

Evaluation of four local plant species for insecticidal activity against *Sitophilus zeamais* Motsch. (Coleoptera: Curculionidae) and *Callosobruchus maculatus* (F) (Coleoptera: Bruchidae)

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ABSTRACT

The study investigated the insecticidal properties of four local medicinal plants—*Ricinus communis* Linn. (castor bean), *Jatropha curcas* Linn. (coral/purging nut), *Anacardium occidentale* Linn. (cashew nut), and *Erythrophleum saueolens* (sassafras)—under laboratory conditions against two storage pests, *Sitophilus zeamais* (Motsch.) and *Callosobruchus maculatus* (F.), using two methods of treatment, topical application of crude powder water extracts and dried powder admixture in grains. The materials were tested at 0, 1, 5, 10, and 20 per cent concentrations. Contact toxicity, repellence, and inhibition of progeny emergence were monitored after treatments. At 20 per cent, *Ricinus* showed the highest repellence of *C. maculatus* (96.08%), followed by *Jatropha* (68.15%), *Anacardium* (62.30%) and *Erythrophleum* (25.40%), respectively. Against *S. zeamais*, *Anacardium* recorded 80.32 per cent repellence, followed by *Ricinus* (60%), *Jatropha* (58%) and *Erythrophleum* (10.02%). At lower concentrations (1, 5 and 10%), repellence of all the plant extracts did not seem to follow any trend. Dried ground seeds or powder water extract of the four plant materials at a dose of 5 per cent (w/w) significantly reduced progeny emergence in treated maize and cowpea grains. From the study, *R. communis* seems to be the best candidate among the four plant species tested. It is highly toxic, repellent, and inhibits progeny emergence to a larger extent than the other three plants. *Jatropha curcas* and *A. occidentale* were also very effective. It is, therefore, concluded that *R. communis*, *J. curcas*, and *A. occidentale* have great potential to develop into botanical pesticides and must be exploited.

RÉSUMÉ

ADABIE-GOMEZ, D. A., MONFORD, K. G., AGYIR-YAWSON, A., OWUSU-BINEY, A. & OSAE, M.: *Evaluation de quatre espèces de plante locale pour l'activité insecticide contre Sitophilus zeamais* Motsch. (Coleoptera: Curculionidae) et *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae). Les propriétés insecticides de quatre plantes médicinales locales: *Ricinus communis* Linn. (haricot de ricin), *Jatropha curcas* Linn. (noix de purge/corail), *Anacardium occidentale* Linn. (noix de cajou), et *Erythrophleum saueolens* (le mançone) étaient étudiées sous les conditions de laboratoire contre deux ravageurs de stockage: *Sitophilus zeamais* (Motsch.) et *Callosobruchus maculatus* (F.) en utilisant deux méthodes de traitement: application actuelle d'extraits de poudre brute en eau et la poudre sèche additionnée engrains. Les matières étaient mises à l'essai à 0, 1, 5, 10, et 20% de concentrations. Toxicité par contact, repoussement et l'inhibition de l'émergence de progéniture étaient suivies de près après les traitements. A 20%, *Ricinus* montrait le repoussement le plus élevé de *C. maculatus* (96.08%), suivi par *Jatropha* (68.15%), *Anacardium* (62.30%) et *Erythrophleum* (25.40%), respectivement. Contre *S. zeamais*, *Anacardium* donnait 80.32% de repoussement, suivi par *Ricinus* (60%), *Jatropha* (58%), et *Erythrophleum* (10.2%). Aux concentrations plus faibles de (1, 5 et 10%), le repoussement de tous les extraits de plantes ne donnaient pas l'impression de suivre aucune tendance. Les grains secs moulus ou l'extrait de poudre en eau des quatre matières de plantes à une dose de 5% (w/w) causaient des réductions considérables dans l'émergence de progéniture de grains de maïs et de dolique traités. D'après l'étude, *R. communis* semble être le meilleur spécimen de quatre espèces de plantes mises à l'essai. Elle est hautement toxique, repoussante et inhibe l'émergence de progéniture dans une plus grande mesure que les trois autres plantes. *Jatropha curcas* et *A. occidentale* étaient également très efficace. Donc, la con-

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clusion est tirée que *R. communis*, *J. curcas* et *A. occidentale* ont beaucoup de potentiel pour le développement en pesticides botaniques et devraient être exploités.

