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EXPERIMENTAL PAPER

Pesticidal activity of certain plant extracts and their isolates against the cowpea beetle *Callosobruchus maculatus* (F.) (Coleoptera: Chrysomelidae: Bruchinae)

NADIA ZIKRY DIMETRY[1*], SOAD EL-GENGAIHI[2], MAHMOUD HAFEZ[3], MAGDY HASSAN ABBASS[1]

[1]Department of Pests and Plant Protection
National Research Centre
Dokki, Cairo, Egypt

[2]Department of Medicinal and Aromatic Plants
National Research Centre
Dokki, Cairo, Egypt

[3]Department of Entomology
Faculty of Science
Cairo University, Egypt

[*]corresponding author: phone:+20 237622603, fax:+20 233370931,
e-mail: nadia_dimetry@yahoo.com

S u m m a r y

Different extracts from seven plant species were assayed against the cowpea beetle (*Callosobruchus maculatus*) in the laboratory. The plants were extracted sequentially with petroleum ether, chloroform and ethyl alcohol. The petroleum ether extract of each was fractionated into sap and unsap, then identified by GC chromatography. Also compounds isolated from chloroform and alcohol extracts of *Citrullus colocynthis* and petroleum ether extract of *Nicandra physaloides* were tested for their toxicity against the adult beetle. The sensitivity of the adults to various crude extracts revealed great variation in effectiveness. Petroleum ether and chloroform extracts of *Nicandra physaloides* proved to be the most toxic in comparison to other extracts tested, while petroleum ether extracts of *Curcuma longa* proved to be the least effective. All the fatty acid fractions of the seven plant species were toxic to the adult beetles at the tested concentrations. *N. physaloides, Schinus terebinthifolius* and *Dodonaea viscosa* resulted in 100% mortality of adults at the highest concentration tested (1.0%). Using 1.0% unsap fraction of *Dodonaea viscosa* resulted in 100% mortality between the adults. The least percentage mortality between the adult beetles recorded (22%) was for unsap fraction of *T. orientalis* at concentration of 0.0625% but increased to 80.0% mortality at concentration of 1.0%. The compounds isolated from chloroform and alcohol extracts of *C. colocynthis* and petroleum ether extract of *N. physaloides* proved to be highly efficient against *C. maculatus* adults.

Key words: *pesticidal activity*, *Citrullus colocynthis, Nicandra physaloides, Sichinus terebinthifolius, Callistemon lanceolatus, Thuja orientalis, Dodonaea viscosa, Curcuma longa* extracts, *Cowpea beetle*, *Callosobruchus maculatus*

INTRODUCTION

Plant extracts has been the subject of age growing interest nearly the last six decades. The most famous extracts known today are those of the neem seed kernel and chinaberry seed extracts.

They contain small amounts of several biologically active compounds [1, 2]. The possibility of their large scale isolation or synthesis for commercial use is still at the beginning. Therefore, from practical point of view, the biologically active crudes are of immediate interest. Many medicinal plants and herbs related to different plant families are of great potential for the pest control [3]. The leaves of *Schinus terebinthifolius, Callistemon lanceolatus, Thuja orientalis* and *Dodonaea viscosa* are of special interest. These plants were examined as a part of a project to manufacture new botanical pesticides funded from the Academy of Scientific Research and Technology. Mishra *et al.* [4] found that the volatile oil of *Callistemon lanceolatus* showed high toxicity to *Callosobruchus maculatus* adults and the LC_{50}value increased with the increasing period of exposure. El-Sayed [5] found that *Dodonaea viscosa* extract has a toxic effect on *Spodoptera littoralis*. Dimetry and El-Hawary [6] showed that *Citrullus colocynthis* extract reduced the reproduction and the life cycle of bean aphid (*Aphis craccivora*). They also added that treatment of *Aphis craccivora* with 70% alcohol extracts of *Nicandra physaloides* and *Curcuma longa* affected the biology of this pest. Yalamanchilli and Punukollu [7] observed that the volatile oil from the leaves of *Curcuma domestica* could effectively protect the seeds against *C. chinensis* at low concentrations.

