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# INFLUENCE OF SEVERAL PLANT EXTRACTS ON THE OVIPOSITION BEHAVIOUR OF *AEDES FLUVIATILIS* (LUTZ) (DIPTERA: CULICIDAE) IN THE LABORATORY

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*Whole, ethanolic, hexanic, lyophilized extracts of several plants and anacardic acid were tested in respect of their influence on the oviposition behaviour of* Aedes fluviatilis *(Lutz) at 100, 10 and 1 ppm concentrations. Extracts of* Allium sativum, Jatropha curcas, Mikania schenkii, Poinciana regia *and* Spatodea campanulata *had a repulsive effect ($\alpha = 0.05$) on females at 100 ppm, those of* Anacardium occidentale, Bidens segetum *and* Caesalpinia peltophoroides *were also repelent at 10 ppm. Extracts of* Coriandrum sativum *(100, 10 and 1 ppm),* Chara zeylanica *(10 ppm),* Cupressus sempervirens *(10 ppm),* Foeniculum vulgare *(10 ppm) and* Spatodea campanulata *(1 ppm) were attractive to the females; 13 (52.0%) of the extracts tested, did not influence the oviposition behaviour.*

Key words: *Aedes fluviatilis* – oviposition behaviour – plant extracts

Most work, relating mosquitoes to plants deals with the larvicidal properties of plant extracts or their mechanical influence on their breeding places (Supavarn et al., 1974; Judd & Borden, 1980; Hobbs & Molina, 1983; Consoli et al., 1988), but little attention has been paid to their chemical influence on oviposition behaviour. Oviposition site selection seems to be the most important factor in determining mosquito breeding places and therefore the distribution of species in nature (Ikeshoji & Mulla, 1970; Ikeshoji et al., 1975). Many works show the selectiveness of mosquito females in choosing their breeding sites and the numerous factors which can affect it (Consoli & Williams, 1978; Hwang & Mulla, 1980; Leite, 1980; Trimble & Wellington, 1980). Chemicals with either repulsive or attractive properties to ovigerous mosquito females would be very useful tools in control programmes, especially since usually females are able to react to small amount of these. *Aedes (Ochlerotatus) fluviatilis* (Lutz, 1904) is a widely distributed neotropical species, found in domestic, peridomestic and silvatic habitats and the present work aims to assess the influence of some plant extracts on its oviposition behaviour.

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## MATERIAL AND METHODS

*Mosquitoes employed:* males and females of *Ae. fluviatilis* were obtained from a colony maintained at Centro de Pesquisas René Rachou – FIOCRUZ, MS, Belo Horizonte, Minas Gerais, Brazil. Routine breeding techniques are described in Consoli & Williams (1978) and Consoli & Williams (1981).

*Plant extracts:* Table I shows the 25 extracts, obtained from 22 different plants, by 5 diverse methods:

- Whole extracts: the fresh plant parts were triturated with an equal volume of distilled water. The resulting mass was strained through a piece of four fold surgical gauze and the obtained liquid constituted the extract.
- Lyophylized extracts: the plant parts were dried and 1 g was boiled for 15 min in 250 ml of distilled water. The resulting liquid was passed through filter paper and 6 ml of it was lyophylized.
- Hexanic extracts: the dried and ground plant parts were extracted with hexane, in a Soxhlet apparatus for 72 h, the solvent being evaporated afterwards (Mendes et al., 1984).

To attain solubility in water, 0.02 ml methanol were added to each 50 mg extract. Previous experiments showed that methanol at this concentration did not affect oviposition behaviour.

- Ethanolic extracts: were prepared in a similar way to hexanic extracts, only changing hexane for ethanol. Previous to solution in water each 50 mg extract were dissolved in 1 ml ethanol. This procedure did not influence oviposition behaviour.

- Anacardic acid: was prepared at the Chemical Laboratory of Centro de Pesquisas René Rachou, accordingly to Tyman (1976).