Introduction

Maize and cowpea grains are stored for a uniform supply of food, to provide reserve for contingencies, and to speculate on high prices (Degage, 1999). However, insect pests cause losses ranging from 20 to 30 per cent during grain storage (Eitcha & Tadesse, 1999). Control of insect pests in the stored product environment has, over the years, been mainly dependent on using chemical insecticides and, to a little extent, on using biological methods. However, this dependence on chemical pesticides has resulted in a complex of pest control problems in the stored product environment; the most important being the effect these chemicals have on the consumer and the environment as well as the cost to the farmer. Thus, the need is to develop alternative control strategies that would reduce or eliminate these problems.

At the Biotechnology and Nuclear Agriculture Research Institute (BNARI) of the Ghana Atomic Energy Commission (GAEC), research into applying gamma radiations in controlling stored product pests has been going on over the years. It has been realized from these researches that no single control strategy can be relied on for sustained, effective control of stored product pests. Therefore, the need is to find alternative control methods that can be combined with irradiation in an integrated system. Attention is now focused on folk medicinal plants that are available in the wild. For subsistence farmers in the developing world including Ghana, medicinal plants could be a cheap and safe alternative to synthetic chemical insecticides.

Plants are a rich source of compounds that have insecticidal activity (Jacobson, 1975). The effectiveness of some plants against stored product insects has already been demonstrated

(Jacobson, 1975; Abbiw, 1990; Kis-Tamas, 1990). Among these, extracts of the neem tree, *Azadirachta indica* A. Juss, has received the most attention (Jacobson, 1975; Saxena, Jilani & Kareem, 1988). *Jatropha curcas* has also been well studied in Ghana and has been established to have pesticidal properties (Cobbinah & Appiah-Kwarteng, 1991; Cobbinah & Tuani, 1992; Owusu-Akyeaw, pers. com.).

This study was aimed at evaluating the insecticidal activity of *J. curcas* and three less studied medicinal plants—*R. communis*, *A. occidentale* Linn. and *E. sauvekens*—on two stored product insects, *C. maculatus* and *S. zeamais*. Characteristics studied included toxicity, repellence, and inhibition of development.

Materials and methods

Collection and preparation of plant materials
Four local plant species were selected for the study. Dried seeds of three plant species, *Ricinus communis*, *Jatropha curcas* and *Erythrophleum sauvekens*, were harvested on the plants from Dodowa, Agomeda and Kwabenya near Accra. Nuts of the fourth plant, *Anacardium occidentale*, were collected from Dome. Seeds of *J. curcas* and *R. communis* were further dried at $27 \pm 1^\circ\text{C}$ for 7 days and then ground with a Hammer laboratory mill to a fine powder. Seeds of *E. sauvekens* and nuts of *A. occidentale* were roasted in an aluminium pan on a hotplate for 1 h, allowed to cool, and then ground with a Hammer laboratory mill to a fine powder. The seed and nut powders were kept in black poly bags in a refrigerator till they were to be used. Some powder for each plant species was used directly as admixture to grains and some used for the powder water extraction. The different treatments of the plant materials were based on their traditional uses in the country.

Crude extraction

Solutions of 20 per cent (w/v) seed powder were prepared to 500 ml in conical flasks for all the four plant species. The conical flasks containing the solutions were placed in a water bath at 40 °C for 3 days and shaken every 6 h. The solutions were then filtered through Whatman filter paper using a vacuum pump. The extracts were diluted to 1, 5, 10 and 20 per cent for use in toxicity tests.

Grains

Grains of maize and white cowpea, purchased from the Madina market near Accra, were sieved to remove any extraneous materials and insects. The grains were then frozen for 2 weeks to kill any insects and also their eggs, larvae or pupae hiding within the grains. Before use, the grains were removed and kept on the laboratory shelf for 24 h to equilibrate.

Culturing of insect pests

Sitophilus zeamais and *Callosobruchus maculatus* were cultured in the laboratory of the Department of Food Science and Radiation Processing, GAEC. Insects used to set up the stock culture were collected from the Malamata and Madina markets near Accra. Kilner jars (22 cm height; 7.5 cm diameter) were filled with 200 g of maize (10 jars) and cowpea (10 jars). Thirty unsexed adult *S. zeamais* and *C. maculatus* were placed in each of the Kilner jars of maize and cowpea, respectively, and covered with pieces of muslin cloth (0.5mm mesh). One week after introducing the insects when they would have laid eggs, they were sieved out of the grains. All the 30 insects placed in each jar were removed carefully. The set-up was placed on laboratory shelves at $27 \pm 1^\circ\text{C}$, 67 ± 10 per cent RH, and 12/12 light/darkness for eggs to develop. Three days after the first adult insects were observed (i.e., when the insects were between 0 and 3 days old), they were sieved out and used for the bioassays or for subcultures.