Protection of stored products against insect pests is of utmost importance to secure a continuous and safe food supply all over the world [8-13]. The study of the effect of plant extracts upon pests will provide chances for finding alternative insecticides. Su [14, 15] and Oji *et al.* [16], determined the insecticidal properties of ethanolic and acetone extracts of *Xylopia aethiopica* and *Piper guineense* against *Callosobruchus maculatus* on the stored cowpeas. Majeed *et al.* [17] tested the toxic effect of acetone, methanol extracts of neem fruits and Coopex25 EC (permethrin+ bioallethrin against *C. analis* (F.) adults. They found that LD_{50} by filter paper impregnation method was found to be 7.8 μg/cm^2 and 18.0 μg/cm^2 for Coopex 25 EC. In Indonesia, Prijono *et al.* [18] exposed seed extracts of 30 species of *Annonaceae, Fabaceae* and *Meliaceae* to *C. maculatus* females (1–2 days old) in Petri dishes. Among the *Annonaceae* species, *Annona squmosa* and *A. glabra* at 0.5% showed a good contact effect against *C. maculatus* (>90%) mortality after three days and 100% after five days. Among *Meliaceae* species, *Dysoxylum caulifolorum* was the only one whose seed extract at 0.5% possessed a good contact effect against *C. maculatus.* None of the *Fabaceae* seed extracts were effective against *C. maculatus.*

The objective of present study is to evaluate the toxicity of seven plant species extracts and to evaluate the unsaponifiable and saponifiable fractions of the petroleum ether extracts of the aforementioned plants and different plant isolates to assess their toxic effect on *C. maculatus* adults in the laboratory.

MATERIAL AND METHODS

Rearing technique of *C. maculatus*

Laboratory stock cultures of *Callosobruchus maculatus* were started by collecting infested mung beanseeds from the Ministry of Agriculture. For purification of the culture, pairs of differentiated males and females of the emerging adults were kept under 29±1°C and 75±5% RH in plastic containers of 1 l capacity, each containing about 200 g of mungbean seeds and covered with muslin. Five generations were produced under these conditions before starting different experiments. Mungbean seeds were heated in an oven at 65-75°C for five hours to kill the microorganisms or any other form of pests.

Plants and extracts used

Seven plant species belong to seven different families were used in present study. They are *Citrullus colocynthis* (Schrad.) seeds *Cucurbitaceae*, *Nicandra physaloides* (Gaertn) herb *Solanaceae*, *Schinus terebinthifolius* (Radd.) leaves *Anacardiaceae*, *Curcuma longa* (L.) rhizomes *Zingiberaceae*, *Callistemon lanceolatus* (D.C.) leaves *Myrataceae*, *Thuja orientalis* (L.) leaves *Cupressaceae*, *Dodonaea viscosa* (Jacq) leaves *Sapindaceae*.

Preparation of different extracts

A known weight of air-dried and fine powder of each plant part (100 g): leaves, rhizome, fruits or seeds were submitted to successive extractions with selective organic solvents of increasing polarity i.e. light petroleum ether (bp. 40–60°C), chloroform and ethyl alcohol (90% *v/v*). The extraction process was continued using a particular solvent until a colourless solution was obtained. Also, no residue was observed on the evaporation of the aliquot from the extract.

Before using the next solvent, the marc (remained) powder of plant was taken out from the extractor, carefully separated on a filter paper, the solvent was allowed to evaporate at room temperature till complete dryness, then repacked in the extractor.

The solvent was evaporated from each extract under reduced pressure at 40°C. The residue was dried to a constant weight and its percentage was calculated. The dried residue was kept in a vacuum desiccators containing calcium chloride to be used in the bioassay tests and also subjected to chemical analysis.

Separation and identification of plant extracts

The residues were phytochemically screened for their components [19]. Saponifiable and unsaponifiable matters of petroleum ether extract were performed according to Farag *et al.* [20].

GLC was used to fractionate and identify the different constituents of the unsaponifiable and saponifiable matters using the following conditions. Apparatus: GCV Pye Unicam equipped with dual flame ionization detector. Column: Coiled glass column (2.8 m x 4 mm) packed with diatomite-C (100–120 mesh) coated with 1% OV-17. Oven temperature from 70 to 270±10°C/min. till 270°C then isothermally at 270°C for 25 min. Detector temperature: 300°. Injector temperature: 280°C. Nitrogen Flow rate: 30 ml/min. Hydrogen flow rate: 33 ml/min. Air flow rate: 330 ml/min. For the unsaponifiable matter, the column used was 10% OV 101. For the fatty acid or saponified: 10% PEGA.

The identification of different constituents was performed by comparing the relative retention time of each component compared with those of standard material. Fatty acids were injected to gas chromatograph, after preparation of their methyl esters [20, 21].

Chemical evaluation of *Citrullus colocynthis* extract

Chloroform extract of *C. colocynthis*

As the chloroform and alcohol extracts of *C. colocynthis* was proved to be highly effective against *C. maculatus*, it is recommended to study in details the chemical constituents responsible for the activity. So, fractionation of the extracts and isolation of its constituents were performed.