TABLE I

Plants: species, family, extracts and parts employed

| Species | Family | Extracts | Parts employed |
|---|---|---|---|
| *Agave americana* L. | Agavaceae | whole | leaves |
| *Allium sativum* L. | Liliaceae | lyophilized<br>whole | stem<br>stem |
| *Ampelozizyphus amazonicus* Duck. | Rhamnaceae | lyophilized | total |
| *Anacardium occidentale* L. | Anacardiaceae | anacardic acid<br>hexanic | rind of fruits<br>rind of fruits |
| *Bidens segetum* Mart. ex Colla | Compositae | ethanolic | total |
| *Caesalpinia peltophoroides* Benth | Caesalpinaceae (Leg.) | ethanolic | stem and leaves |
| *Chara zeylanica* A. Brown | Characeae | lyophilized | stem and leaves |
| *Coriandrum sativum* L. | Umbeliferae | lyophilized<br>lyophilized | fruits<br>leaves |
| *Cupressus sempervirens* L. | Cupressaceae | lyophilized | leaves |
| *Dieffenbachia picta* Schott | Araceae | lyophilized | leaves |
| *Eucalyptus saligna* Smith | Myrtaceae | lyophilized | leaves |
| *Euphorbia cotinifolia* L. | Euphorbiaceae | lyophilized | leaves |
| *Foeniculum vulgare* Mill. | Umbeliferae | lyophilized | leaves |
| *Jatropha curcas* L. | Euphorbiaceae | ethanolic | fruits and leaves |
| *Mikania hisurtissima* DC. | Compositae | hexanic | stem and leaves |
| *Mikania schenkii* Hieron | Compositae | ethanolic | stem and leaves |
| *Nerium oleander* L. | Apocinaceae | ethanolic | stem and leaves |
| *Petroselium sativum* L. | Umbeliferae | lyophilized | flowers and leaves |
| *Poinciana regia* Bojer | Caesalpinaceae (Leg.) | ethanolic | flowers and leaves |
| *Ruta graveolens* L. | Rutaceae | ethanolic | stem and leaves |
| *Spatodea campanulata* P. Beauv. | Bignoniaceae | ethanolic | flowers |
| *Vernonia salzmanni* DC | Compositae | ethanolic | stem and leaves |

*Experiments:* twenty-five experiments, each repeated three times, were carried out. For each replicate 200 males and 200 females, aged between 4 and 5 days, were put into a cage build of "eucatex" and nylon netting (40 x 40 x 40 cm). A supply of 5% honey solution was provided. Five days after females had taken a blood meal on anaesthetized mice *(Mus musculus)* experimental and control dishes were put into the cage for 24 h, being the number of eggs laid in each dish recorded. For each experiment, a different plant extract was used in solutions of 100, 10 and 1 ppm in distilled water and placed inside the cages in transparent glass dishes (150 ml/9.5 cm diameter). Always a similar dish, containing only distilled water was added as control. The position of the dishes was different inside each replicate cage.

*Statistical evaluation:* the differences between means were evaluated using Ducan's test (Levin, 1978) and a significance level of $\alpha = 0.05$ (5%) was adopted.

## RESULTS AND DISCUSSION

Table II shows totals, means and standard deviations of eggs laid in the diverse experimental media and in control dishes. Twelve (48.0%) of the 25 extracts tested influenced oviposition behaviour in an attractive or repulsive way and 13 (52.0%) were indifferent to females at oviposition.

*Extracts with a repulsive effect:* of all extracts tested, 8 (32.0%) repelled females significantly at 100 ppm concentration: *A. sativum* (2), *A. occidentale* (2), *B. segetum, C. peltophoroides, J. curcas, M. schenkii, P. regia* and *S. campanulata.* At 10 ppm only 3 (12.0%) extracts maintained such properties *(A. occidentale* (2), *B. segetum* and *C. peltophoroides)* and none was repulsive to females at 1 ppm. Consoli (1987) and Consoli et al. (1988) observed in *Ae. fluviatilis* that at 100 ppm solutions the extracts of *A. occidentale* (2) and *S. campanulata* enhanced larval mortality and *A. sativum* (2) did so at 1 ppm but similar extracts of *B. segetum, J. curcas, M. schenkii* and *P. regia* were innocuous to larval at 100 ppm concentrations. No references were found on the influence of these plants on the oviposition behaviour of other mosquitoes, but Heal & Roger (1950) mention the larvicidal properties, in higher concentrations, of extracts of other species of genera *Bidens, Caesalpinia* and *Jatro-*

TABLE II

Totals, means and standard deviations of eggs laid in 100, 10, 1 ppm of plant extracts and control dishes

