Efficacy of some botanical extracts against *Callosobruchus maculatus* in cowpea seeds and an evaluation of their toxicity

S.I. Ahmed[A] and **A.A. Gazzy**[B]
[A] Pesticides Department, Faculty of Agriculture, Kafr El-Sheikh University, 33516, Egypt.
[B] Biology Department, Faculty of Science, Kafr El-Sheikh University, 33516, Egypt.

Abstract
Extracts of seven plant species were tested under laboratory conditions for their ability to protect cowpea seeds against the insect *Callosobruchus maculatus*. The insect was reared and tested on whole cowpea seeds with respect to the adult's mortality, hatchability of laid eggs and emergence. Gas chromatography-mass spectrometry (GC–MS) analysis was carried to identify the chemical components of the most effective plant extracts against *C. maculatus*. Furthermore, the safety of the most effective plant extracts was evaluated with respect to biochemical and histological changes in rats.

The results revealed that *Euonymus japonicus* and *Cassia fistula* extracts were the most effective against *C. maculatus* with respect to insect mortality and progeny relative to the control. The GC-MS analysis of these extracts showed the presence of different bioactive compounds. These extracts also showed low toxicity on treated rats. The results suggest that these extracts may be a safe alternative to insecticides.

Keywords: stored products, malathion, cowpea, extract.

Introduction
Most research in stored-product protection focuses on cereals, the most common commodity in storage, and there are several insects and moulds that can reduce their quality and quantity. Beans, including cowpea, *Vigna unguiculata* (L.) Walp., are an important staple worldwide (Rajapakse and van Emden 1997) providing the second most important source of human dietary protein and the third most important source of calories (Pachico 1993).

A key pest of cowpea is the cowpea weevil, *Callosobruchus maculatus* (F.), a bruchid that infests both pods in the field and seeds in storage (Stoll 1988). Females lay their eggs on the surface of the seeds. Approximately 4–5 days later the egg hatch (26–28°C). The first instar larvae burrow through the seed coat and into the seed. The development of the larvae and pupation is completed entirely in the seed. Adults emergence from the seeds after 25–30 days at 25°C (Fox and Tattor 1994).

According to Singh *et al.* (1978), 100% of cowpea seeds are infested after 3 ± 5 months of storage. Tanzubil (1991) found that this insect can damage 100% of stored seeds causing weight losses of up to 60%. After six months of storage, losses in terms of perforated seeds can reach 90% (Seck *et al.* 1991).

The application of insecticides and fumigants to grain in storage can lead to the development of insecticide resistance, possible health hazards especially to mammals, and the risk of environmental contamination (Cox 2004). Therefore, effort has recently focused on the screening of natural products as alternatives to conventional insecticides for stored-grain insect pest control (Weaver and Subramanyam 2000, Cox 2004, Rajendran and Sriranjini 2008). At present, there are two natural alternatives to chemical control that are increasingly being investigated for integrated pest management (IPM) in stored products, namely, plant essential oils and diatomaceous earths. Essential oils from plants have already been used as raw materials in many fields, including perfumes, cosmetics, phytotherapy and nutrition. They also have potential as a source of insecticides with environmental compatibility (Katz *et al.* 2008).

Recently, many studies have focused on the possibility of using plant essential oils for protection of stored grain from insect pests (Collins 2006, De Carvalho and Da Fonseca 2006). However, research should also focus on the safety of botanical extracts with regard to human health. An assessment of enzymatic activity in the blood is generally a more sensitive measure of a compound's toxicity than assessment of histopathological changes; although the latter can be assessed within a shorter time period. Nevertheless, observation of tissue alterations is considered to have a confirmatory and supporting diagnostic role for detecting certain blood abnormalities, and so may have potential relevance as a preliminary test for the toxicity of botanical extracts (Cornelius *et al.* 1959).

Therefore, this study aims to evaluate insecticidal activity of some plant extracts (*Cassia senna*, *Caesalpinia gilliesii*, *Thespesia populnea* var. *acutiloba*, *Chrysanthemum frutescens*, *Euonymus japonicus*, *Bauhinia purpurea* and *Cassia fistula*) against *C. maculatus* in cowpea seeds with the respect to progeny and mortality of adult insects, to identify the chemical components of the most effective plant extracts and finally to evaluate their toxicity to rats with respect to biochemical and histological changes.

Materials and methods
The insect
Callosobruchus maculatus (Egyptian strain) was obtained from the Department of Stored Product Pests Control, Research Institute of Plant Protection, Sakha Kafr El-sheikh. This strain was continuously reared free of insecticidal contamination for several years at 30 ± 2°C and 70 ± 5% relative humidity (r.h.). The culture was raised by adding about 60 newly emerged *C. maculatus* adults to 500 g of cowpea seeds in large box and kept under laboratory condition in the Pesticide Department. After, 35 days newly emerged (F1) adults were collected and used to infest the cowpea samples.

