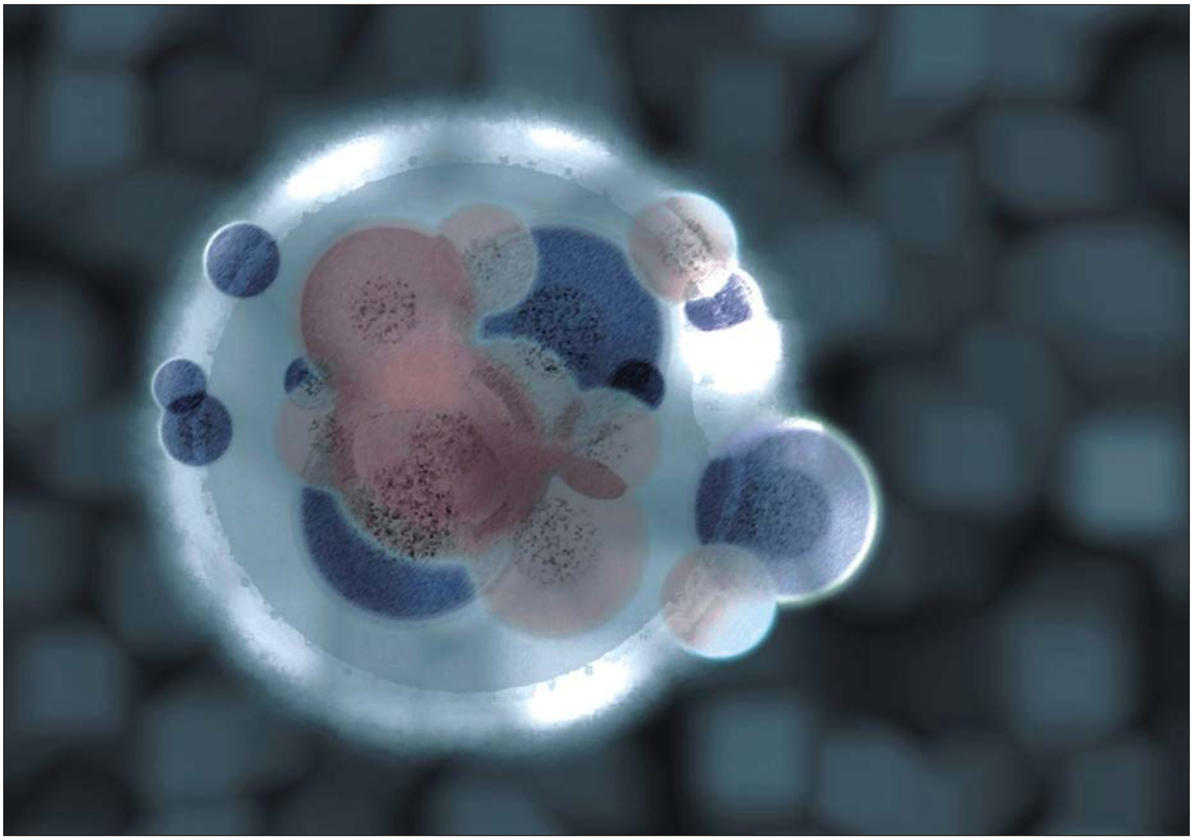

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Effects of Six Common Plant Seed Oils on Survival, Eggs Lying and Development of the Cowpea Weevil, *Callosobruchus maculatus* (F.) (Coleoptera:Bruchidae)

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Abstract: This research reported the effects of 6 common plants extracts including neem and some others which have been never tested on the longevity of adults, the number of eggs laid and adult emergence of *Callosobruchus maculatus*. Six seed oils extracted by methylene chloride were tested: *Azadirachta indica*, *Ricinus communis*, *Thevetia nerifolia*, *Balanites eagyptiaca*, *Moringa oleifera* and *Kaya senegalensis*. All these oils reduce significantly survival of adults, oviposition and total emergence of adults. The most efficient is that of *Thevetia nerifolia*. At higher concentrations the treatment affects slightly the germination of seeds which remains over 50%. Seed oil of *Thevetia nerifolia* can be used as a good alternative to pesticides against *Callosobruchus maculatus* in addition with that of *Azadirachta indica* which effects are well established by many former works.

