BOTANICAL EXTRACTS EXHIBIT DUAL ACTION AGAINST *CULEX PIPIENS* LARVAE AND *BIOMPHALARIA ALEXANDRINA* SNAILS

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Abstract—Some extracts of *Euphorbia helioscopia* [Euphorbiaceae], *Calendula micrantha* [Compositae] and *Azadriachta indica* [Meliaceae] were screened for the control of *Culex pipiens* larvae, the vector of Filariasis and *Biomphalaria alexandrina* snails the vector of Schistosomiasis in Egypt. These plants exhibit dual effect on both pests which share the same aquatic breeding habitat and are of medical importance. *B. alexandrina* snails were more susceptible than first instar larvae of *C. pipiens* toward all extracts of the plants tested. The acetone extracts of the three tested plants were the most active. The similarity of data of all fractions tested against both species revealed that these plant extracts probably exhibit the same mode of action toward the pests tested.

INTRODUCTION

The mosquito *Culex pipiens* and the snail *Biomphalaria alexandrina* are two major pests of medical importance as vectors of Filariasis (Khalil *et al.*, 1932, and Price, 1984) and Schistosomiasis (WHO 1986) which represent a public health problem in Egypt. Both pests inhibit the same fresh water ecosystem. Control of both diseases can be achieved by the control of their vectors. Economical and ecological considerations significantly govern the use of plants with biological activities that are relatively specific, inexpensive and readily available in the affected areas (Hostettmann, 1984).

Several plants have been screened for the insecticidal and molluscicidal activities (Marston *et al.*, 1993). *Agave americana* was reported to be toxic against the mosquito species (Dharmshaktu 1977). Moreover, the treatment of the late third instar larvae with ethanolic extract of the herb *Descurania sophia* inhibited the emergence and caused larval mortality (Mohsen, 1990). On the other hand, the first report of plant molluscicides may be that in 1933 on *Balanites aegyptiaca* (Archibald, 1933) and *B. Maughamii* (Wagner, 1933). Thereafter numerous plant species were reported as strong molluscicidal agents (Ahmed *et al.*, 1984 and Ayoub *et al.*, 1986). *Phytolacca dodecandra, Tetrapleura tetraptera, Swartzia madgascariensis* were the most commonly studied (WHO, 1980).

The present work is an attempts to evaluate the effect of some extracts of three plants *Euphorbia helioscopia, Calendula micrantha* and *Azadriachta indica* on both *Biomphalaria alexandrina* snails and *Culex pipiens* first instar larvae.

MATERIALS AND METHODS

Collection of materials

Euphorbia helioscopia (Euphorbiaceae), *Calendula micrantha* (Compositae) and *Azadriachta indica* (Meliaceae) were collected from Giza governorate in March 1995. These plants were kindly identified by Dr. El-Hadidi, Professor of plant Taxonomy, Faculty of Science, Cairo University. The plants were shade dried and finally powdered by electrical mill.

Culex pipiens larvae as well as *Biomphalaria alexandrina* snails were collected from their natural development sites from the irrigation schemes in Giza Governorate, Delta Valley in plastic whirl-pack bags containing natural breeding water and transported in a portable ice-box to be adapted in the laboratory.

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Preparation of plant extracts

Known weights (100 gm) of the dry powder of each of the tested plants were separately extracted with petroleum ether, benzene, acetone, chloroform, ether, ethyl acetate and methanol. Each extract was dried under reduced pressure using rotavapor and stored as a stock until use. Each extract was diluted on the basis of weight/volume in distilled water to a series of dilutions that would permit the computation of LC_{50} and LC_{95}.

