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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.

بعض التاثيرات الحيوية لمستخلص بذور الانيسون *Pimpinella anisum* L. في البعوض *Culex pipiens molestus* F.

الملخص

أختبر تأثير تراكيز مختلفة من مستخلص بذور الانيسون *Pimpinella anisum* L. على يرقات العمر الثاني للبعوض *Culex pipiens molestus F.* اعطى المستخلص نسبة موت بلغت 100 % عند التركيز 10 مك/مل. وبلغت قيمة LC50 7 مك/مل وذلك بعد 48 ساعة من التعريض. وبينت النتائج ان مستخلص البذور ضل فعالا" بعد فترة خزن بلغت 30 يوما". اظهر مستخلص البذور فاعلية منظمة للنمو، فقد سبب عدة تشوهات مظهرية، اذ لوحظ ان معظم اليرقات الميتة كانت غير قادرة على الانسلاخ، وكانت العذارى الميتة فاقدة لصبغة الميلانين أو ذات صبغة. وكانت البالغات الناتجة غير قادرة على تخليص لواحق الجسم من جلد انسلاخ العذراء.

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 and growth inhibitory activity has also been reported to be present in *Coriandrum sativum* (Matthews, 1981).

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 on 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 extarcted 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 crud 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).

	Concentrations (µg/ml)					
Days after exposure	**0**	**2**	**4**	**6**	**8**	**10**
1	0	10	15	25	60	80
2	0	20	24	33	70	100
3	0	22	33	40	74	100
4	0	25	34	48	76	100
5	2	28	45	55	81	100
6	2	32	58	71	80	100
7	3	40	70	80	85	100

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).

Concentration (µg/ml)	Larvae	Pupae	Adults	Total	Adults Emerged
0	2	1	0	3	97
2	10	10	20	40	60
4	16	27	27	70	30
6	40	30	10	80	20
8	60	13	12	85	15
10	100	0	0	100	0