The chloroform extract was dissolved in 90% ethanol. The soluble fraction was extracted with petroleum ether to take any pigments and fats. The remainder chloroformic extract was fractionated on preparative thin layer plates to resolve five spots by ellution with toluene-ethyl acetate (3:2). Each substance eluted from the plate was checked for its purity by rechromatography with solvents (toluene-ethyl acetate (3:2) and chloroform methanol (9:1). The five substances were identified by co-chromatography with authentic standards, m.p. and their reaction with triphenyl tetrazolium chloride reagent [22]. Three fractions of the chloroform extract were obtained and named CHl 1, II, III.

Alcohol extract of *C. colocynthis*

Alc-1 and Alc-2

Alc-1 and Alc-2 are two fractions from the alcoholic extracts of *C. colocynthis* seeds. The alcoholic extract of *C. colocynthis* seeds was evaporated till dryness, then dissolved in distilled water. The water portion was extracted with butanol saturated with water in a separating funnel. The butanol layer was collected, evaporated, then dissolved in methanol and precipitated with ether. The precipitate was dissolved in methanol and precipitated again with ether. The precipitate was dried over calcium chloride to obtain a yellowish amorphous powder termed Alc-1. The powder obtained give positive Molisch test and red colour with triphenyl tetrazolium chloride. The residue obtained after butanol extract was collected, evaporated under vacuum (Alc-2) and screened phytochemically to reveal its nature.

Chemical evaluation of *Nicandra physaloides* extract

N. physaloides, its petroleum ether extract was proved to have an insecticidal effect against *C. maculatus.* The petroleum ether extract was fractionated to its saponifiable and unsaponifiable matter, the latter revealed potent effect against cowpea beetle. The unsaponifiable was investigated by TLC which indicates the separation of three spots. These spots were isolated in appreciable amounts using preparative TLC with solvent system benzene-ethyl acetate (6:2 *v*/*v*) and anisaldehyde as a reagent.

Pesticidal action of some plant extracts against adult stage of *Callosobruchus maculatus*

The treatment was applied by anesthetizing newly emerged adults (less than 24 hours old) *C. maculatus* with few drops of diethyl ether, picking them individually with a vacuum tweezer and applying 0.5 μl of different acetone solution at the concentrations selected on the dorsal meso-thorax with micro-applicator. Control insects were anesthetized and treated with 0.5 μl of acetone. Ten beetles (5 males and 5 females) were treated with different concentrations (0.5, 0.25, 0.125, 0.0625). Four replicates were performed for each treatment. After treatment, the beetles were held in 10 cm diameter Petri-dishes (5 males and 5 females/dish). The dishes were examined daily for dead insects. Mortality was recorded after 24, 48, 72 and 96 hours. Mortality was corrected using Abbott's formula (1925) [23]. Potency levels of the test toxicants were determined at a given level such as LC_{50} or LC_{90} as an order of toxicity was expressed in a number of folds or times of potency of the test extract compared with the least toxic one. The following equation was employed to determine the toxicity index according to Sun [24].

$$\text{Sun's toxicity index} = \frac{LC_{50} \text{ or } LC_{90} \text{ of the standard material} \times 100}{LC_{50} \text{ or } LC_{90} \text{ of the tested material}}$$

Toxicity of standard toxic material used in any study is always considered equivalent to 100%. On the other hand, the potency level of the test toxicant was determined at a given level such as LC_{50} or LC_{90}, as an order of toxicity expressed in a number of folds or times of potency of the test extracts is compared with the least toxic one.

RESULTS

Insecticidal activity of different crude successive extracts against *Callosobruchus maculatus* adults

The sensitivity of adults to various crude extracts revealed great variation in effectiveness (tab. 1). Petroleum ether and chloroform extracts of *Nicandra physaloides* proved to be the most toxic extracts in comparison to the other extracts tested where the percentage mortality of the adults after 96 hours varied from 100% for those individuals treated with petroleum ether extracts of *N. physaloides* to 86.7% for these adults treated with chloroform extracts of the same plant at 0.5%. The least effective extracts on the other hand, were found to be the different successive extracts of *Schinus terebinthifolius* where the percentage mortalities between treated adults decreased greatly in comparison to other extracts tested.

The sensitivity of the adults to various extracts revealed great variations in effectiveness at LC_{50} level ranged from 0.00056% in case of ethyl alcohol extract of *Dodonaea viscosa* to 18.5938% in case of petroleum ether extract of *C. longa*.