Rearing & maintenance of *Culex pipiens*

Larvae were reared in white enamel pans, 30 cm diameter, and 10 cm. depth, containing a mixture of tap water and natural breeding water (1:1). Larvae were fed on Tetramine (Tropical fish-food, Tetra Werke W. Germany). Larvae and developing pupae were maintained in a walk-in insectary under controlled conditions of temperature (27±2°C), 70-80% R.H. and 16:8 h light : dark photoperiod. Developing pupae were collected daily, transferred to small plastic containers ¾ filled with tap water and then introduced into 30 cm^3 screened wooden cubic cages for adult emergence. Emerging adults were maintained under the same environmental conditions and had access to a 10% sucrose solution soaked in cotton pads as a carbohydrate source, and tap water as an oviposition medium. Upon hatching neonate larvae were collected and used for bioassay experiments.

Rearing & maintenance of *Biomphalaria alexandrina*

Freshly collected Biomphalaria alexandrina snails were examined successively in the laboratory for natural trematode infection. Positive snails were discarded. Adult healthy snails of a moderate size (7–10 mm diameter) were adapted in dechlorinated tap water under normal laboratory conditions (25±2°C) with diurnal alteration, pH 7–7.8 and fed on dried lettuce leaves for three weeks before being used for susceptibility experiments.

Susceptibility tests

Both pests were exposed to a series of extract dilutions ranging from 500–10 ppm using dipping technique.

Twenty five neonate 1st instar larvae of *C. pipiens* with four replicates per concentration and 10 snails with 3 replicates were used to establish the mortality regression lines. The exposure was extended to 24 hrs followed by another 24 hrs as recovery period. The percentage mortality was counted and compared with the control mortalities where individuals received only 0.05% ethanol.

The mortality data were corrected (Abbott, 1925) and submitted to probit analysis. LC_{50}, LC_{95} (ppm.), and the slope function with fiducial limits at 95% significance level were computed using probit analysis software (Raymond, 1985) according to Finney (1971).

RESULTS AND DISCUSSION

One approach to the control of tropical diseases is the elemination of the vectors responsible for their transmission. Filaria requires mosquito larvae as intermediate host of *Wuchereria bancrofti,* while schistosomiasis requires aquatic snails as intermediate host of *Schistosoma mansoni.* By the use of plant-derived molluscicides and insecticides, an attempt can be made to control the spread of these diseases.

The toxicity of *Euphorbia helioscopia, Calendula micrantha* and *Azadriachta indica* extracts were evaluated against *Biomphalaria alexandrina* snails and *Culex pipiens* first instar larvae to determine the suitable solvent used for extracting the active ingredients of the three tested plants. Results are presented in Tables 1, 2 and 3.

It should be mentioned that first instar larvae of *C. pipiens* were used in susceptibility tests to avoid the tedious and possibly inaccurate selection of successive instars since variation in size,

Table 1. The larvicidal and molluscicidal activities of some extracts of *Euphorbia helioscopia* against 1st instar *Culex pipiens* larvae and *Biomphalaria alexandrina* snails.

Extract	*Culex pipiens* larvae				*Biomphalaria alexandrina* snails			
	LC_{50}ppm	LC_{90}ppm	Slope function	χ^2	LC_{50}ppm	LC_{90}ppm	Slope function	χ^2
Acetone	50.58 (47.71–54.06)	80.79 (72.9–92.84)	6.3±0.55	2.70	10.13 (6.47–15.96)	23.28 (10.85–52.81)	3.54±0.55	7.94
Methanol	66.45 (51.02–86.98)	113.3 (72.58–184.52)	5.53±0.79	6.70	19.54 (17.95–21.4)	39.1 (34.10–46.77)	4.26±0.35	0.74
Chloroform	60.13 (54.54–66.64)	134.69 (115.6–164.13)	3.66±0.29	4.10	16.94 (15.32–18.83)	38.69 (33.03–47.47)	3.57±0.28	3.61
Ethyl acetate	84.94 (80.22–90.3)	136.1 (124.22–153.2)	6.26±0.49	4.31	23.74 (22.14–25.58)	23.74 (37.77–48.83)	5.12±0.41	3.74
Ether	61.26 (55.49–67.98)	138.33 (118.02–170.7)	3.62±0.31	3.60	17.22 (16.15–18.47)	29.03 (26.01–33.40)	5.65±0.47	0.17
Petroleum ether	57.07 (51.33–64.02)	135.77 (114.2–170.34)	3.4±0.28	1.10	13.98 (12.98–15.12)	25.65 (22.78–29.97)	4.86±0.40	1.19
Benzene	98.01 (85.94–113.31)	285.5 (228.4–384.86)	2.76±0.24	1.08	30.10 (27.89–32.66)	56.12 (49.63–65.97)	4.74±0.39	0.56

Table 2. The larvicidal and molluscicidal activities of some extracts of *Calendula micrantha* against 1st instar *Culex pipiens* larvae and *Biomphalaria alexandrina* snails.

