Some Biological Effects of *Pimpinella anisum* L. Seed Extract on *Culex pipiens molestus* F.

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**ABSTRACT**

Different concentrations of seed extract of anise *Pimpinella anisum* L. were tested against 2nd instar larvae of mosquito *Culex pipiens molestus* F. One hundred percent mortality was observed at 10 µg/ml and the LC50 was 7 µg/ml after 48h following exposure. Results showed that seed extract was effective up to 30 days of storage. The seed extract exhibited growth-inhibiting activity. Various morphogenetic aberrations was found in the mosquito after treatment of 2nd instar larvae. Most of the treated larvae died in the larval exuvium. Unmelanized and melanized pupae were also noted and the adults were unable to free their appendages.

**INTRODUCTION**

The search and application of insect growth regulators (IGRs) of plant origin have received considerable attention in recent years, because these materials could be used to solve the serious problems of insect resistance to chemical insecticides. The IGRs are generally selective in their toxicity and slow in their killing action to insects, safe to the
environment and to the natural enemies of insect pests. It has been reported that there
are more than 1600 plant species known to possess bioactive properties (Grainge et al.,
1985; Schmutterer, 1995).

The investigations on the presence of insect growth regulators have been extended
to plant species of the family Umbelliferae. Hartzell and Wilcoxon (1941) reported
insecticidal effect by extract of *Carum carvi* to a number of mosquito species.Insecticidal
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The first published information available on anise *Pimpinella anisum* L. is by Heal
et al. (1950), who reported insecticidal and antifeedant activity of *P. anisum* against the
*Oncopeltus fasciatus*.

Shaaya and Pisarev (1991), Sarac and Tunc (1995) and Tunc et al. (2000) reported
that the essential oil of *P. anisum* has high toxicity to adults and eggs of different stored
grain insects. However, no information is yet available on the effect of *P. anisum* extract
don dipteran insects, especially on mosquitoes. The present paper reported the biological
activity of *P. anisum* seeds extract as a larvicide in the second instar larvae of *Culex
pipiens molestus* (Forskal).

**MATERIALS AND METHODS**

**Rearing method:**

Second instar larvae of *C. pipiens molestus* were collected from a stock colony and
kept in pyrex storage jars (80 by 100 mm) containing 200 ml of tap water as previously
described (Marcard, et al., 1986). The containers were held at 27±2°C and photoperiod of
16: 8(L :D). Daily larvae were supplied with rabbit food and water was replaced every
four days.

**Preparation of extract:**

Seeds of *Pimpinella anisum* were pulverized to a fine powder by means of a
hammer mill. The powder was extracted with 80 % methanol in a blender (50 g
seeds/100 ml solvent), stirred for 1h and filtered under reduced pressure. The residue was
extracted once again with fresh methanol. The extracts were combined, filtered,
concentrated *invacuo* and lyophilized for 24h. The dry crude extract was redissolved in
80% methanol and washed with the same volume of petroleum ether (b.p 30–50°C) by
stirring for half an hour.

After repetition of the procedure, the separated methanol extract was dried using a
rotary evaporated and freeze dryer and then partitioned between the solvent water and
ethyl acetate (1:1). The water layer was washed once again with fresh ethyl acetate. The
ethyl acetate extract was concentrated in a rotary evaporator and redissolved in 80%
methanol, giving a final concentration of 10%(w/v), which were stored in a refrigerator
at 5°C to be used as the basic solution for various concentrations in biological tests
(Breuer and DeLoof, 2000).

Additional stock solution 10% (w/v) was stored in a refrigerator for 30 days for
studying the persistence of the seed extract.
Bioassay:
The tests were conducted on second instar larvae of *C. pipiens molestus*. Newly ecdysed larvae were exposed to 5 concentrations ranging from 2 to 10 µg/ml until their emergence. Control larvae were reared in jars containing water and 80% methanol. The bioassay was conducted with three replicates each of 20 larvae. The insects were examined daily until adult emergence. Abnormal specimens were grouped according to morphogenetic abnormalities for quantitative assessment (Zebitz, 1984).