Comparing the slope values, it is very low (0.1913) in case of ethyl alcohol extract of *D. viscosa*, and the highest slope recorded (2.1127) was for *C. longa* chloroform extract. The other plant extracts come between these two values (tab. 2).

Table 1.

Insecticidal activity of different crude plant extracts against *C. maculatus* adults

Solvent used	Concentrations (%)	% Corrected accumulated mortality after 96 h						
		C. colocynthis	*N. physaloides*	*S. terebinthifolius*	*C. longa*	*C. lanceolatus*	*T. orientalis*	*D. viscosa*
Petroleum ether	0.5	59.9	100.0	26.8	33.4	13.4	40.0	33.4
	0.25	53.2	100.0	26.7	20.0	0.0	26.7	13.4
	0.125	53.2	100.0	13.4	26.7	6.7	26.7	6.7
	0.0625	53.3	67.7	6.7	20.0	0.0	13.4	6.7
Chloroform	0.5	33.3	86.7	20.1	62.7	46.7	13.4	33.3
	0.25	13.4	66.6	6.7	26.8	26.7	13.4	33.3
	0.125	20.1	40.0	6.7	6.7	20.0	6.7	20.0
	0.0625	0.0	26.7	6.7	6.7	13.4	0.0	20.1
Ethanol	0.5	73.4	20.1	13.3	40.1	26.8	26.7	73.4
	0.25	60.0	20.1	13.3	26.7	20.1	26.6	66.7
	0.125	60.0	20.1	13.40	13.4	20.0	13.4	66.6
	0.0625	46.7	6.7	0.0	13.4	13.3	13.4	66.7

Table 2.

Efficacy of successive extracts of different plants against *C. maculatus* adults

Plants used	Solvent	LC_{50}	Slope	Toxicity index at LC_{50}	No of folds compared with *C. longa* pet. ether
C. colocynthis	Pet. ether	0.6364	0.1634	0.08815	29.217828
	Chloroform	0.6735	1.8999	0.83296	27.607706
	Ethyl alchol	0.0608	0.3861	0.92206	305.6078
N. physaloides	Pet. ether	0.0177	1.8626	3.17397	1051.9824
	Chlorofom	0.1450	1.9540	0.38682	128.21004
	Ethyl alcohol	5.7963	0.6617	0.0097	3.207854
S. terebinthifolius	Pet. ether	1.4658	1.0472	0.03827	12.685374
	Chloroform	18.4022	0.6617	0.00304	1.0104107
	Ethyl alcohol	2.6285	1.2093	0.02134	7.0739597
C. longa	Pet. ether	18.5938	0.3372	0.00301	1.0
	Chloroform	0.4337	2.1127	0.12934	42.871184
	Ethyl alcohol	1.0056	1.0222	0.05578	18.489362
C. lanceolatus	Pet. ether	15.1461	0.9404	0.0037	1.2276272
	Chloroform	0.7156	1.0933	0.07839	25.98295
	Ethyl alcohol	8.8011	0.5027	0.00637	2.112667
T. orientalis	Pet. ether	0.9129	0.897	0.06145	20.367961
	Chloroform	2.3526	1.3451	0.02384	7.9034731
	Ethyl alcohol	3.7624	0.6517	0.01491	4.9420576
D. viscosa	Pet. ether	1.5655	1.1987	0.03583	11.876993
	Chloroform	2.6415	0.5405	0.02123	7.0391294
	Ethyl alcohol	0.00056	0.1913	100.0	33144.01

Insecticidal activity of saponified and unsaponified matter from different plants against *C. maculatus* adults

The data in table 3 show the effect of petroleum ether fractions (sap. and unsap.) from seven plant species before against *C. maculatus* adults. For the sap. fractions, the data showed that all the fatty acid fractions of the seven plant species were toxic to the adult beetles at tested concentrations. *N. physaloides, S. terebinthifolius* and *D. viscosa* resulted in 100% mortality of adult beetles at the highest concentration tested (1.0%). The sap. fraction of *S. terebinthifolius* was superior to all other extracts tested as it gave mortalities varied between 90.0–100% at all the concentrations tested. *C. lanceolatus* sap. fraction was found to be the least effective as it induced lower mortalities at all the tested concentrations, only 45.0% of the adults died after 96 hours at concentration of 0.0625%. At the highest concentration (1.0%) tested, *C. colocynthis* gave high mortality (85%) but on using the lowest concentration (0.0625%), only 40% mortality occurred between the adults after the tested period. Table 4 shows that the least steepness of the seven sap. fractions are *C. longa* and *C. lanceolatus*, which means that their effect is not concentration dependent. The highest steepness recorded was for *D. viscosa* (slope 1.628852), as the toxicity is concentration dependent.