Mortality and progeny emergence bioassays

The experiments for *S. zeamais* and *C. maculatus* were run concurrently. One hundred grams (100 g) of equilibrated grains were weighted into jam jars (12 cm height \times 5.5 cm diameter). Seed powder was added to the jars at 0, 1, 5, 10 and 20 g to give 0, 1, 5, 10 and 20 per cent seed powder concentrations for maize and cowpea. Each concentration was replicated three times. Twenty 0 to 3-day-old adult *S. zeamais* and *C. maculatus* were introduced into each jar of maize and cowpea, respectively, and covered with pieces of muslin cloth. The set-up was kept on the laboratory shelves for 7 days. The insects were then sieved out and the number of dead and living insects were counted and percentage mortality determined. All the insects were removed and the set-up kept till progeny emergence. The number of progeny emerging were counted and removed from the set-ups from the 30th day and 25th day after introduction for *S. zeamais* and *C. maculatus*, respectively; and thereafter, every 24 h for 12 days when no emergence was recorded in the treatments.

Topical application bioassay

A total of 20 adults, comprising 10 females and 10 males of *S. zeamais* and *C. maculatus*, were used for each concentration. Concentrations of 0, 1, 5, 10 and 20 per cent (v/v) crude extract preparations were used with three replications. The head of an insect pin was dipped into the solution and the first drop allowed to fall off; the second drop was applied to the mid-thoracic region of the insect. The insects were then placed individually in 1.5 cm \times 1.5 cm \times 1.5 cm-glass vials with a grain of maize for *S. zeamais* and cowpea for *C. maculatus*. The vials were then covered and kept on the laboratory bench for 7 days after which mortality was determined.

Repellency bioassay

Food preference chambers were prepared by joining two GA-7-3 vessels (3" \times 3" \times 4") together at the base by a T-shaped transparent glass tube

with the tail end connected to a hole drilled at the base of a plastic Petri dish (90 mm diameter). One hundred grams of equilibrated maize for *S. zeamais* and cowpea for *C. maculatus* were put into each of the two GA-7-3 vessels. The grains in one of the vessels were mixed with seed powder at 1, 5, 10 and 20 per cent, and those in the other vessel were untreated and served as control. Each concentration was replicated three times. Fifty adult *S. zeamais* for maize and *C. maculatus* for cowpea were introduced into the Petri dish for each set-up. The end of the long arm of the T-shaped tube connected to the Petri dish was opened after 1 h when the insects had stabilized. The set-up was left for 24 h and the number of insects in the control and treated vessels were counted. Percentage repellence was determined using the formula: $Pr = (Nc - Nt) / (Nc + Nt) \times 100$, where Pr = percentage repellence, Nc = number of insects in control, and Nt = number of insects in treated grains.

Data analysis

All mortality counts were corrected for natural mortality using Abbot's formula (Abbot, 1925). Means were compared using ANOVA in SPSS ver10. Probit analysis in SPSS was used to determine LC_{50} .

Results

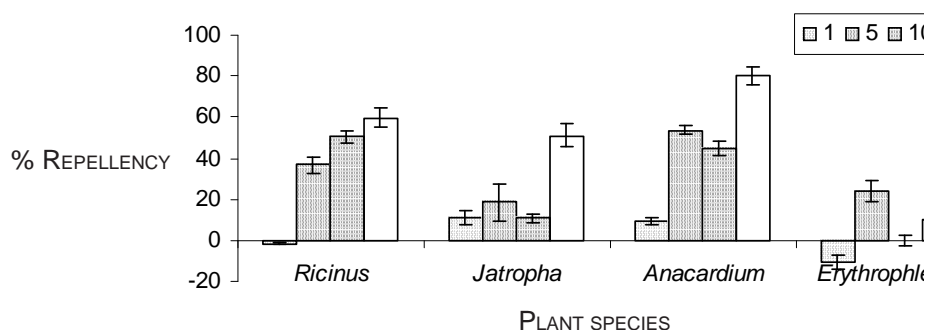
Repellence of *S. zeamais* and *C. maculatus* by plant materials