The stored product
Cowpea seeds were used to culture *C. maculatus* and to evaluate the efficacy of tested plant extracts and malathion against the insect. Clean uninfested cowpea seeds were stored in airtight tins until required for experiments. The experiments were carried out in a room kept at a constant temperature of 25°C and 70% r.h.

Plants and preparation of crude extracts
The leaves of seven medicinal plant species (*Cassia senna*, *Caesalpinia gilliesii*, *Thespesia populnea* var. *acutiloba*, *Chrysanthemum frutescens*, *Euonymus japonicus*, *Bauhinia purpurea* and *Cassia fistula*) were collected from a local nursery at Kafr El-Sheikh, Monofia, Gharbia and Alexandria Governorates, Egypt. *Cassia senna* (Alexandrian senna) (Fabaceae) is native to tropical Africa and is cultivated in Egypt and Sudan. *Caesalpinia gilliesii* (bird of paradise) (Fabaceae) is native to tropical America, mainly Argentina and Uruguay. *Thespesia populnea* var. *acutiloba* (wild tulip tree) (Malvaceae) is native to South Africa. *Chrysanthemum frutescens* (marguerite daisy) (Asteraceae) is native to the Canary Islands. *Euonymus japonicus* (Japanese spindle) (Celastraceae) is native to Japan, Korea and China. *Bauhinia purpurea* (purple camel's foot) (Fabaceae) is native to South China. *Cassia fistula* (golden

shower) (Fabaceae) is native to southern Asia.

Leaf samples were oven dried for 24 h at 70°C and then, finely powdered using a blender. Each sample (25 g) was extracted twice with 300 mL of methanol at room temperature for two days. The extracts were filtered through Whatman filter paper (No.15). The combined filtrate was concentrated to dryness by rotary evaporation at 40°C.

Effect of plant extracts and malathion on emergence of C. maculatus adults

Twenty grams of cowpea seeds were treated with an aqueous solution of the plant extract at concentrations of 100, 200 and 300. For malathion, cowpea seeds were treated at concentrations of 5, 10 and 20 ppm. The seeds were treated by dipping them twice into water solutions of malathion and botanical extracts at tested concentrations for five seconds and then spreading them on plastic sheets to dry for 90 min. The control treatment used water only. All treatments were replicated three times.

The treated cowpea seeds were then transferred to 85 × 75 mm plastic jars and 10 *C. maculatus* adults were added and kept at 30 ± 2°C and 70 ± 5% r.h. according to the method described by Kestenholz *et al*. (2007). Hatchability, emergence and reduction percentages were calculated as shown in equations 1, 2 and 3 as described by El-Lakwah *et al*. (1992).

% Hatchability = (Mean no. of hatched eggs/Mean No. of egg laid) × 100 (eq. 1)

% Emergence = (Mean no. of emerged adults/Mean No. of eggs laid) × 100 (eq. 2)

% Reduction = ((MNEC-MNET)/ MNEC) × 100 (eq. 3)

MNEC = Mean no. of emerged in control

MNET = Mean no. of emerged in treatment

Effect of plant extracts and malathion on mortality of C. maculatus

Twenty grams of cowpea seeds were treated with aqueous solutions of the plant extract and malathion and transferred to plastic jars as above. The number of dead insects in each jar was counted twice at one and two weeks after treatment and the percentage of insect mortality was recorded and corrected using the Abbott formula (1925).

Chemical composition of the most effective plant extracts

Gas chromatography/mass spectrometry (GC/MS) analysis was carried to identify the chemical components of the most effective plant extracts (*Euonymus japonicus* and *Cassia fistula*) against *C. maculatus* according to the method described by Durate-Almeida *et al.* (2004). The samples were injected three times for confirmation. The analysis was conducted on HP 6890 GC system coupled with a 5973 network mass selective detector with a capillary column of HP-5MS (60 m × 0.25 mm, film thickness 0.25 μm). The oven temperature program was turned on at 50°C, held for 2 min then raised to 200°C at a rate of 5°C min^{-1}. Helium was used as the carrier gas at a flow rate 1.0 mL min^{-1}, with a split ratio equal to 1/50. The detector and injector temperatures were 250 and 200°C, respectively.

Some of the detected compounds in the tested plant extracts were identified by comparison of their retention indices (RI) and mass spectra fragmentation with those of the available analytical standards (1,8 cineole; linalool; n-hexadecanoic acid and tetradecanoic acid) and mass spectra fragmentation stored in Wiley 7n.1 and NIST libraries in GC-MS. The other detected compounds were identified only by comparison of their retention indices (RI) and mass spectra fragmentation with those stored in Wiley 7n.1 and NIST libraries in GC-MS. The samples were analyzed at the central laboratory for pesticides, Agriculture Research Centre, Egypt.