Key words: *Callosobruchus maculatus*, cowpea, protection, seed oils, toxicity

INTRODUCTION

Callosobruchus maculatus (F.), commonly known as the cowpea weevil is the major pest of wild range of stored legume seeds, especially of the cowpea *Vigna unguiculata* (L.). In many countries of western and central Africa, cowpea is a major dietary staple.

It is often the main source of protein of the families. But the stocks are rapidly broken down by the cowpea weevil within 3 to 5 months after harvest (Singh *et al*., 1978). During the storage, the cowpea weevil causes heavy qualitative and quantitative losses. Caswell (1981) reported a loss of approximatively 50% of cowpeas in 3 to 4 months in northern Nigeria. Tanzubil (1991) found that loss can reach 60% in northern Ghana. The damaged seeds are unsuitable for human and animal consumption and they can not be used for planting. So, preservation of the quality of the seeds for the following year is one of the worrying problems of farmers.

Modern control methods are too costly and technically difficult of application in an Africa farm. Pesticides also may cause health risks because of the inadequate use by the farmers. The farmers generally use alternatives such as shaking of the stocks once a month, solarisation, fumigation and admixture of the stocks with ash, plant powders or plant oils to prevent them. Some authors recommend the solarisation and heating of stocks to prevent attacks (Chauman and Ghaffar, 2002; Lale and Vidal, 2003).

The admixture of oils extracted from common plants to cowpea stocks have been studied by many authors. Keita *et al*. (2000, 2001) have tested essential oils of five West African plant species and found that the plants of the genus *Ocimum* can be used as a good alternative to synthetic insecticides. Raja *et al*. (2001) reported the effect of volatile oils from *Mentha arvensis*, *Mentha spicata* and *Cymbopogon nardus* on *Callosobruchus maculatus*. They observed significant reduction of the number of eggs laid, adult survival, adult emergence and seed damage compared to controls. Lale and Abdulrahman (1999) have evaluated the effect of neem seed oil obtained by acetone extraction using three methods on the cowpea weevil. They found all the three extracts reduced the number of eggs laid and adult emergence. Rajapakse and Van Emden (1997) have tested four vegetable oils (corn, groundnut sunflower and sesame) on bruchids including *Callosobruchus maculatus*. They found the four oils significantly reduce the oviposition and the longevity of adults. Ketoh *et al*. (2002) have studied the susceptibility of *C. maculatus* and its parasitoid *Dinarmus basalis* to essential oils of 3 plants of Togo (*Ocimum basilicum* L., *Cymbopogon nardus* L. and *Cymbopogon schoenanthus* L.). They found that essential oil of *Cymbopogon schoenanthus* was the most efficient but was also toxic to the parasitoid. Tapondjou *et al*. (2003) used the volatiles oils extracted by steam distillation of the leaves of *Eucalyptus saligna*

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and *Chenopodium ambrosioides* on this beetle. They found these extracts and the powder of leaves efficient. Adedire and Akinneye (2004) tested the powder and ethanol extract of *Tithonia diversifolia* leaves for their efficacy at five different concentrations (0.0, 0.5, 1.0, 1.5 and 2.0%) on the mortality, oviposition and adult emergence of cowpea seed beetle. The extract and the powder caused significantly the mortality of adults and reduced oviposition and the development of the beetle. Kellouche and Soltani (2004) have evaluated the biological activity of powders from dry leaves of four plant species: *Ficus carica*, *Eucalyptus globulus*, *Olea europaea*, *Citrus limon* and a powder from cloves of myrtle, *Syzygium aromaticum*, on this beetle. They found them slightly efficient compared to control. Abdullahi and Muhamed (2004) used powders of *Guirea senegalensis*, *Piliostigma reticulatum* and dried fruit powder of *Piper guineense*, compared to the effect of a conventional insecticide, Actellic-2-Dust. They found that *P. guineense* had the highest larvicidal effects and lower productivity result and *P. reticulatum* had the lowest mortality rate and higher productivity. Ketoh *et al.* (2005a, b) have evaluated the effects of extracts obtained by steam distilltallion from *Cymbopogon nardus, cymbopogon schoenantus* and *Ocimum basilicum* on the cowpea weevil combined with the exposure to its natural enemy, *Dinarmus basalis*. Boeke *et al.* (2005) have screened 33 African plants commonly used to protect crops for their toxic and repellent effects against this beetle. They found that powders of *Nicotiana tabacum, Tephrosia vogelii* and *Securidaca longepedunculata* significantly reduced the number of progeny. *Clausena anisata*, *Dracaena arborea*, *T. vogelii*, *Momordica charantia* and *Blumea aurita* were repellent to the beetle, whereas *Chamaecrista nigricans, Azadirachta indica* and *Hyptis suaveolens* were attractive.