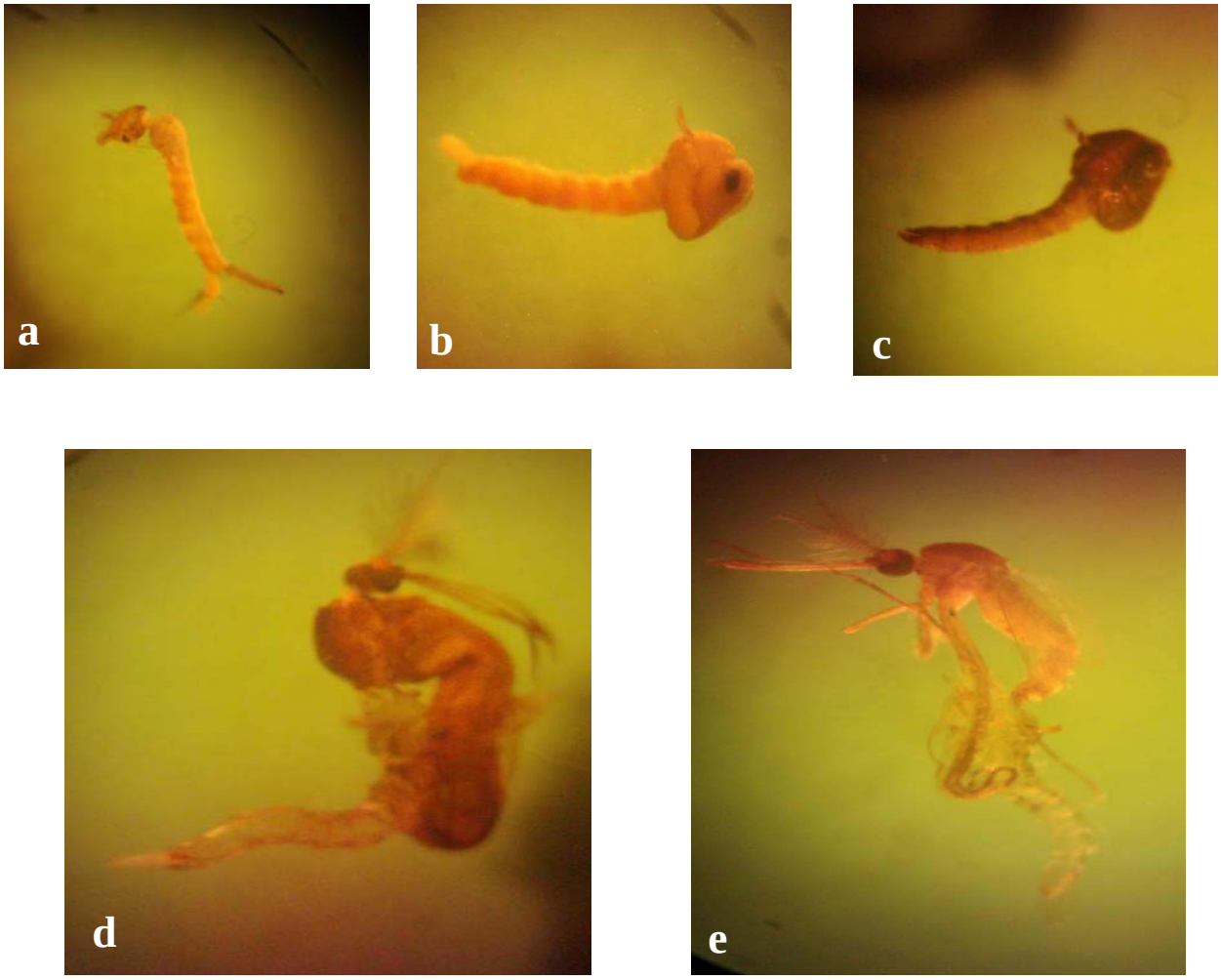

Fig.1: Morphogenetic aberrations induced by *P. anisum* seed extract after the exposure of *C. pipiens molestus* 2nd instar larvae: a,larva in 2nd instar exuvium; b, pupa unmelanized; c, pupa melanized; d, adult liberated anterior part but wings, abdomen and legs remained within the pupal skin; and e, 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).

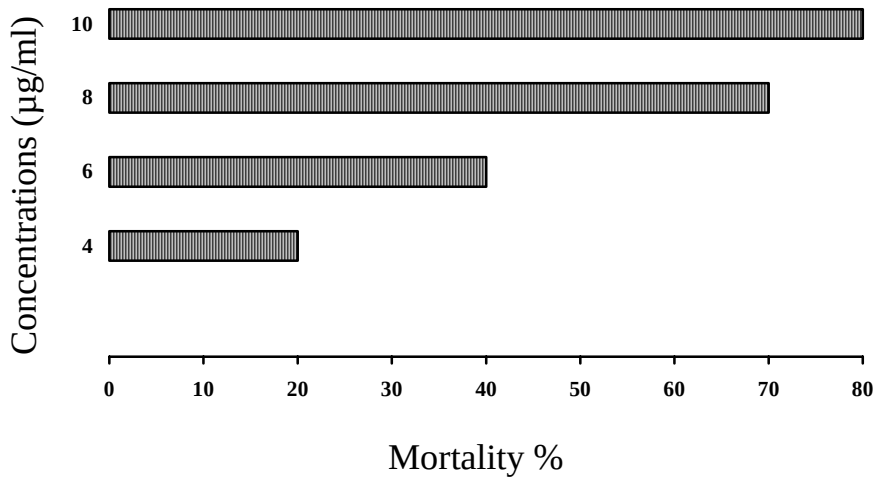

Fig. 2: Mortality percentages of *C. pipiens molestus* larvae exposed to seed extract of *P. anisum* after 30 days of storage.

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 castanium*, *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 kuelzniella.*

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.

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