| Plants extracts | Experiments ppm 100 | | 10 | | 1 | | Control | |
|---|---|---|---|---|---|---|---|---|
| | N | $\bar{x} \pm s$ | N | $\bar{x} \pm s$ | N | $\bar{x} \pm s$ | N | $\bar{x} \pm s$ |
| Anacardic acid | 1496 | 498.7/ 369.8 | 2563 | 854.3/ 519.1 | 2565 | 855.0/162.7 | 3283 | 1094.3/103.6 |
| *A. americana* | 1143 | 381.0/ 239.4 | 2123 | 707.7/ 554.0 | 1796 | 598.7/ 61.8 | 1492 | 497.3/435.5 |
| *A. sativum 1* | 1932 | 644.0/ 201.5 | 2982 | 994.0/ 212.8 | 3111 | 1037.0/516.3 | 2951 | 983.7/565.3 |
| *A. sativum 2* | – | – | 3453 | 1151.0/ 466.3 | 4744 | 1581.3/718.2 | 2552 | 850.7/116.4 |
| *A. amazonicus* | 2667 | 889.0/ 112.1 | 2133 | 711.0/ 181.2 | 3435 | 1145.0/323.0 | 2984 | 994.7/539.1 |
| *A. occidentale* | – | – | 1768 | 589.3/ 446.1 | 2780 | 926.7/822.3 | 4962 | 1654.0/311.6 |
| *B. segetum* | – | – | 216 | 72.0/ 64.2 | 1596 | 532.0/175.8 | 1510 | 503.3/329.9 |
| *C. peltophoroides* | 179 | 59.7/ 100.8 | 561 | 187.0/ 40.6 | 1379 | 459.7/124.8 | 527 | 371.7/183.5 |
| *C. zeylanica* | 1092 | 364.0/ 264.2 | 1831 | 610.3/ 105.5 | 317 | 105.7/ 98.3 | 712 | 237.3/220.2 |
| *C. sativum 1* | 1281 | 427.0/ 322.4 | 2163 | 721.0/ 305.8 | 1308 | 436.0/433.4 | 1106 | 368.7/215.9 |
| *C. sativum 2* | 4952 | 1650.7/ 216.8 | 4890 | 1630.0/1098.7 | 4998 | 1666.0/168.6 | 1208 | 402.7/190.6 |
| *C. sempervirens* | 847 | 282.3/ 198.0 | 5331 | 1777.0/ 865.2 | 1979 | 659.7/289.3 | 614 | 204.7/274.1 |
| *D. picta* | 1223 | 407.7/ 365.0 | 1212 | 404.0/ 449.1 | 1083 | 361.0/527.8 | 1005 | 335.0/252.9 |
| *E. saligna* | 3355 | 1118.3/1185.6 | 1370 | 856.7/ 537.7 | 2300 | 766.7/159.5 | 2573 | 857.7/456.7 |
| *E. cotinifolia* | 3623 | 1207.7/ 417.5 | 3312 | 1104.0/ 306.4 | 2701 | 900.3/240.4 | 2061 | 687.0/367.8 |
| *F. vulgare* | 334 | 111.3/ 65.2 | 965 | 321.7/ 145.3 | 303 | 101.0/ 38.0 | 48 | 16.0/ 27.7 |
| *J. curcas* | 180 | 60.0/ 103.9 | 644 | 214.7/ 206.1 | 1265 | 421.6/432.6 | 2328 | 776.0/432.4 |
| *M. hisurtissima* | 4467 | 1489.0/ 692.0 | 6250 | 2083.3/ 595.8 | 3990 | 1330.0/632.2 | 2900 | 966.7/160.7 |
| *M. schenkii* | 129 | 43.0/ 13.9 | 687 | 229.0/ 199.0 | 1261 | 420.3/191.6 | 1411 | 470.3/194.3 |
| *N. oleander* | 1070 | 356.7/ 122.3 | 2041 | 680.3/ 425.2 | 2872 | 957.3/579.3 | 2260 | 753.3/196.0 |
| *P. sativum* | 1857 | 619.0/ 358.0 | 2122 | 707.3/ 414.8 | 1747 | 582.3/386.4 | 3013 | 1004.3/175.0 |
| *P. regia* | 104 | 34.7/ 60.0 | 1811 | 603.7/ 211.7 | 1697 | 565.7/383.2 | 686 | 228.7/180.4 |
| *R. graveolens* | 68 | 22.7/ 39.3 | 881 | 293.7/ 195.0 | 1090 | 363.0/250.3 | 653 | 217.7/257.6 |
| *S. campanulata* | – | – | 759 | 253.0/ 228.2 | 1533 | 511.0/357.3 | 820 | 273.0/175.8 |
| *V. salzmanni* | 171 | 57.0/ 52.8 | 487 | 162.3/ 99.8 | 1002 | 334.0/171.5 | 878 | 292.7/266.7 |

*pha*. Also Amonkar & Reeves (1970) showed the larvicidal effect of *A. sativum* extracts on several species of *Aedes* and *Culex*.