RESULTS
The seed extract of *Pimpinella anisum* was lethal to 80% of *C. pipiens molestus* larvae within 24h at 10 µg/ml (Table 1). After 2-days of exposure to the extract at the concentrations of 8 and 10 µg/ml, 70 and 100% larvae died, respectively. Those exposed to 6 µg/ml were 80% dead after 7 days. Mortality percentage for the larvae treated with 2 µg/ml was 40%, even by the seventh day. It was estimated that the LC50 value of the extract to the 2nd instar larvae was 7 µg/ml after 2-days.

When comparing the growth inhibiting and larvicidal effects of the concentrations to *P. anisum*, a stage specific difference in the biological activity becomes visible (Table 2). The concentration of 10 µg/ml caused larvicidal effect only, the larvae died at exuviate to the next instar (Fig.1, Plate a). Larval and pupal mortalities were high at 6 and 8 µg/ml, the larvae were unable to emerge from the exuvium and have died at this intermediate stage, and the died pupae were unmelanized with abdomen recurved (Fig.1, Plate b). The concentration of 4µg/ml caused mortality 16 in pupal and adult stages. Pupae which have died at 4µg/ml concentration partly melanized (Fig.1, Plate c), but when a similar concentration was given to 2nd instar larvae some of them were pupated, but produced defective adults. Such adults were able to liberate their anterior parts, but not their wings, abdomen and legs, its remained with pupal skins, other adults were attached to the pupal skins by their legs (Fig.1, Plate d and e). But at concentration of 2 µg/ml a high number of adults were emerged.

Table 1: The % mortality of 2nd instar larvae of *C. pipiens molestus* at various concentrations of seed extract of *P. anisum* (Initial number of larvae 60, at each concentration).

<table>
<thead>
<tr>
<th>Days after exposure</th>
<th>Concentrations (µg/ml)</th>
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<tbody>
<tr>
<td></td>
<td>0</td>
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<td>1</td>
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<td>32</td>
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<td>7</td>
<td>40</td>
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</table>
Table 2: Percente mortality of the different developmental stages following the exposure of the 2nd instar larvae of *C. pipiens molestus* to *P. anisum* seed extract (Initial number of larvae 60, at each concentration).

<table>
<thead>
<tr>
<th>Concentration (µg/ml)</th>
<th>Larvae</th>
<th>Pupae</th>
<th>Adults</th>
<th>Total</th>
<th>Adults Emerged</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>3</td>
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Fig. 1: Morphogenetic aberrations induced by *P. anisum* seed extract after the exposure of *C. pipiens molestus* 2nd instar larvae: a, larva in 2nd instar exuvium; b, pupa unmelanized; c, pupa melanized; d, adult liberated anterior part but wings, abdomen and legs remained within the pupal skin; and e, adult attached to pupal skin by legs.
Figure (2) revealed that after storage of extract at 8 and 10 µg/ml for a period of 30 days, exposure of the 2nd instar larvae to these concentrations gave 70 and 80% mortality, respectively. Generally, a sharp decline in mortality occurred at the lower concentrations (4 and 6 µg/ml).

**DISCUSSION**

It can be seen from the results that *P. anisum* extract possesses insecticidal activity to the 2nd instar larvae after 2 days of exposure. Mortality could be due to inhibition of development or to failure in ecdysis during the different stages of *C. pipiens molestus*. Embryo genesis Seed extract of *P. anisum* was more effective on the larval stage, since the LC50 value against larvae after 2 days exposure were found to be about 7 µg/ml. Shaaya and Pisarev (1991) also found that the essential oil of *P. anisum* caused 100, 75 and 12% mortality in adults of *Tribolium castanum*, *Rhyzopertha dominica* and *Sitophilus oryzae* with 24h, respectively. Tunc et al. (2000) showed that the fumigant activity of essential oil from *P. anisum* resulted in 100% mortality of the eggs of *T. confusum* and *Ephestia kuehniella*.

Mortality in treated larvae is high, especially during moulting and the pupae which are short-lived, dying even before they melanize. Also adults were partly emerged and appendages were deformed. It seems that seed extract of *P. anisum* have some compounds that could interfere with the hormonal balance, It resembles the effect of *Azadirachta indica* (Warthen, 1979; Zebitz, 1984 and Al-Sharook et al., 1991) and has the same effect to that insect growth regulator methoprene (Busvine, 1978).
There is, however, a need to investigate the biological effects seed extract of *P. anisum* against other insects. Research is now underway to formulate enriched products from plants with high insect growth regulator properties for practical applications.

REFERENCES