Animal treatment

Adult Wistar male rats (*Rattus norvegicus*), eight weeks old and 80–100 gm in weight were obtained from Faculty of Medicine, Tanta University. Rats were housed in wire cages under standard conditions with free access to drinking water and food in a temperature controlled room with 14 h light and 10 h dark cycle. The rats were given a standard diet as describe by Romestaing *et al.* (2007). Before treatment, rats were left two weeks for adaptation. The animals were randomly divided into three groups of three. Two groups were treated with the most effective plant extracts and the third group was the control. Extracts of *Euonymus japonicus* and *Cassia fistula* were administered once orally at a concentration of 500 mg kg^{-1} body weight. The control group rats were administrated once orally with an equal amount of almond oil. After 21 days the rats were sacrificed under anaesthesia. Then, blood samples were taken, under anaesthesia, by cardiac puncture and stored in vials containing heparin. Specimens from kidney and liver were taken from each treatment and kept in neutral buffered formalin 10% for histopathological tests.

Enzymes assays

Blood samples were centrifuged at 4500 rpm for 15 min at 4°C and the blood serum was used to determine the Glutamate Pyruvate Transaminase (GPT), creatinine and alkaline phosphatase (ALP) according the methods described by Barham and Trinder (1972), Reitman and Frankel (1957) and Wilkinson *et al.* (1969), respectively.

Histopathological test

The histopathology test was carried out at Histopathology Laboratory, Department of Histopathology, Faculty of Veterinary Medicine, Kafr El-Sheikh University, according to the method described by Bancroft and Stevens (1996).

Statistical analysis

Data from the experiments were statistically analyzed using one-way repeated measurement analysis of variance. Duncan's multiple range test (Duncan 1955) were used to separate means using SAS program (Version 6.12, SAS Institute Inc., Cary, USA).

Results

Effect of plant extracts and malathion on the emergence of C. maculatus

The effect of malathion and the plant extracts on some biological aspects of *C. maculatus* were shown in Table 1. The numbers of eggs laid, hatchability and emergence as a percentage of the control, were significantly decreased in most treatments and the reductions were dose dependent. Malathion followed by *Cassia fistula* and *Euonymus japonicus* extracts recorded the highest reduction of emergence as a percentage of the control while *Chrysanthemum frutescens* extract was the least effective.

Malathion and *Euonymus japonicus* extracts were the most effective treatments in reducing eggs laid and *Cassia senna* extract was the least effective. Malathion and *Cassia fistula* extract were the most effective treatments in reducing egg hatchability and *Chrysanthemum frutescens* extract was the least. *Cassia fistula* and malathion were the most effective treatments in reducing emergence while *Caesalpinia gilliesii* was the lowest. Generally, malathion, *Cassia fistula* and *Euonymus japonicus* extracts were the most effective treatments in controlling the progeny of *C. maculatus* adults.

Effect of plant extracts and malathion on mortality of C. maculatus adults

The efficacy of the plant extracts and malathion in killing *C. maculatus* adults are presented in Table 2. Malathion and all botanical extracts showed significance differences in the morality of adult C. *maculatus* relative to control treatment. Malathion, *Euonymus japonicus* and *Cassia fistula* extracts were the most effective treatments followed by *Bauhinia purpurea*, *Thespesia populnea* var. *acutiloba*, *Cassia senna*, *Caesalpinia gilliesii* and *Chrysanthemum frutescens* respectively. The mortality of *C. maculatus* significantly increased in the second week relative to the first week in all tested treatments and was concentration dependent.

Table 1. Effect of the tested plant extracts and malathion on some biological aspects of *C. maculatus*.

Treatments	Concentration (ppm)	No of laid eggs	Hatchability (%)	Emergence (%)	Reduction (%)
Cassia senna	100	381 a[A]	70.0 d	58.5 c	40.0
	200	376 a	68.0 ef	55.3 e	45.8
	300	360 b	65.5 g	43.2 j	60.8
Caesalpinia gilliesii	100	330 c	89.5 b	55.0 b	35.0
	200	320 e	87.5 c	47.0 f	49.0
	300	300 g	85.6 e	42.0 i	58.5
Thespesia populnea var. *acutiloba*	100	293 h	80.2 g	54.5 g	50.8
	200	285 i	73.7 i	50.5 i	59.2
	300	253 l	71.0 l	48.3 l	66.5
Chrysanthemum frutescens	100	280 j	90.0 f	58.7 d	43.0
	200	266 k	82.7 r	55.0 r	53.5
	300	260 l	77.0 j	53.5 i	58.8
Euonymus japonicus	100	320 de	83.4 d	39.7 i	59.2
	200	220 n	75.0 m	44.2 m	71.9
	300	164 q	72.0 o	5.0 o	79.2
Bauhinia purpurea	100	316 f	80.6 ef	63.5 b	38.0
	200	248 m	78.6 k	60.5 r	54.6
	300	166 q	71.7 o	58.5 m	73.0
Cassia fistula	100	362 a	69.6 f	37.7 k	63.5
	200	263 k	63.0 m	36.0 n	76.9
	300	215 o	58.0 n	33.6 p	83.8
Malathion	1	177 p	56.5 p	89.0 l	65.8
	2	101 r	44.5 q	71.0 q	76.0
	3	62 s	40.3 r	60.0 r	87.7
Control	0.0	325 cd	100.0 a	100.0 a	0.00
Significance					
Treatment type (TT)		***	***	***	
Treatment concentration (TC)		***	***	***	
Interaction (TT × TC)		***	***	***	

[A] Letters indicate differences between means at P = 0.05 using Duncan's multiple range test (Duncan 1955).