Table 3.

Insecticidal activity of saponifiable and unsaponifiable matter of different plants against *C. maculatus*

Concentration (%)	*C. colocynthis*		*N. physaloides*		*S. terebnthifolius*		*C. longa*		*C. lanceolatus*		*T. orientalis*		*D. viscosa*	
	Sap.	Un-sap.	Sap.	Un-sap.	Sap.	Un-sap.	Sap.	Un-sap.	Sap.	Un-sap.	Sap.	Un-sap.	Sap.	Un-sap.
1.0	85.0	75.0	100.0	95.0	100.0	90.0	90.0	87.5	70.0	77.5	85.0	80.0	100.0	100.0
0.5	90.0	75.0	95.0	90.0	100.0	80.0	90.0	80.0	80.0	72.5	90.0	67.5	90.0	95.0
0.25	65.0	72.5	95.0	90.0	100.0	77.5	80.0	77.5	70.0	60.0	80.0	40.0	85.0	90.0
0.125	65.0	70.0	90.0	85.0	95.0	60.0	85.0	60.0	65.0	62.5	70.0	25.0	70.0	82.0
0.0625	40.0	60.0	90.0	75.0	90.0	55.0	80.0	60.0	45.0	55.0	55.0	22.0	60.0	80

Concerning the toxicity of the unsap. fractions, it is obviously clear from table 3 that the highest percentage mortalities of the adults *C. maculatus* resulted from its treatment with *D. viscosa, N. physaloides, S. terebinthifolius* and *C. longa* unsap. fractions. Using 1.0% unsap. fraction of *D. viscosa* resulted in 100% mortality between the adults. Decreasing the concentration from 1.0 to 0.0625% of the same fraction, the percentage mortality of the adults decreased i.e. that the mortality of the adults is concentration dependent.

N. physaloides unsap. fraction also caused 95.0% mortality between adult beetles at a concentration of 1.0 and 75.0% mortality at a concentration of 0.0625%. The least percentage mortality between adult beetles recorded (22.0%) was for unsap. fraction of *T. orientalis* at a concentration of 0.0625%, however, it increased to 80.0% mortality at a concentration of 1.0%.

Table 4 shows that the least slope recorded was for *C. colocynthis* unsap. (0.328871) and the highest steepness was (1.443378) for *T. orientalis* indicated that in this case the toxicity is concentration dependent.

Table 4.

Efficacy of petroleum ether sap. and unsap. portions of different plants against *C. maculatus*

Plants used	Sap.		Unsap.		Toxicity index at LC_{50}		No of folds compared with	
	LC_{50}	Slope	LC_{50}	Slope	Sap.	Unsap.	Sap. (*C. colocynthis*)	Unsap. (*T. orientalis*
C. colothnthis	0.080067	1.152709	0.005611	0.328871	$2.4988e^{-8}$	84.384	1.0	50.093
N. physaloides	0.003327	0.879204	0.004777	0.678787	$6.011e^{-7}$	100.0	24.066	59.363
S. terebinthifolius	0.002211	0.957823	0.044492	0.806912	$9.046e^{-7}$	10.737	36.213	6.374
C. longa	$1.99e^{-11}$	0.096336	0.038252	0.797263	100.0	12.488	0.040	7.413
C. lanceolatus	0.045889	0.581337	0.038781	0.506594	$4.358e^{-8}$	12.318	1.745	7.312
T. orientalis	0.030497	0.789411	0.0283578	1.443378	$6.558e^{-8}$	1.685	2.625	1.0
D. viscosa	0.054261	1.628852	0.01709	1.169621	$3.686e^{-7}$	0.408	1.476	16.593

Phytochemical studies of *Nicandra* herb

A petroleum ether extract of *N. physaloides* (family *Solanaceae*) was proved to be insecticidally active against *C. maculatus.* The petroleum ether extract was fractionated to its sap. and unsap. fractions. The latter revealed potent to cowpea beetle.

The unsap. was investigated by TLC which indicates the separation of three spots which were isolated in appreciable amounts using preparative TLC with solvent system, benzene-ethyl acetate (6:2 *v*/*v*) and *p*-anisaldehyde as a reagent. The three compounds isolated induce high activity against the cowpea beetle.