Fig. 1 presents results of repellence bioassays for extracts of the four plants on *S. zeamais* and *C. maculatus*. Against *S. zeamais* (Fig. 1a), *Anacardium* performed best, giving 80.32 per cent repellence, while *Erythrophleum* showed significantly least repellence (10.02%). At 20 per cent, *Ricinus* seemed to show significantly highest repellence of *C. maculatus* (96.08%), followed by *Jatropha* (68.15%) and *Anacardium* (62.30%), respectively; while *Erythrophleum* showed the lowest repellence of *C. maculatus* of 25.40 per cent (Fig. 1b). At lower concentrations (1, 5 and 10%), repellence of all the plant extracts did not seem to follow any trend. Repellency of *C. maculatus* was significantly higher ($P < 0.05$) than that of *S. zeamais* for all the plants, except for *Anacardium*.

Mortality effect of plant materials on *S. zeamais* and *C. maculatus*

Fig. 2 shows mortality of *S. zeamais* and *C. maculatus* due to topical application of extracts from the four plants and powder admixture treatment of grains. Generally, *Ricinus* caused higher mortality in both insects for topical application and admixture treatment. With the topical application bioassay on *S. zeamais*, *Jatropha*, *Anacardium* and *Erythrophleum*

(a) *Sitophilus zeamais*



(b) *Callosobruchus maculatus*

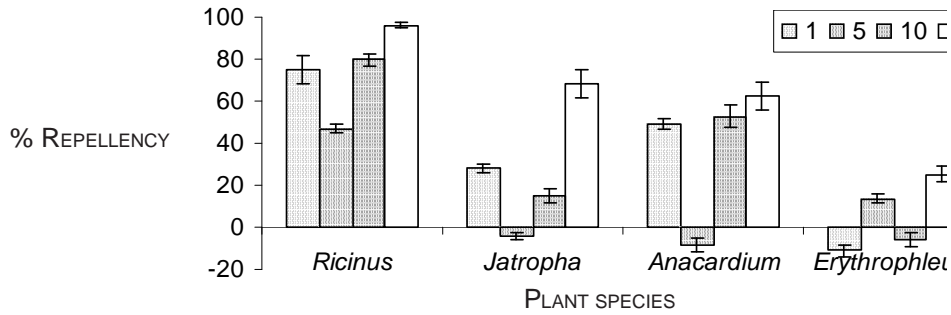


Fig. 1. Repellence of *S. zeamais* and *C. maculatus* by extracts of four plant species.