Composition of the most effective plant extracts

The identified chemical components of *Euonymus japonicus*, *Cassia fistula*, the most effective extracts against *C. maculatus*, are listed in Tables 3 and 4. Twenty six compounds were identified from *Euonymus japonicus* and 16 compounds from *C. fistula* extracts. The identified compounds were fatty acids and their derivatives (aldehydes, esters, alcohols).

Toxicity evaluation

Effect of the most effective plant extracts on liver enzymes The activities of the liver function enzymes ALP and GPT were determined to evaluate the toxicity of *Euonymus japonicus* and *Cassia fistula* extracts. The data in Table 5 showed that there were no significant differences in the activity of ALP and GPT after 21 days of treatment with the most effective plant relative to the control.

Effect of *Euonymus japonicus* and *Cassia fistula* extracts on kidney function There were no significant differences in creatinine level in rats administrated with *Euonymus japonicus* and *Cassia fistula* extracts relative to the control (Table 5). which suggests that the rats had normal kidney function. Moreover, the histology of kidney tissue treated with the tested plant extracts relative to control confirmed this explanation.

The histopathological changes in the kidney The normal structure of kidney tissue is shown in Figure 1a. For the rats treated with *Euonymus japonicus* extract the tissue was somewhat like the control but for small vaculation and degeneration in renal tubules (Figure 1b). For the rats treated with *Cassia fistula* extract kidney tissue was also normal as control except for some collecting tubules (Figure 1c).

The histopathological changes in the liver The normal structure of liver tissue is shown in Figure 2a. For the rats treated with *E. japonicus* extract, blood vessels appeared to be engorged with blood and hepatocytes contained vacuolated cytoplasm (Figure 2b). However, the treated livers were similar to controls. In the case of rats treated with *Cassia fistula* extract the liver appeared to be normal but for some blood vessels engorged with blood, few lymphocytic infiltrations and activation in Kopffer cells (Figure 2c).

Discussion

The results of this study implied that the tested plant extracts were effective in controlling *C. maculatus* adults and their progeny in stored cowpea. The efficacy of plant extracts against *C. maculatus* in stored cowpea has been reported by many researchers (Tapondjou *et al.* 2002, Ketoh *et al.* 2005, Kestenholz *et al.* 2007, Iboudo *et al.* 2010). However, the efficacy of the plant extracts tested here, especially those most effective against *C. maculatus*, have not been reported before.

The efficacy of extracts from *Cassia fistula* and *Euonymus japonicus* was similar to the recommended chemical compound malathion. This is suggests that the use of these products may be an alternative to the chemical control of *C. maculatus*. This could reduce the use of pesticides, minimizing their hazards to the environment and human health, and also help to overcome the problem of resistance development in *C. maculatus* to chemical insecticides.

GC-MS analysis of *Euonymus japonicus* and *Cassia fistula* extracts identified compounds, such as 1,8-cineole, linalool, tetradecanoic acid, octadecatrienoic acid and hexadecanoic acid and hexadecanoic acid methyl ester at high percentages relative to other detected compounds. The insecticidal activity of these extracts against *C. maculatus* may be due to the presence of these fatty acids and their derivatives (Negahban *et al.* 2006, Rozman *et al.* 2007, Ogendo *et al.* 2008, Lopez *et al.* 2008).

Although, the insecticidal activity of the plant extracts is attributed mainly to its major compounds, the synergistic or antagonistic effect of other compounds in the mixture has to be considered (Ragas *et al.* 2005). Each of the plant extract components makes its own contribution to biological activity of the extract against the tested insect.

The mode of action of the bioactive natural monoterpinoids (hydrocarbons, alcohols and ketones) isolated from plant extracts oils may be due to inhibition of acetylcholinesterase (Miyazawa *et al.* 1997, Lee *et al.* 2000). Lee *et al.* (2000) reported that, 1,8-cineole was the most potent inhibitor of AChE among the monoterpenes tested and this may be a mode of action for

Table 2. Effect of the tested plant extracts and malathion on percent mortality of *C. maculatus* adults.