Compound 1 having the R_f values in the region of sterols has also the colour characteristic with sterols. Acetate derivatives were made for this compound and chromatographically fractionated on TLC and then impregnated with silver nitrate 10%. The compound was proved to be a mixture composed of β-sitosterol, stigmasterol, and cholesterol. These compounds were quantitatively determined using GLC which reached 61.76, 4.15 and 3.13%, respectively.

Compound 2 has the R_f value of 0.54, isolated by PTLC, and identified as *β*-amyrin by acetate derivative and by interpretation of EL-MS m/z: 426 (M^+), 218 (100%), 207 ($\{C_{14}H_{28}O\}^+$), 203 ($\{218 - CH_3\}^+$), 189 ($\{207 – H_2O\}^+$). The third compound was fractionated by GLC and was proved to be a mixture of hydrocarbons (C_{16} to C_{32}) (tab. 7).They were characterized by saturated hydrocarbons C18 (n-Octadecane and C22 (n-Docosane) as trace compounds.

Insecticidal activity of different compounds isolated from *C. colocynthis* and *N. physaloides* against *C. maculatus* adults

The chloroformic and alcoholic extracts of *C. colocynthis* and petroleum ether extract of *N. physaloides* were proved to be highly efficient against *C. maculatus* adults, so detailed studies were carried out to isolate the most efficient compounds and tested their insecticidal effect against the adult beetles.

a-Compounds isolated from chloroform extract of *C. colocynthis*

Three chloroformic fractions (Chl-1, Chl-2, Chl-3), when checked on preparative thin layer chromatography, reveal the same spots. So they were combined and fractionated by each of two solvents mentioned before.

Five substances were identified by co-chromatography with authentic standards and by melting points. The five substances were: cucurbitacin D, I, B, E and L. Their R_F values were as follows: 0.12, 0.24, 0.36, 0.61 and 0.76, respectively. Also, the melting points were 151-152, 148-149, 180-184, 234-236 and 122-126°C in the same order.

The data obtained in tables 5 and 6 show that the LC_{50} value of CH-1 (0.00067) was the most efficient toxic fraction isolated from chloroform extract of *C. colocynthis* against *C. maculatus* in comparison with fractions CH-2 and CH-3 which gave maximum percentage of mortality between the adults (93.33%) at 0.05% in spite of this fact they are less toxic than fraction Chl-1.

Table 6 shows that the least slope recorded was for fraction Chl-1 isolated from *C. colocynthis* chloroform extract (0.239179) indicated that mortality is not concentration-dependent. The highest slope for the fraction Chl-2 isolated from *C. colocynthis* chloroform extract indicates that in this case the toxicity is a concentration dependent.

Table 5.

Insecticidal activity of different isolated compounds against *C. maculatus*

% Concentration	% Corrected accumulated mortality after 96 h							
	C. colocynthis CH-1	*C. colocynthis* CH-2	*C. colocynthis* CH-3	*C. colocynthis* Alc-1	*C. colocynthis* Alc-2	*N. physaloides-1*	*N. physaloides*- 2	*N. physaloides* – H.C.
0.05	66.64	93.33	93.33	80.0	80.0	66.67	86.67	80.0
0.025	66.65	86.67	66.67	73.33	66.67	73.0	80.0	66.67
0.0125	59.99	73.33	53.34	66.67	66.67	53.32	53.33	40.0
0.00625	59.98	53.33	73.33	59.99	53.34	40.0	53.33	39.99

Table 6.

Efficacy of different isolates against *C. maculatus* adults

Isolated compounds	LC_{50}	Slope	Toxicity index at LC_{50}	No of folds compared with *N. physaloides* H.C.
C. colocynthis CH-1	0.00067	0.239179	100.0	18.495522
C. colocynthis CH-2	0.0052	1.552448	12.884615	2.3830769
C. colocynthis CH-3	0.003748	0.97111	17.8762	3.3062966
C. colocynthis Alc-1	0.002665	0.651098	25.140712	4.6499061
C. colocynthis Alc-2	0.004492	0.750756	14.915405	2.75821
N. physaloides-1	0.006835	0.652205	9.8024871	1.8130212
N. physaloides-2	0.006759	1.273406	9.9127089	1.8334073
N. physaloides-H.C.	0.012392	1.32877	5.406714	1.0

b-Compounds isolated from alcohol extract of *C. colocynthis*

The precipitate obtained from the alcoholic extract gave positive Molish test and red colour with triphenyl tetrazolium chloride which means that this precipitate contains cucurbitacen as glycoside. The other fraction, when shaked with water, gives resistant froth which means that it is a saponin. The two alcoholic fractions need further investigations.