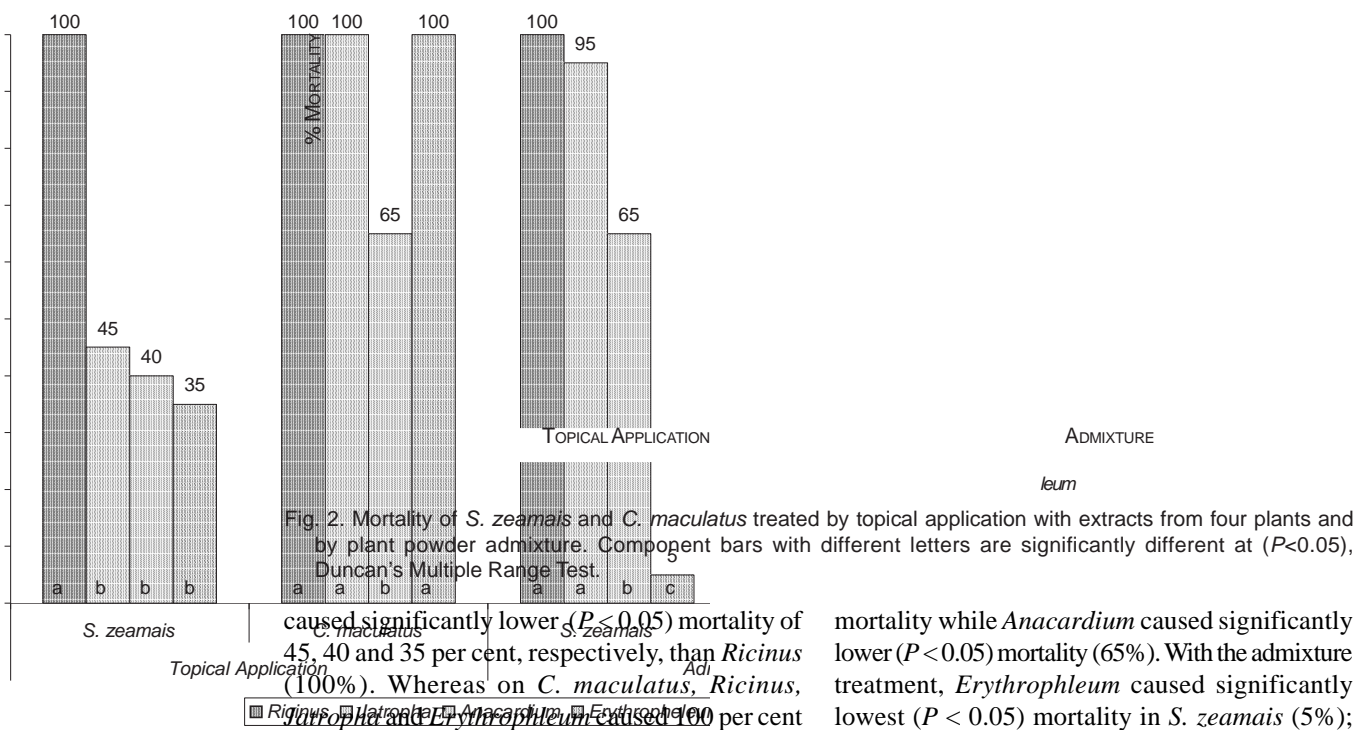


Fig. 2. Mortality of *S. zeamais* and *C. maculatus* treated by topical application with extracts from four plants and by plant powder admixture. Component bars with different letters are significantly different at ($P < 0.05$), Duncan's Multiple Range Test.

caused significantly lower ($P < 0.05$) mortality of 45, 40 and 35 per cent, respectively, than *Ricinus* (100%). Whereas on *C. maculatus*, *Ricinus*, *Jatropha* and *Erythrophleum* caused 100 per cent

mortality while *Anacardium* caused significantly lower ($P < 0.05$) mortality (65%). With the admixture treatment, *Erythrophleum* caused significantly lowest ($P < 0.05$) mortality in *S. zeamais* (5%);

Erythrophleum and *Anacardium* caused significantly lowest ($P < 0.05$) mortality in *C. maculatus* (30 and 40%, respectively).

Using probit analysis in SPSS, median lethal concentrations (LC_{50} s) were estimated for all four plant materials applied topically and as admixtures to *S. zeamais*, and then for all four plants applied as admixture to *C. maculatus*. The LC_{50} s for the plant materials applied to *C. maculatus* topically could not be determined because the data were unsuitable for probit analysis. Table 1 shows the LC_{50} s. *Erythrophleum* had the largest LC_{50} against *S. zeamais* for both treatments (111.64 g for topical application and 132.85 g for admixture application). *Ricinus* had the lowest LC_{50} for *S. zeamais* treated topically (0.79 g), and *Jatropha* had the lowest

in the 1 per cent treatment, 3 in the 5 per cent treatment, and completely preventing emergence in the 10 per cent treatment. *Jatropha* did not perform too well against *C. maculatus* at 1 per cent, but completely prevented progeny emergence at 5 per cent. *Anacardium* could not completely inhibit progeny emergence of *S. zeamais*, but reduced the number of progeny from 178 in the control to 25 in the 20 per cent treatment. It could also not reduce emergence of *C. maculatus* to any significant level. *Erythrophleum* could also not reduce progeny emergence of any of the two insects; it rather seemed to significantly increase the number of progeny emergence in *C. maculatus*.

TABLE 1

Median Lethal Concentration (LC_{50}) of Materials From Four Plants Applied Topically and as Admixture to *S. zeamais* and *C. maculatus*

Plant	S. zeamais						C. maculatus		
	Admixture			Topical application			Admixture		
	LC_{50}	LFL	UFL	LC_{50}	LFL	UFL	LC_{50}	LFL	UFL
<i>R. communis</i>	5.85	2.90	10.05	0.79	0.01	3.58	3.55	1.63	6.08
<i>J. curcas</i>	3.51	1.63	6.21	41.21	9.13	2206.20	6.21	3.22	10.64
<i>A. occidentale</i>	20.32	11.05	45.67	48.33	10.45	8996.46	47.94	20.40	151.36
<i>E. sauevelens</i>	132.85	11.91	2088.63	111.64	19.91	99315.22	46.28	17.16	143.97

Key: LFL – Lower fiducial limit; UFL – Upper fiducial limit

for *S. zeamais* treated by admixture (3.51g). For *C. maculatus* treated by admixture, *Anacardium* had the highest LC_{50} (47.94 g) and *Ricinus* the lowest.