Treatments	Concentration (ppm)	Mortality after one week (%)	Mortality after two weeks (%)
Cassia senna	100	23.3 k[A]	83.3 d
	200	40.0 hi	97.5 d
	300	53.3 g	100.0 d
Caesalpinia gilliesii	100	30.0 j	84.0 d
	200	33.3 ij	93.3 d
	300	40.0 hi	100.0 d
Thespesia populnea var. *acutiloba*	100	30.0 j	90.0 c
	200	40.0 hi	93.5 b
	300	43.0 h	100.0 b
Chrysanthemum frutescens	100	40.0 hi	73.3 e
	200	46.7 h	83.3 d
	300	67.6 de	97.6 d
Euonymus japonicus	100	53.3 g	100.0 d
	200	77.6 bc	100.0 d
	300	80.0 b	100.0 d
Bauhinia purpurea	100	57.6 ef	90.0 c
	200	70.0 de	93.5 b
	300	77.6 bc	100.0 b
Cassia fistula	100	60.0 fg	93.7 b
	200	63.3 ef	100.0 b
	300	73.3 cd	100.0 b
Malathion	1	100.0 a	100.0 b
	2	100.0 a	100.0 b
	3	100.0 a	100.0 b
Control	0	0.00 l	0.00 a
Significance			
Treatment type (TT)		***	***
Treatment concentration (TC)		***	***
Interaction (TT × TC)		***	***

[A] Letters indicate differences between means at P = 0.05 using Duncan's multiple range test (Duncan 1955).

essential oils and monoterpenes against stored grain insect pests. Also, the mode of action of the tested extracts may be largely attributable to its fumigant action (Shaaya *et al.* 1997, Park *et al.* 2003).

Botanical extracts as pest control agents present two main characters: the first is their safety to humans and the environment, and the second is a lower likelihood of resistance developing within the pathogen of concern. Also, the rat tests are often more sensitive and may not reflect human sensitivity. Moreover, the exposure levels may be far greater than what would actually be experienced or detected in cowpea seeds after they are grown and processed.

Regarding resistance development, it is believed that it is difficult for insects to develop resistance to such a mixture of bioactive components with, apparently, different mechanisms of insecticidal activity (Wei *et al.* 2008).

This study is considered the first step toward more investigations into the use of botanical extracts as alternative for controlling of stored products pests. This will help to reduce the environmental pollution and the adverse effect on human health from insecticide use. Since these botanical extracts have no significant toxicity relative at the high dosage that was given orally it is unlikely that humans will be adversely affected by residues.

Conclusions

The insecticidal activity of the tested plant extracts against *C. maculatus* indicated the potential of some plant species to be a natural source of insecticidal material. Insecticidal activity was confirmed in all the tested plant species, although the results showed that extracts varied in their effectiveness against *C. maculatus*. It is possible to use botanical products as an alternative to chemical control of *C. maculatus* and this may contribute to reducing the amount of insecticides applied and subsequently minimizing their hazards to the environment and human health. Work should continue on other invasive species to isolate insecticidal compounds and on field trials with promising extracts or compounds. Further research is needed in order to evaluate the practical effectiveness of essential oils in protecting stored products.

References

Abbott, W.S. (1925). A method of computing the effectiveness of an insecticide. *Journal of Economic Entomology* 18, 265-7.

Bancroft, J.D. and Stevens, A. (1996). Theory and practice of histopathological techniques, fourth edition. (Churchill Livingstone, USA).

Barham, D. and Trinder, P. (1972). A colorimetric method for the determination of creatinine in serum. *Analyst* 97, 142-5.

Fox, C.W. and Tattor, M. (1994). Oviposition substrate affect adults mortality independent of reproduction in the seed beetle *Callosobruchus maculates*. *Ecological Entomology* 19, 108-110.

Collins, D.A. (2006). A review of alternatives to organophosphorus compounds for the control of storage mites. *Journal of Stored Products Research* 42, 395-426.

Cornelius, C.E., Charles, W. and Arhode, E. (1959). Serum and tissue transaminase activities in domestic animals. *Cornell Vet.* 49, 116-21.

Cox, P.D. (2004). Potential for using semiochemicals to protect stored products from insect infestation. *Journal of Stored Products Research* 40, 1-25.

De Carvalho, C.C.R. and Da Fonseca, M.M.R. (2006). Carvone: why and how should one bother to produce this terpene. *Food Chemistry* 95, 413-22.

Duncan, D.B. (1955). Multiple range and multiple F tests. *Biometrics 11, 1*-42.

Durate-Almeida, J.M.D., Negri, G. and Salatino A. (2004). Volatile oils in leaves of *Bauhinia* (Fabaceae Caesalpinioideae). *Biochemical Systematics and Ecol*ogy 32, 747-53.

El-Lakwah, F.A., Darwish, A.A. and Khaled, O.M. (1992). Effectiveness of dill seed powder on stored products insects. *Annals of Agricultural Science, Moshtohor* 34, 2031-7.

Iboudo, Z.L.C.B., Dabiré, R.C.H., Nébié, I.O., Dicko, S., Dugravot, A. M. and Cortesero, A. (2010). Biological activity and persistence of four essential oils towards the main pest of stored cowpeas, *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae). *Journal of Stored Products Research* 46, 124-8.

