

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 *Azadirachta 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 inhabit 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 (Hostéttmann, 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 madagascariensis* were the most commonly studied (WHO, 1980).

The present work is an attempt to evaluate the effect of some extracts of three plants *Euphorbia helioscopia*, *Calendula micrantha* and *Azadirachta 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 *Azadirachta 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.

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 ($27 \pm 2^\circ\text{C}$), 70-80% R.H. and 16:8 h light : dark photoperiod. Developing pupae were collected daily, transferred to small plastic containers $\frac{3}{4}$ filled with tap water and then introduced into 30 cm³ 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 ($25 \pm 2^\circ\text{C}$) 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 elimination 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 *Azadirachta 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,