Extract	*Culex pipiens* larvae				*Biomphalaria alexandrina* snails			
	LC_{50}ppm	LC_{90}ppm	Slope function	χ^2	LC_{50}ppm	LC_{90}ppm	Slope function	χ^2
Acetone	59.22 (52.02–68.04)	171.1 (138.64–225.7)	2.78±0.23	0.51	44.36 (42.05–47.52)	72.82 (66.25–82.47)	6.03±0.50	2.36
Methanol	83.88 (76.55–92.66)	117.15 (152.06–217.1)	3.95±0.34	1.16	69.41 (64.37–75.53)	127.63 (111.95–152.4)	4.85±0.42	3.11
Chloroform	88.96 (70.96–111.94)	130.75 (87.97–198.9)	6.67±1.31	9.34	77.33 (70.70–85.76)	157.89 (134.42–196.8)	4.13±0.37	4.78
Ethyl acetate	112.33 (106.8–118.62)	170.31 (156.96–189.4)	7.09±0.56	4.11	96.05 (90.28–102.94)	158.8 (142.61–183.64)	5.87±0.51	3.47
Ether	117.92 (111.27–125.51)	193.155 (175.14–220.17)	5.98±0.51	1.63	102.27 (94.78–110.9)	190.98 (168.97–224.4)	4.73±0.39	1.51
Petroleum ether	107.93 (99.74–117.71)	204.81 (179.4–244.07)	4.61±0.38	0.57	99.31 (92.49–107.49)	179.85 (156.5–208.45)	5.12±0.44	1.89
Benzene	95.53 (80.74–113.38)	135.94 (101.2–189.3)	8.37±1.28	6.17	83.33 (75.56–93.25)	182.18 (153.08–300.6)	3.28±0.27	3.79

Table 3. The larvicidal and molluscicidal activities of some extracts of *Azadriachta indica* against 1st instar *Culex pipiens* larvae and *Biomphalaria alexandrina* snails.

Extract	*Culex pipiens* larvae				*Biomphalaria alexandrina* snails			
	LC_{50}ppm	LC_{90}ppm	Slope function	χ^2	LC_{50}ppm	LC_{90}ppm	Slope function	χ^2
Acetone	52.98 (42.18–64.41)	35.53 (225.55–574.3)	1.60±0.14	3.44	13.01 (12.05–14.12)	24.56 (21.74–28.76)	4.65±0.34	3.98
Methanol	62.76 (55.16–72.17)	180.34 (146.1–237.46)	2.80±0.23	1.78	23.88 (22.67–25.22)	36.71 (33.85–40.75)	6.87±0.54	5.94
Chloroform	85.09 (72.72–100.81)	317.38 (244.95–445.9)	2.2±0.18	1.08	37.18 (32.56–42.52)	40.29 (38.76–64.36)	10.50±1.70	6.52
Ethyl acetate	46.09 (41.48–51.48)	109.72 (92.88–136.55)	3.4±0.28	2.98	17.52 (16.52–18.64)	28.49 (25.99–32.07)	6.07±0.48	5.33
Ether	No Mortality up to 1000 ppm				No Mortality up to 1000 ppm			
Petroleum ether	No Mortality up to 1000 ppm				No Mortality up to 1000 ppm			
Benzene	No Mortality up to 1000 ppm				No Mortality up to 1000 ppm			

weight and physiological age of larvae segnificantly enhance variation in bioassay data (Ibarra & Federici 1987), while neonate larvae are conditionally uniform since provide a uniform response and reduce the variation in data (Hughes and Wood, 1981) and respond to lower concentration of testing materials (Davidson 1982 and Dharmshaktu *et al.*, 1987).