Katz, T., Miller, J., and Hebert, A. (2008). Insect repellents: historical perspectives and new developments. *The Journal of the American Academy of Dermatology*. 58, 865-71.

Kestenholz, C., Philip, C., Steven, S. and Belmain, R. (2007). Comparative study of field and laboratory evaluations of

Table 3. The main constituents of *Euonymus japonicus* plant extract under GC-MS analysis.

No.	Identified compounds	Retention time	% area
1	1,8-cineole	4.99	3.99
2	1,6-octadien-3-ol 3,7 dimethyl (Linalool)	5.71	5.52
3	Cyclohexen-1-ol 4-methyl 1-1 (1-methylethyl)	6.57	1.92
4	3-Cyclohexene-1-methanal alpha alpha 4-methyl	6.73	2.22
5	1,6 octadien-3-ol 3,7 dimethyl 2-aminobezoate	7.2	0.69
6	Cyclohexasiloxane dodecamethyl	7.74	1.44
7	Cyclohexene-1-methanol alpha alpha 4-trimethyl acetate	8.14	13.83
8	2,6 octadien-1-ol 3,7 dimethyl	8.38	1.90
9	Copaene	8.42	1.93
9	2H-1-benzopyran-2-one 3,4 dihydro	8.75	1.49
10	Caryphllene	8.84	1.26
11	1,6,10 dodecantriene 7,11, dimethyl-3-methylene	9.02	4.62
12	Cycloheptasiloxane tetradecamethyl	9.25	4.30
13	Naphthalene	9.38	1.28
14	1,3, benzodioxole 4-methoxy-6-(2-propenyl)	9.73	1.10
15	Torreyal (aldhyde)	9.66	0.73
16	Nerolidol	9.95	1.02
17	Tetradecanoic acid (Myristic acid)	11.68	0.5
18	Neophytodiene	12.19	0.54
19	Cyclohexadecane	16.65	2.42
20	Hexadecanoic acid methyl ester (methyl palmitate)	13.07	1.44
21	Cyclohexadecane	13.98	0.73
22	9,12 octadecadien-1-ol (linoleyl alcohol)	14.59	5.62
23	Ethanol 2-(9,12 octadecadienyloxy)	16.08	1.22
24	6,8 dichloro-2-{4-chlorophenyl-4-bromoacetylquinoline (Quinolinic acid derivatives)	17.21	2.81
25	Tetracosamethyl cyclododecasiloxane	19.18	3.26
26	Iron, monocarbonyl (1,3 butadiene 1,4 dicarbonic acid diethyl ester a,a dipyridyl	24.97	1.01

Table 4. The main constituents of *Cassia fistula* plant extract under GC-MS analysis.

No.	Name	Retention time	% Area
1	Cyclohexene-1-methanol alpha apha 4-trimethyl acetate	8.14	0.38
2	Cycloheptasiloxane tetradecamethyl	9.25	4.17
3	2 (4H) Benzofuranone 5,6,7,7a tetrahydro-4,4,7a trimethyl	9.94	1.09
4	Silane	10.6	4.96
5	Tetradecanoic acid (Myristic acid)	11.6	0.37
6	6,8 dichloro-2- {4-chlorophenyl-4-bromoacetylquinoline (quinolinic acid derivatives)	11.79	6.02
7	2 ethylthiolane	12.42	1.56
8	Mome inositol	12.61	5.33
9	Hexadecanoic methyl ester (Palmitic acid methyl ester)	13.07	0.84
10	Hexadecanoic acid (Palmitic acid)	13.64	3.80
11	Cyclodecasiloxane eicosamethyl	15.32	5.72
12	9,12,15 octadecatrienoic acid methyl ester (methyl linolinoate)	15.85	6.79
13	Octadecatrienoic acid (Alpha-linolenic acid)	16.02	1.9
14	7 chloro-1,3-dihydro-3-(trimethylsiloxy)-1-(trimethylsilyl)-5-phenyl-2h-1,4 benzodiazepien-2-one	21.17	7.60
15	Cyclododecasiloxane tetracosamethyl	23.11	7.30
16	Iron, monocarbonyl (1,3 butadiene 1,4 dicarbonic acid diethyl ester a,a dipyridyl	24.98	7.17

the ethnobotanical *Cassia sophera* L. (Leguminosae) for bioactivity against the storage pests *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae) and *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae). *Journal of Stored Products Research* 43, 79-86.

Ketoh, G.K., Koumaglo, H.K., Isabelle, A. and Glitho, I.A. (2005). Inhibition of *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae) development with essential oil extracted from *Cymbopogon schoenanthus* L. Spreng. (Poaceae), and the wasp *Dinarmus basalis* (Rondani) Hymenoptera: Pteromalidae). *Journal of Stored Products Research* 41, 363-71.