*Extracts with an attractive effect:* only 1 (4.0%) extract was found to attract the females at all three concentrations employed: *C. sativum* (2); in 3 (12.0%) solely 10 ppm concentrations were attractive *(C. zeylanica, C. sempervirens* and *F. vulgare)* and *S. campanulata* extract was attractive exclusively at 1 ppm. All these extracts were innocuous to *Ae. fluviatilis* larvae, except *S. campanulata* at 100 ppm (Consoli, 1987; Consoli et al., 1988). There are numerous references on the influence of genus *Chara* on mosquitoes: Caballero (1919) ascribed larvicidal properties to *Chara foetida*, but McGregor (1924) observed that *Chara foetida* and *Chara hispida* were unable to deter larval development; Matheson & Hinman (1929) stated that in aquatic habitats containing *Chara fragilis* mosquito larvae did not complete their development and females of genera *Aedes* and *Culex* did not lay eggs. Amonkar & Reeves (1970) and Furlow & Hays (1972) also refer to the toxic properties of this genus to mosquitoes, but Angerilli (1980a) observed that *Chara globularis* was the predominant vegetation in some mosquito breeding sites, and that the water which previously contained this species was attractive to ovigerous *Ae. aegypti* females (Angerilli, 1980b). In previous experiments, it was asserted that *Ae. fluviatilis* larvae develop normally in dishes where *C. zeylanica* growned, and the actual presence of this species in the water was indifferent to its females at oviposition. No references were found on the effects of *C. sempervirens* and *F. vulgare* on mosquitoes, but Cruz (1979) refers to the popular reputation of the former as a mosquito repelent.

*Extracts that were indifferent to females:* the remaining 13 ( 52.0%) extracts did not influence oviposition in *Ae. fluviatilis*. Among these, the extracts of *A. americana, N. oleander* and *V. salzmanni* showed larvicidal activity at 100 ppm and *A. occidentale* (1) at 10 ppm (Consoli, 1987; Consoli et al., 1988). Sometimes the oviposition behaviour of *Ae. fluviatilis* toward an extract seems to be detached from the extract's toxicity for its larval: the extracts of *A. americana, C. sativum* (1) and *N. oleander* did not repel females, despite being toxic to the larval (Consoli et al., 1988). Maw (1970) observed that capric acid was simultaneously attractive to the females and toxic to the larval of *Culex restuans* and Murphey & Burbutis (1967) stated that female *Culex salinarius* were able to lay eggs in various solutions lethal to eggs and larval. On the other hand, in spite of being repulsive to females of *Ae. fluviatilis*, the extracts of *B. segetum, J. curcas, M. schenkii* and *P. regia* are harmless to the larvae of the same species (Consoli, 1987; Consoli et al., 1988). The concentration of some extracts offered seems to be important, since at 100 ppm oviposition was completely inhibited by *S. campanulata* and at 1 ppm ovigerous females were attracted. Different parts of the same plant may also have different effects: the leaf extract of *C. sativum* was attractive to females and innocuous to larvae but the fruit extract of the same plant resulted as indifferent to females but toxic to larvae (Consoli et al., 1988). Furlow & Hays (1972), Judd & Borden (1980) and Hobbs & Molina (1983) described physical and chemical ways plants can interfere with mosquito oviposition and development. Lewis et al. (1974) described the efficacy of ovitraps containing alfafa infusion for *Culex pipiens quinquefasciatus* and Sucharit et al. (1982) showed the oviposition preference of three species of *Mansonia* for water where *Pistia stratiotes* was present. The diversity of methods adopted by different authors make precise comparisons difficult, but it seems evident that numerous plants and plant products can have important influence on the reproductive behaviour of mosquitoes.

RESUMO

**Influência de diversos extratos vegetais sobre o comportamento de oviposição de *Aedes fluviatilis* (Lutz) (Diptera: Culicidae) em laboratório** — Extratos brutos, etanólicos, hexânicos, liofilizados de diversos vegetais e ácido anacárdico foram testados quanto a sua influência sobre o comportamento de oviposição das fêmeas de *Aedes fluviatilis* (Lutz), nas concentrações de 100, 10 e 1 ppm. Os extratos de *Allium sativum, Jatropha curcas, Mikania schenkii, Poinciana regia* e *Spatodea campanulata* mostraram-se repelentes ($\alpha = 0,05$) para as fêmeas na concentração de 100 ppm e os de *Anacardium occidentale, Bidens segetum* e *Caesalpinia peltophoroides* também na de 10 ppm. Os extratos de *Coriandrum sativum* (100, 10 e 1 ppm), *Chara zeylanica* (10 ppm), *Cupressus sempervirens* (10 ppm), *Foeniculum vulgare*

(10 ppm) e *Spatodea campanulata* (1 ppm) atrairam a oviposição das fêmeas; 13 (52,0%) dos extratos testados foram indiferentes às fêmeas nas concentrações utilizadas.

Palavras-chave: *Aedes fluviatilis* – comportamento de oviposição – extratos vegetais

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