Results in Table (1) showed that the acetone extract of *E. helioscopia* was the most toxic extract against both *C. pipiens* larvae and *B. alexandrina* snails with LC_{50} of 50.58 and 10.13 ppm respectively, whereas the benzene extract showed the lowest activity with LC_{50}=98.01 and 30.1 ppm against both pests respectively. Other extracts showed moderate toxicity towards the two pests. The molluscicidal activity of the acetone extract was previously reported by Shoeb and El-Sayed (1984).

Comparing the LC_{50} values of *C. micrantha* extracts against *C. pipiens* larvae and *B. alexandrina* snails (Table 2), revealed that the acetone extract is the most active (LC_{50}=59.22 and 44.63 ppm) respectively. Also, the remaining extracts showed reducing toxicity starting with ethanol, followed by chloroform, benzene, ethyl acetate, petroleum ether and finally ether extract which exhibited the lowest potency toward the two organisms.

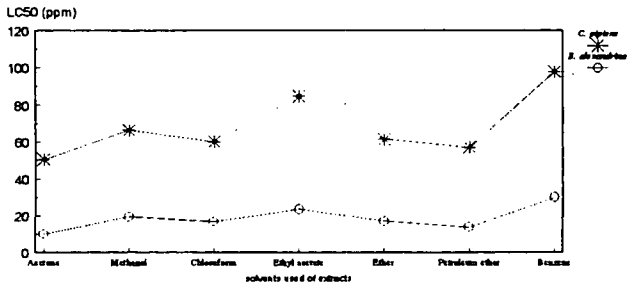
In the case of *A. indica* (Table 3), the acetone extract also recorded higher activity against *C. pipiens* larvae and *B. alexandrina* snails with LC_{50}=52.98 and 13.01 ppm respectively, whereas ether, petroleum ether and benzene extracts showed no activity up to 500 ppm and 300 ppm against *C. pipiens* and *B. alexandrina* respectively.

From the above data it can be concluded that:

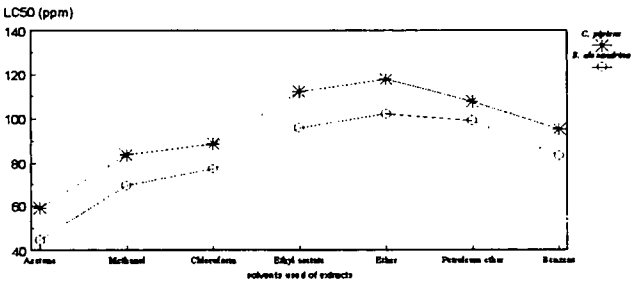
- The three tested plants exhibited dual toxic effect against both *C. pipiens* larvae and *B. alexandrina* snails and these results are in full agreement with previous studies on *Fagara macrophylla, Agave americana* and saponins of other plants which showed both molluscicidal and larvicidal activities (Kubo *et al.*, 1984; Dharmshaktu *et al.*, 1987; Maillard *et al.*, 1993 and Marston *et al.*, 1993).

- Acetone extracts of the three plants were the most active against both pests and this observation could be attributed to the presence of diterpene compounds as the toxic constituents in *E. helioscopia* and *A. indica* (Yamamura *et al.*, 1981; Evans 1978 and Ara *et al.*, 1990), and sesquiterpene compounds in *C. micrantha* which are characteristic of family Compositae (Garcia *et al.*, 1976; Nakanishi 1982 and Rodriguez *et al.* 1976).
- It was also noticed that both *E. helioscopia* and *A. indica* are more active than *C. micrantha.* This is in full agreement with high biological activity of the diterpene compounds present in the two plants (Evans, 1978 and Sakata *et al.*, 1971)
- *B. alexandrina* snails were found to be more susceptible than *C. pipiens* larvae towards all extracts of the tested plants where *B. alexandrina* recorded higher mortality. This observation was consistent with Gebremedlin *et al.* (1994) who reported that mosquito larvae were more resistant than *B. glabrata* snails to *Tetrapleura tetraptera* and *Phytolacca dodecandra* plants.
- The molluscicidal activity of *C. micrantha* extracts against *B. alexandrina* snails agreed with that previously reported by Shoeb and Refahy (1986).
- The similarity in response data of both organisms toward all extracts tested revealed the possibility that extracts act by similar mode of action in both organisms regardless the mode of entry which may differ from one species to another. Pattern of LC_{50}s of both pests treated with different solvent extracts of *Euphorbia helioscopia, Calendula micrantha* and *Azadriachta indica* are presented in the Figure 1 (a, b, c) respectively.