Lee, S.E., Choi, W.S., Leec, H.S. and Park, B.S. (2000). Cross-resistance of a chlorpyrifos-methyl resistant strain of *Oryzaephilus surinamensis* (Coleoptera: Cucujidae) to fumigant toxicity of essential oil extracted from *Eucalyptus globulus* and its major monoterpene, 1,8-cineole. *Journal of Stored Products Research* 36, 383-9.

Lopez, M.D., Maria, J., Jordan, M.J. and Villalobos, J. (2008). Toxic compounds in essential oils of coriander, caraway and basil active against stored rice pests *Journal of Stored Products Research* 44, 273-8.

Miyazawa, M., Watanabe, H. and Kameoka, H. (1997). Inhibition of acetylcholinesterase activity by monoterpenoids with a p-menthane skeleton *Journal of Agricultural and Food Chemistry* 45, 677-9.

Negahban, M., Moharramipour, S. and Sefidko, F. (2006). Chemical composition and insecticidal activity of *Artemisia-scoperte* essential oil against three Coleoptera stored-product insects. *Journal Asia-Pacific Entomology* 9, 381-8.

Ogendo, J.O., Kostyukovsky, M., Ravid, U., Matasyoh, J.C., Deng, A.L. and Omolo, E.O. (2008). Bioactivity of *Ocimum gratissimum* L. oil and two of its constituents against five insect pests attacking stored food products. *Journal of Stored Products Research* 44, 328-34.

Pachico, D. (1993). The demand for bean technology. *In* 'Trends in CIAT commodities', ed. G. Henry, pp. 60-73 (Centro Internacional de Agricultura Tropical, Cali, Colombia).

Park, Il-Kwon, Lee, Sang-Gil, Choi, Don-Hwa, Park, Ji-Doo and Ahn, Young-Joon (2003). Insecticidal activities of constituents identified in the essential oil from leaves of *Chamaecyparis obtusa* against *Callosobruchus chinensis* (L.) and *Sitophilus oryzae* (L.). *Journal of Stored Products Research* 39, 375-84.

Ragas, C.Y., Hofilena, J.G. and Rideout, J.A. (2005). New furanoid diterpenes from *Caesalpinia pulcherrima*. *Journal of Natural Products* 65, 1107-10.

Rajapakse, R. and Van-Emden, H.F. (1997). Potential of four vegetable oils and ten

Table 5. Effect of *Euonymus japonicus* and *Cassia fistula* extracts on serum GPT, ALP and creatinine of rates at a dose of 500 mg kg^{-1} body weight.

Treatments	GPT (IU/L[B] ± SE[A])	ALP (IU/L ± SE)	Creatinine (mg dl^{-1}[C] ± SE)
Control	3.39 ± 55.2	5.57 ± 101	0.07 ± 0.175
Euonymus japonicus	4.47 ± 59.1	5.1 ± 111	0.09 ± 0.169
Cassia fistula	5.8 ± 62	10.2 ± 112	0.08 ± 0.179

[A] Standard Error. [B] International Units per Litre. [C] mg dl^{-1} milligram per decilitre.

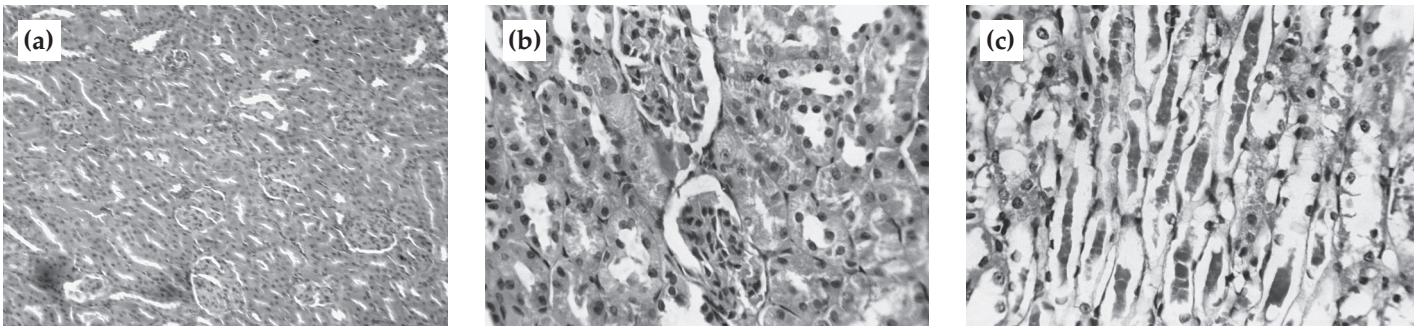

Figure 1. Section from rat's kidney 21 days after treatment with extract of *Euonymus japonicus* (b) and *Cassia fistula* (c) at dose level of 500 mg kg^{-1}. Treatment is shown relative to the control (a).