Effect of plant powder on progeny emergence for *S. zeamais* and *C. maculatus*

Fig. 3 shows the effects of powders of all four plants on the emergence of progeny for *S. zeamais* and *C. maculatus*. *Ricinus* seemed to be the most effective against *C. maculatus*; it completely inhibited progeny emergence at 1 per cent, whereas there were 460 progenies in the control. *Jatropha* was best against *S. zeamais*, reducing progeny emergence from 211 in the control to 10

Discussion

Plants produce and store varied secondary metabolites that have been found to play a protective role against herbivores (Weaver et al., 1991; Wink, 1993). These metabolites are exploited for insect pest control in stored products. However, the insecticidal activities vary from plant to plant, and these variations depend largely on the chemical composition of the plant species (Kis-Tamas, 1990; Wink, 1993). By their effect on insects, natural plant compounds may be grouped as toxic substances or behaviour-modifying substances (Chaudhury, 1990). The toxic substances may be ovicidal, larvicidal, or

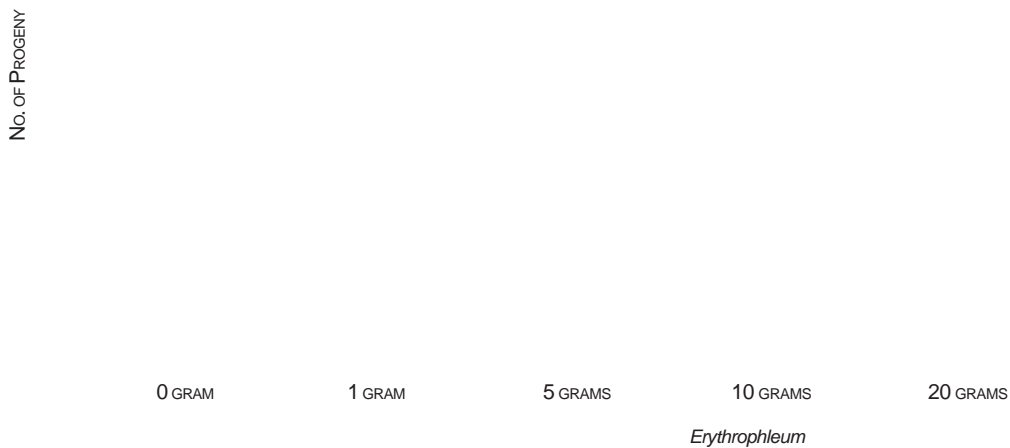


Fig. 3. Effect of powders of four plants applied to grains as admixtures at different concentrations on progeny emergence of *S. zeamais* and *C. maculatus*.

adulticidal. They include nicotine, rotenone, ryania, pyrethrins, phenols, and quassin. The behaviour-modifying compounds such as asarones, ajugarins, some pyrethrins, sparteines, cytosine, lupinine and angustifoline may act as antigonadals, oviposition deterrents, growth and development inhibitors, antifeedants or repellents or both (Chaudhury, 1990; Kis-Tamas, 1990; Wink, 1993). All the four plant species studied seem to have toxic and behaviour-modifying properties to varying degrees. A good candidate for botanical pesticide that is effective against stored product insects is a plant that combines as many of these toxic and behaviour-modifying properties as possible (Kis-Tamas, 1996).

From the study, *R. communis* seems to be the best plant among the four species tested. It is highly toxic, repellent, and inhibits progeny emergence to a larger extent than the other three

plant species. *Jatropha curcas* and *A. occidentale* were also very effective, but *E. sauveiens* was the least repellent, least toxic and rather seemed to enhance progeny emergence instead of inhibiting it. The high toxicity of the plant materials to the two insects could be attributed to the constituent secondary metabolites reputed for insecticidal activity. The inhibition of progeny production by *R. communis*, *J. curcas* and *A. occidentale* suggests that these plants have ovicidal and repellent properties (Udo, Owusu & Obeng-Ofori, 2004). The combined low repellence and lack of anti-reproduction properties shown by *E. sauveiens* make repellence a possible means of inhibiting progeny production in the other three plants studied.

Conclusion

Ricinus communis, *J. curcas* and *A. occidentale*

have great potential to develop into botanical pesticides and must be exploited. *Erythrophleum sauevelens* must be studied further to determine which specific part has the insect control properties and against which insects it is most effective. Further studies into extracting, isolating, characterizing and formulating these plant species is recommended.

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