Most of the earlier research the effect of aromatic plant extracts on this beetle and focused on neem extracts which are the most efficient. Neem seeds are not available everywhere and at all seasons. Looking for common and available seeds which can be easily used by farmers, in addition to that of neem become a challenge for the research.

This research reported the effects of 6 common plants extracts including neem and some others which have been never tested on the longevity of adults, the number of eggs laid and adult emergence of *Callosobruchus maculatus*.

MATERIALS AND METHODS

The insects: The insects were collected on cowpea sold at rural markets near N'djamena on October 2002, at the end of the rainy season. They were, first identified then, reared for several months on untreated cowpea seeds at 30°C and 70% r.h. at laboratory before tests. Under these conditions, total development from eggs to adults occurs in about 18 to 25 days. The insects are sexed by examination of the elytral pattern (Southgate *et al.*, 1957; Halstead, 1963). Females are maculated with four elytral spots. Males are plain with less distinctly spots.

Cowpea seeds: Cowpea seeds were bought at Koundoul, a rural market near N'Djamena, after ensuring they were not treated. The seeds are than kept in hermetic flasks at -20°C for 5 days before used.

Oil extraction: The seeds used are from *Azadirachta indica* (Meliaceae), *Ricinus communis* (Capparidaceae), *Thevetia nerifolia* (Apocynaceae), *Balanites eagyptiaca* (Balanitaceae), *Moringa oleifera* (Moringaceae) and *Kaya senegalensis* (Meliaceae). They were collected from plants at Farcha in the vicinity of the Faculty of Sciences. Seeds of *Balanites eagyptiaca* and *Thevetia nerifolia*, because of their hard coat, are first dehulled. All seeds are grounded in warring Blendor grinder type for about 20 min. One hundred grams (100 g) of each grounded seed were extracted at laboratory temperature for seven days with 400 mL of methylene chloride in hermetic flasks. The supernatant is then filtered on wathman paper N°1. The solutions were kept in flasks, under hood, at laboratory temperature until complete methylene chloride evaporation. The obtained oils were used in the experiments.

Bioassays: Five milliliters (5 mL) of tested oil were added to 1 kg of cowpea seeds. The cowpeas were shaken thoroughly to obtain umiform oil coverage.

Adult longevity tests: Three replicates of 50 g of treated cowpeas with each oil were placed in 8.5×5×5 cm plastic boxes. Ten pairs of newly emerged adults were introduced in each box. Three replicates without oil treatment were used as control. The number of dead adults was counted daily. The most toxic oil was tested over a range of concentration between 2 and 10 mL kg^{-1}.

Number of eggs laid: Five pairs of adults were placed on 40 treated cowpea seeds in 3 replicated. Untreated cowpeas were used as control. The total numbers of eggs laid were counted daily for four days when generally there were no more adults alive. The seeds were then kept to observe the emergence of adults.

Germination tests: The treatments with oils were done at the end of the rainy season (the end of November 2003) just after the harvest. At the beginning of the rainy season (June 2004), germination tests have been carried. Fifty of each treated and untreated but attacked cowpea seeds are placed in petridish containing humidified filter paper. A control with untreated and non attacked seeds has been done too. The petri dishes are placed in laboratory temperature. Germinating seeds are counted daily until the third day.

Statistical analysis: Means were compared using student t-test and the level of significant difference was determined at $p<0.05$

RESULTS

Longevity of adults: Table 1 and Fig. 1 show the mortality of insects on treated and non-treated seeds.

All tested oils significantly reduce the longevity of adults on treated cowpea. All adults died in less than 5 days. The less efficient oil is from *Moringa oleifera*. Adults died in four days and this was not significantly different from the control. Oil of *Thevetia nerifolia* is the most efficient because all adults were dead on the second day, therefore the effect of this oil at different the concentration from 2 to 10 mL kg^{-1} was studied (Table 5).

Number of eggs laid: All tested oils reduced the number of eggs laid. *Thevetia nerifolia*, here again, reduced more the number of laid eggs. That can be explain by the fact that all adults died only at the second day (Table 2). Females didn't have the time to laid eggs before dying. In some boxes with treated cowpeas (*Moringa*, *Balanites* and *Ricinus*) eggs laid on the seeds but also on the box surface were observed. It seems that oil created an adherence problem of eggs on seeds thus they fall down in the box.