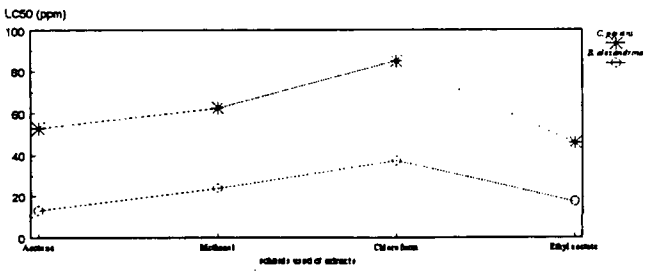
Figure 1(a) Pattern of LC_{50}s of *C. pipiens* larvae and *B. alexandrina* snails treated with extracts of *E. helioscopin.*



(b) Pattern of LC_{50}s of *C. pipiens* larvae and *B. alexandrina* snails treated with extracts of *C. micrantha.*



(c) Pattern of LC_{50}s of *C. pipiens* larvae and *B. alexandrina* snails treated with extracts of *Azadriachta indica.*



REFERENCES

Abbott, W. S. (1925). A method of comparing effectiveness of insecticides. *J. Econ. Entomol.* 18: 365–367.

Ahmed, E. M. Bashir, A. K. and El-Kheir, Y. M. (1984). Investigations of Molluscicidal activity of certain Sudanese plants used in folk medicine. *Plant Med.* 10(1): 74–77.

Ara, I., Bina S., Siddiqui S., Shaleen F. and Salimuzzamen S. (1990). Tricyclic diterpenes from the stem bark of Azadriachta indica. *Planta Medica* 56: 84–86.

Archibald, R. G. (1933). The use of the three Balanites aegyptiaca in the control of schistosomiasis in the Sudan. *Trans. Roy. Soc. Trop. Med. Hyg.* 27: 207–211.

Ayoub, M. and Yankov, L. K. (1986). On the molluscicidal activity of the plant Phenolic. *Fitoterapia.* 56(4): 223–226.

Davidson, E. W. (1982). Purification and properties of soluble cytoplasmic toxin from the mosquito pathogen Bacillus sphaericus strain 1593. *J. Invert. Pathol.* 39: 6–9.

Dharmshaktu, N. S., P. K. Prabhakaran and P. K. M. Menon. (1987). Laboratory study on the mosquito larvicidal properties of leaf and seed extract of the plant Agave americana. *J. Trop. Med. Hyg.* 90(2): 79–82.

Evans, F. J. (1978). The Tigliene, Daphnane and Ingenane Diterpenes, their chemistry, distribution and biological activities . *J. Lloydia* 193–233.

Finney, D. J. (1971). Probit analysis, *Cambridge University Press,* 3rd Edition.

Gebremedhin, G., Adewunmi, C. O., Becker, W., Agbedahunsi, J. M. and Dorfler, G. (1994). Hirudinicidal activities of some natural molluscicides used in schistosomiasis control. *J. ethnopharmacol.* Ireland: Elsevier Science, Ireland Ltd. 41(1/2):127–132.

Hostettmann, K. (1984). The use of plants and plants derived compounds for the control of schistosomiasis. *Naturwissenschaften* 71(5): 247–251.