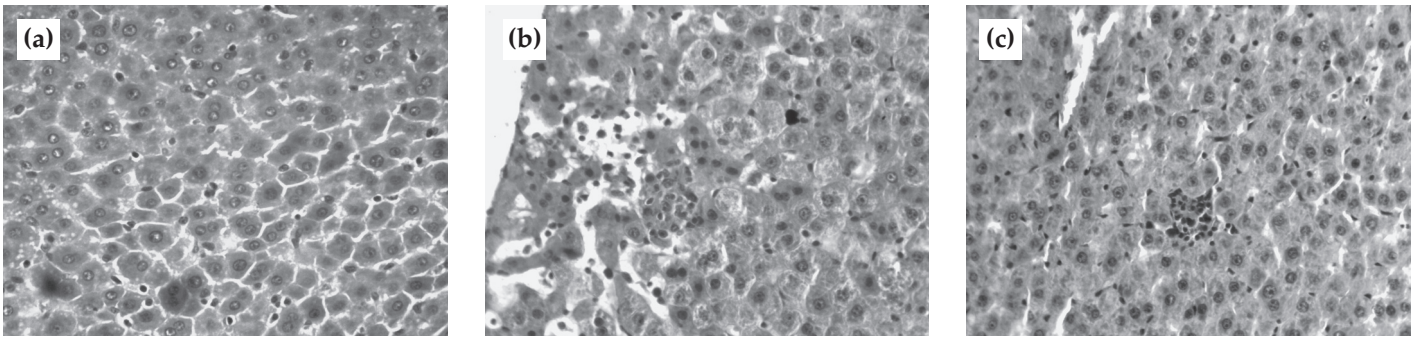

Figure 2. Section from rat's liver, 21 days after treatment with extract of *Euonymus japonicus* (b) and *Cassia fistula* (c) at dose level of 500 mg kg^{-1}. Treatment is shown relative to the control (a).

botanical powders for reducing infestation of cowpea by *Callosobruchus maculatus*, *C. chinensis* and *C rhodesianus*. *Journal of Stored Products Research* 33, 59-68.

Rajendran, S. and Sriranjini, V. (2008). Plant products as fumigants for stored-product insect control. *Journal of Stored Products Research* 44, 126-35.

Reitman, S.M. and Frankel, D. (1957). A colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminase. *American Journal of Clinical Pathology* 28, 56-64.

Romestaing, C., Piquet, M., Bedu, E., Rouleau, V., Dautresme, M., Ollivier, I.H., Filippi, C., Duchamp, C. and Sibille, B. (2007). Long term highly saturated fat diet does not induce NASH in Wistar rats. *Nutrition and Metabolism* 4, 4.

Rozman, V., Kalinovica, I. and Korunic, Z. (2007). Toxicity of naturally occurring compounds of Lamiaceae and Lauraceae to three stored-product insects. *Journal of Stored Products Research* 43, 349-55.

Seck, D., Sidibé, B., Haubruge, E., Hemptinne, J.L. and Gaspar, C. (1991). La protection chimique des stocks de niébé et de maïs contre les insectes au Sénégal. Medelingen van de Faculteit Landbouwwetenschappen Rijksuniversiteit Gen 56/3b, 1225-34.

Shaaya, E., Kostjukovski, M., Eilberg, J. and Sukprakarn, C. (1997). Plant oils as fumigants and contact insecticides for the control of stored-product insects. *Journal of Stored Products Research* 26, 43-53.

Singh, S.R., Luse, R.A., Leuschner, K. and Nangju, D. (1978). Groundnut oil treatment for the control of *Callosobruchus maculatus* (F.) during cowpea storage. *Journal of Stored Products Research* 14, 77-80.

Stoll, G. (1988). Protection naturelle des végétaux en zones tropicales. (CTA, AGRECOL) 177 pp.

Tanzubil, P.B. (1991). Control of some insect pests of cowpea (*Vigna unguiculata*) with neem (*Azadirachta indica* A.Juss.) in Northern Ghana. *Tropical Pest Management* 37, 216-17.

Tapondjou, L.A., Adler, C., Bouda, H. and Fontem, D.A. (2002). Efficacy of powder and essential oil from *Chenopodium ambrosioides* leaves as post-harvest grain protectants against six-stored product beetles. *Journal of Stored Products Research* 26, 43-53.

Weaver, D.K. and Subramanyam, B. (2000). Botanicals. *In* 'Alternatives to pesticides in stored-product IPM', eds B. Subramanyam and D.W. Hagstrum, pp. 303-320. (Kluwer Academic Publishers, Massachusetts).

Wei, L.W., Wei, M., Bing-yu, Z., You-chen, D. and Feng, I. (2008). Antagonistic activities of volatiles from four strains of *Bacillus* spp. and *Paenibacillus* spp. against soil-borne plant pathogens. *Agricultural Sciences in China* 7, 1104-14.

Wilkinson, J.H., Joseph, A. and Seymour, W. (1969). Evaluation of new system for kinetic measurement of serum alkaline phosphatase. *Clinical Chemistry* 15, 487-95.