Development from egg to adult: Adults begin to emerge from seeds at 18th to 25th days.

Table 1: Total mortality of insects on treated cowpea seeds within days

Plant	24 h	48 h	72 h	96 h
Kaya senegalensis	9	14	19	20
Thevetia nerifolia	14	20	-	-
Azadirachta indica	12	16	20	-
Ricinus communis	7	13	17	20
Moringa oleifera	5	9	14	20
Balanites eagyptiaca	10	16	18	20
Control	3	8	12	20

Table 2: Number of eggs laid within four days

Plant	24 h	48 h	72 h	96 h
Kaya senegalensis	8	16	18	18
Thevetia nerifolia	3	3	3	3
Azadirachta indica	4	7	7	7
Ricinus communis	7	17	19	19
Moringa oleifera	9	15	15	15
Balanites eagyptiaca	16	16	16	16
Control	13	32	71	77

Table 3: Total emergence of adults on treated seeds

Plant	Essai 1	Essai 2	Essai 3	Average
Kaya senegalensis	13	12	13	12,66
Thevetia nerifolia	0	2	1	1
Azadirachta indica	2	4	3	3
Ricinus communis	11	14	13	12,66
Moringa oleifera	11	13	11	11,66
Balanites eagyptiaca	11	10	9	10
Control	48	53	56	52,33

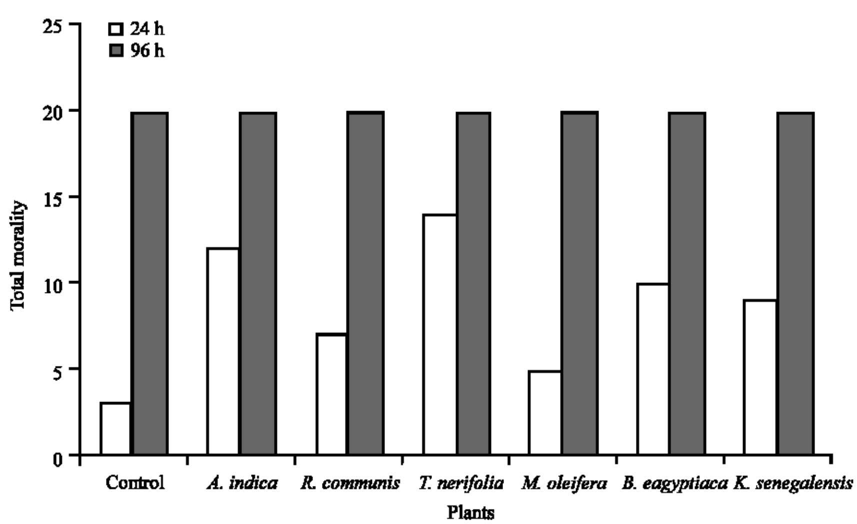

Fig. 1: Insects morality after 24 and 96 h on treated cowpea seeds

Table 4: Total germinated seeds and germination percent

Treatment	1st day	2nd day	3rd day	Germination (%)
Control (untreated and non infested)	36	45	47	94
Untreated but infested	7	12	12	24
Kaya senegalensis	25	34	34	68
Thevetia nerifolia (5 mL kg^{-1})	18	25	36	72
Thevetia nerifolia (10 mL kg^{-1})	17	17	17	34
Azadirachta indica	18	26	31	62
Ricinus communis	22	30	33	66
Moringa oleifera	8	13	13	26
Balanites eagyptiaca	18	24	25	50

Table 5: Total mortality of adults reared on different concentrations of *Thevetia* oil

Concentration of *Thevetia nerifolia* oil	24 h	48 h	72 h	96 h
2 mL kg^{-1}	5	12	18	20
4 mL kg^{-1}	12	20	-	-
6 mL kg^{-1}	14	20	-	-
8 mL kg^{-1}	17	20	-	-
10 mL kg^{-1}	20	-	-	-

Total emergence from control is about 53 insects. Emergence from seeds treated with *Thevetia* oil is the least with an average of 1 adult only. From seeds treated with neem oil only 3 adults emerged. *Moringa, Ricinus, Kaya* and *Balanites* reduced total development too but less than *Thevetia* and neem. Total emergences from cowpea treated with these oils are in average 12 for *Moringa*, *Ricinus* and *Kaya* and 10 for *Balanites* (Table 3).