Hughes, P. R. and H. A. Wood. (1981). A synchronous peroral technique for the bioassay of insect viruses. *J. Invert. Pathol.* 37: 154–159.

Ibarra J. E. and B. A. Federici. (1987). An alternative bioassay employing neonate larvae for determining the toxicity of suspended particles to mosquitoes. *J. of the American Mosquito control Association* 3(2): 187–192.

Khalil, M., A. Halawani and I. S. Hilmy. (1932). On the transmission of filariasis bancrofti in Egypt. *J. Egypt. Med. Ass.* 15: 317–322.

Kubo, I., Matsumoto, T., Klocke, J.A. and Kamikawa, T. (1984). Molluscicidal and insecticidal activities of isobutylamides isolated from Fagara macrophylla. *Experientia.* Basel : Birkhauser 40(4): 340–341.

Maillard, M., Marston, A. and Hostettmann, K. (1993). Search for molluscicidal and larvicidal agents from plants. ACS-Symp-Ser. Washington, D.C.: American Chemical Society (534):256–273.

Marcos G., Silva A. J. R., Paul M. B., Gillbert B. and J. A. Rabi (1976). Absolute stereochemistry of Eremanthine, A schistosomicidal sesquiterpene lactone from Eremanthus elaegnus. *Phytochemistry* 15: 331–332.

Marston, A., Maillard, M. and Hostettmann, K. (1993). Search for antifungal, Molluscicidal and larvicidal compounds from African medicinal plants. *J. Ethno-Pharmacol.* Limerick: Elsevier Scientific Publishers 38(2/3):215–223.

Mohsen, Z., H., Abdel-Latif, M. Jawad, M. Al-Saadi and A. Al-Naib. (1990). Mosquito larvicidal and ovipositional activity of Descurania sophia extract. *Int. J. Crude Drug. Res.* 28(1): 77–80.

Nakanishi, K. (1982). Recent studies on bioactive compounds from plants. *J. Nat. Prod.* 45: 15–26.

Nitao, J. K., Nair, M G., Thorogood, D. L., Johnson, K. S. and Scriber, J. M. (1991). Bioactive neolignans from the leaves of Magnolia virginiana. *Phytochemiestry.* Oxford, Pergamon Press 30(7): 2193–2195.

Raymond, M. (1985). Presentation d'un programme d'analyse log-probit pour micro-ordinnateur. Cah. Orstom, Ser. Ent. med et Parasitol., Vol. 22(2): 117–121.

Rodriguez E., G.H.N. Towers and J.C. Mitchell. (1976). Biological activities of sesquiterpene lactones. *Phytochemistry* 15: 1573–1580.

Sakata, K., Kawazu, K. and Mitsui, T. (1971). Studies on a pescicidal constituents of Hura vepitans part I : Isolation and characterization of Huraloxin and its piscicidal activity. *Agr. Biol. Chem.* 35:1084–1091.

Sharma, V. P. and M. A. Ansari. (1994). Personal protection from mosquitoes (Diptera:Culicidae) by burning neem oil in kerosene. *J. Med. Entomol.* 31(3): 505–507.

Shauke Y.; Seiji K.; Shigeru O.; Masatoki I.; and Yoshihiko S. (1981). The isolation and structures of Euphoscopins A and B. *Tetrahedron letters* 22(52): 5315–5318

Shoeb, H.A. and L.A. Refahy (1986). The molluscicidal properties of Compositae; Calendula micrantha. *Helminthologia* 23: 105–111.

Shoeb, H.A. and M.M. El-Sayed. (1984). A short communication on the molluscicidal properties of some plants from Euphorbiaceae and Agavaceae. *Helminthologia* 21: 33–54.

Wagner, V.A. (1933). The possibility of Eradicating Bilharzia by extensive planting of the tree Balanities. *South Africa Med. J.* 10: 10

WHO (1986). Molluscicides. WHO, Tech. Rep. Ser. 737,7.

WHO (1980). Epidemiology and control of schistosomiasis, WHO Tech. Rep. Ser. 643