The data presented in tables 5 and 6 show that both fractions named Alc-1 and Alc-2 isolated from alcohol extract of *C. colocynthis* gave high toxicity to *C. maculatus* adults. The fraction Alc-1 was found to be more toxic (LC_{50}=0.002665) than Alc-2 (LC_{50}=0.004492). Concerning the percentage mortality between the adults, it was found that both fractions gave almost equal percentage mortalities between adults.

c-Compounds isolated from petroleum ether extracts of *N. physaloides* against *C. maculatus* adults

The data presented in tables 5 and 6 show that triterpenes isolated from petroleum ether extract of *N. physaloides* (*N. physaloides*-2) gave the highest percentage mortality (86.67%) of *C. aculatus* adults at a concentration of 0.05% compared to 80% mortalities for those individuals treated with the isolated hydrocarbons (tab. 7) and 66.67% for sterols (*N. physaloides*-1) at the same concentration.

Table 7.

Hydrocarbons fractionated by GLC from the unsaponified matter of *Nicandra physaloides*

Carbon No	Compound identified	Percentage
C_{16}: O	n-Hexdecane	3.26
C_{17}: O	n-Heptadecane	1.29
C_{18}: O	n-Octadecane	0.55
C_{19}: O	n-Nonadecasone	46.16
C_{20}: O	n-Eicosane	0.45
C_{21}: O	n-Hencosane	1.13
C_{22}: O	n-Docosane	0.31
C_{24}: O	n-Teracosane	1.86
C_{26}: O	n- Hexacosane	0.47
C_{28}: O	n-Octacosane squalene	6.23
C_{30}: O	n-Triacontane	15.47
C_{32}: O	n-Dotriacontane	15.68

Comparing the LC_{50}values it was found that triterpenes were the most efficient in toxicity towards the adult beetles (LC_{50}=0.006759). Slopes recorded show that the least steepness of the lines was for fraction Chl-1 isolated from *C. colocynthis* chloroform extract (slope=0.239179) and the highest steepness was for fraction Chl-2 isolated from *C. colocynthis* chloroform extract (slope=1.552448). This indicated that the toxicity is concentration dependent. The identification of different fractions will be the subject of further investigation.

DISCUSSION

In search for possible substituent for toxic and persistent insecticides a great attention has been directed towards the insecticides occurring naturally in plants. For this purpose, seven plant species were selected in the present study for their constituents, and also for their availability in Egypt.

The results of the present study show that the sensitivity of the adults *C. maculatus* to various extracts revealed great variations in effectiveness at LC_{50} level ranged from 0.00056% in case of ethyl alcohol extract of *D. viscosa* to 18.5938% in case of petroleum ether extract of *C. longa*. Concerning the slope values, the steepness is very low (0.1913) in case of ethyl alcohol extract of *D. viscosa* and the highest slope recorded (2.1127) was for *C. longa* chloroform extract. The other extracts tested came between these two plant extracts.

Several authors screened the effect of different plant species extracts from different families against several stored product pests. The results of the present findings are in agreement with those of Su [25-27, 14, 15] and Nawrot *et al.* [28].

Mbata and Ekpendu [24] showed that *Piper guineense* extracts caused mortality to adult beetles even at low concentrations. Also Pemonge *et al.* [30] found that topical applications of *Trigonella foenum-graecum* extracts resulted in a high degree of mortality against *Tribolium castaneum* and *Acanthoscelides obtectus.* Dimetry *et al.* [11] stated that different formulations of *C. colocynthis* either active ingredients in powder or emulsifiable concentrate were effective biopesticides against *C. maculatus* adults. They secured considerable protection for the stored seeds for different periods depending on the kind of storage sacks and kind and formulation rate used. Adedir *et al.* [31] found that Cashew kernels were extracted with six different solvents. The different extract products were evaluated on *C. maculatus* adults mortality, ovicidal and adult emergence effect. They found that the tested extracts drastically reduced oviposition and they found that the extracts of n-hexane, petroleum ether and acetone extracts completely prevented infestation and damage of the treated cowpea seeds for a period of 3 months. On the other hand, Jibrin *et al.* [2] stated that neem seed oil is of proven potency as compared to the other plant extracts tested (*Aloe vera*, flame seeds and *Trema orientalis* for the control of cow pea weevil *C. maculatus*).