Seed germination: A large difference can be observed between germination percent of control and that of untreated but infested seeds. The percent of the control is 94% and that of untreated but an infested seed is only 24% (Table 4). The germination of treated seeds is fewer than the percent of control but better than that of untreated and infested seeds. All the treated seeds have a germination percent above 50% except that of *Moringa oleifera*. The best germination percent of treated seeds is that of *Thevetia nerifolia* with 72% and the lowest is that of *Moringa oleifera* with only 26%. A significant difference can be observed too between the germination of seeds treated with *Thevetia* oil at 5 and 10 mL kg^{-1}. At 10 mL kg^{-1} the germination is fewer (34%) than that of seeds treated at 5 mL kg^{-1} (72%).

DISCUSSION

These results confirm that seed oils can be used to protect cowpea against *Callosobruchus maculatus*. Keita *et al.* (2000), Rajapakse and Van Emden (1997), Singh *et al.* (1978), Bhaduri *et al.* (1985), Ketoh *et al.* (2001), Lale and Mustapha (2000), Uvah and Ishaya (1992) reported the effect of different oils, extracted by different methods, on the longevity of *Callosobruchus maculatus* on treated cowpea seeds. Neem oil has been the most studied. It have been demonstrated that neem oil contains azadirachtin, which is toxic to this beetle. The present study shows that seed oil of *Thevetia nerifolia* is more efficient than that of neem in reducing longevity of the adults of *Callosobruchus maculatus*. Pandey *et al.* (1986) reported the effect of this oil on the longevity of *Callosobruchus chinensis* adults reared on seeds of green gram (*Phaseolus aureus*). Seeds oil of *Thevetia nerifolia* seems to be a good alternative to synthetic insecticides.

The effect of concentration of this oil was tested between 2 and 10 mg kg^{-1}. It can be seen on Table 5 that only at the end of the first day all adults died on treated seeds at the concentration of 10 mL kg^{-1}. No eggs have been laid at this concentration. 10 mL kg^{-1} is the concentration generally used to prevent attacks of *Callosobruchus maculatus*. At lower concentrations, all adults died at the second day only (4 to 8 mL kg^{-1}).

About the development from eggs to adults, these results confirm the works done by other researchers. Credland (1992) explain the reduction of emergence by the ovicidal action of the oils. Oils occlude the funnel, which permit gas exchange with outside creating asphyxiation of the eggs. Daniel and Smith (1994) effectively have shown that eggs had an increase in oxygen uptake between the first to 7th days after oviposition. If oils reduce the oxygen uptake, eggs will starve.

These works explain the reduction of total emergence by the ovicidal action of oils. Occluding the funnel by which there is gas exchange between eggs and the outside is a physical action. If it's the only explanation, emergence of the adults will be approximately the same for all oils but table 3 shows differences between oils' actions. The chemical components of oils probably act also on the survival of eggs. The toxic action of neem oil is explained by the presence of a compound, Azadirachtin A.

The reductions of germination of treated seeds can be explained by the problem of water absorption by seeds. Water and oil are not miscible. Seeds' coat is covered of oil after treatment thus they cannot absorb enough water which is necessary for the germination. This situation can create also gas exchange problems. It has been effectively observed that at the 3rd day much of treated seeds, which haven't germinated, rotted away. The treatment of cowpea seeds with oil reduces slightly the germination but it still better than untreated and infested seeds. This study shows that *Thevetia nerifolia* oil, at a concentration of 5 mL kg^{-1}, is the best to protect the cowpea seeds. At higher concentration the treatment affect more the germination of seeds.

The results confirm the efficiency of seed oil of neem which is well established and have been reported by others researchers before. It reveals that the seed oil of *Thevetia nerifolia* extracted by methylene chloride is more efficient than that of neem. All the 6 seed oils tested affect slightly the germination of the treated cowpea seeds. Some of these oils can be used to prevent attack of cowpea by *Callosobruchus maculatus* at concentrations not exceeding 5 mL kg^{-1} (upper concentrations affect the germination of seeds). Seed oil of *Thevetia nerifolia* seems a good alternative to commercial pesticides for the poor African farmers in order to protect their cowpea seeds. This plant is common in tropical Africa where cowpea is the most used.

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