In present study, all sap. fractions of seven plants tested were toxic to adult beetles at all tested concentrations. *N. physaloides and S. terebinthifolius* imposed 100% mortality at 1.0% concentration. Testing of unsap. fraction of *D. viscosa* resulted in 100% mortality between adult beetles *C. maculatus*. Decreasing the concentration from 1.0 to 0.0625% of the same fraction the percentage mortality of the adults decreased. That means that the mortality of adults is concentration-dependent. These results are in consistent with [32] who found that the unsaponifiable matter of *D. viscosa* was the most toxic while the saponifiable matter of *C. longa* was the most potent against *Tetranychus urticae.*

Present investigation show again that CHl-1 fraction ranked the first between the fractions isolated from chloroform and alcohol extracts of *C. colocynthis.* It has high toxiceffect against adults (LC_{50} values=0.00067) as the concentration decreased, the percentage mortality decreased. However, the triterpenes β-amyrin isolated from *N. physaloides* petroleum ether extract proved to be the most efficient in toxicity against the adult beetles (LC_{50}=0.0067).

CONCLUSION

From the foregoing results, it could be concluded that chloroform and alcohol extracts of *C. colocynthis* and petroleum ether extract of *N. physaloides* are the most efficient extracts against *C. maculatus* adults, so further studies have been carried out concerning the isolation of the active compounds from these extracts and studied their potency against the adult beetles.

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DZIAŁANIE PRZECIWSZKODNIKOWE WYCIĄGÓW Z WYBRANYCH GATUNKÓW ROŚLIN I ICH IZOLATÓW PRZECIWKO STRĄKOWCOWI CZTEROPLAMEMU *CALLOSOBRUCHUS MACULATUS* (F.) (COLEOPTERA: CHRYSOMELIDAE: BRUCHINAE)

NADIA ZIKRY DIMETRY[1*], SOAD EL-GENGAIHI[2], MAHMOUD HAFEZ[3], MAGDY HASSAN ABBASS[1]

[1]Department of Pests and Plant Protection
National Research Centre
Dokki, Cairo, Egypt

[2]Department of Medicinal and Aromatic Plants
National Research Centre
Dokki, Cairo, Egypt

[3]Department of Entomology
Faculty of Science
Cairo University, Egypt

[*]autor, do którego należy kierować korespondencję: tel.:+20 237622603,
faks:+20 233370931,e-mail: nadia_dimetry@yahoo.com

S t r e s z c z e n i e

W niniejszych badaniach przetestowano wpływ wyciągów z siedmiu gatunków roślin na śmiertelność strąkowca czteroplamego. Materiał roślinny ekstrahowano stosując kolejno: eter naftowy, chloroform oraz alkohol etylowy. Wyciągi eterowe były frakcjonowane do fazy zmydlającej i niezmydlającej się, a następnie identyfikowano je za pomocą chromatografii gazowej. Testowano również toksyczność związków wyizolowanych z chloroformowego i alkoholowego wyciągu z *Citrullus colocynthis* oraz eterowego wyciągu z *Nicandra physaloides*. Wrażliwość dorosłych osobników strąkowca na działanie poszczególnych surowych wyciągów roślinnych wykazywała duże zróżnicowanie. Wyciąg eterowy i chloroformowy z *Nicandra physaloides* był najbardziej toksyczny w porównaniu z innymi badanymi ekstraktami, natomiast wyciąg eterowy z *Curcuma longa* miał najniższą skuteczność. Wszystkie frakcje kwasów tłuszczowych siedmiu badanych gatunków roślin były toksyczne dla dorosłych chrząszczy w analizowanych stężeniach. Zastosowanie wyciągów z *N. physaloides, Schinus terebinthifolius* i *Dodonaea viscosa* skutkowało 100% śmiertelnością dorosłych osobników w najwyższym testowanym stężeniu (1,0%). 1,0% frakcja niezmydlająca się uzyskana z *Dodonaea viscosa* powodowała 100% śmiertelność dorosłych osobników. Najniższą procentową śmiertelność dorosłych chrząszczy (22%) zanotowano przy zastosowaniu frakcji niezmydlającej się z *Thuja orientalis* w stężeniu 0,0625%, ale wzrosła ona do 80,0% przy stężeniu 1,0%. Wykazano, że składniki wyizolowane z chloroformowego i alkoholowego wyciągu z *C. colocynthis* i eterowego ekstraktu z *N. physaloides* są bardzo skuteczne przeciw dorosłym osobnikom *C. maculatus.*

Słowa kluczowe: *działanie przeciwszkodnikowe, wyciągi z Citrullus colocynthis, Nicandra physaloides, Sichinus terebinthifolius, Callistemon lanceolatus, Thuja orientalis, Dodonaea viscosa, Curcuma longa* extracts, *Cowpea beetle*, *strąkowiec czteroplamy (Callosobruchus maculatus)*