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Full Length Research Paper

Larvicidal effects of aqueous extracts of *Balanites aegyptiaca* (desert date) against the larvae of *Culex pipiens* mosquitoes

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The effect of aqueous extracts of the fruit pulp, seed kernel, roots, bark, and leaves of *Balanites aegyptiaca* Del. (Zygophyllacea) against the larvae of the *Culex pipens* mosquito was investigated. Early fourth instars larvae of *C. pipiens* mosquitoes were exposed, for up to three days, to a dilution of 0, 0.1, 0.25, 0.5, 1.0, and 2.0% aqueous extracts of fruit pulp, seed kernel, roots, bark, and leaves. All tested extracts showed larval mortality, however, larval mortality was greatest with the aqueous root extract. The lowest concentration of root extract (0.1%) showed 100% larval mortality after three days, whereas a 0.5% concentration of aqueous bark extract was needed for 100% larval mortality. Aqueous extracts of leaf, fruit pulp, and seed kernel showed less larval mortality compared to the root and/or bark extracts. It is suggested that all parts of the *B. aegyptiaca* contain larvicidal properties that could be developed and used as natural insecticides for mosquito control.

Key words: *Culex pipens*, mosquito, desert date, *Balanites aegyptiaca*.

INTRODUCTION

Mosquitoes constitute a major public health menace as vectors of serious human diseases (El Hag et al., 1999). *Culex pipiens* (northern house mosquito) is the vector of the West Nile Virus (WNV) that causes encephalitis or meningitis. The disease affects the brain tissue and the most serious cases can result in permanent neurological damage and be fatal (Hubalek and Kaluuzka, 1999). WNV is distributed throughout Africa, the Middle East, and southern temperate and tropical Eurasia, and was recently introduced into North America as well (Campbell et al., 2002). There is no vaccine to prevent this infection, nor are there drugs to combat the disease in infected persons, so vector control is the most prevalent solution available so far for reducing morbidity. Most of the widely used vector interruption methods are synthetic insecticide-based. These synthetic insecticides not only affect the non-target population, but also constantly increase resistance to the vector (Wattal et al., 1981). The search for natural insecticides which do not have any ill effects on the non-target population and are easily degradable remains the top priority (Redwane et al., 2002).

Balanites aegyptiaca Del.*,* also known as 'desert date' in English, a member of the family Zygophyllaceae, is the one of the most common but neglected wild plant species of the dryland areas of Africa and South Asia (Hall and Walker, 1991). The tree can grow to 6–10 meters in height, is highly resistant to stresses such as sandstorms and heat waves, and grows with minimal available moisture. The tree has thick, tough glossy leaves, spiny branches, and a double root system, and produces date-like fruits. The plants grow extensively even when neglected. One estimate is that more than 400,000 tons of Balanites fruit are produced in Sudan alone (Mohamed et al., 2000). It can successfully grow in a marginal sand dune with saline and sewage water (data not yet published). Various parts of the Balanites tree have been used for folk medicines in many regions of Africa and Asia (Hall and Walker, 1991; Iwu, 1993; Newinger, 1996; Mohamed et al., 2002). A literature

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survey has revealed antifeedent, antidiabetic, molluscicide, antihelminthic, and contraceptive activities in various *Balanites* extracts (Liu and Nakanishi, 1982; Jain and Tripathi, 1991; Kamel et al., 1991; Ibrahim, 1992; Rao et al., 1997). Most of the studies reported the active compounds to be saponins. Saponins are amphiphilic glycosidic compounds with lipophilic nuclear aglycone and a hydrophilic sugar chain.

Saponins are freely soluble in both organic solvents and water (Hostettman and Marston, 1995). During the course of last two decades, many plant extracts have been evaluated for their larvicidal activities (Jang et al., 2002), in order to find a method of biological control of mosquitoes; very few studies were done about the larvicidal effect of saponin-rich plants (Pelah et al., 2002). However, most of the studies focused on organic solvent extracts. Taking the bioactivity of saponin compounds into consideration, and the presence of these compounds in *B. aegyptiaca*, along with the severity of mosquito-borne diseases and the socioeconomic situation in Balanites grown areas, this study was carried out on the larvicidal effect of the various extracts using simple procedures, of the Balanites plant against *C. pipens* larvae.

MATERIALS AND METHODS

Extracts of fruit pulp, seed kernel, leaf, root, and bark of *B. aegyptiaca* were prepared from plant material collected at the Ben-Gurion University of the Negev's Balanites plantation site at Kibbutz Samar, Israel. Extraction of all the parts was carried out using as simple a method as possible, thinking that it could be repeated any remote areas of the world where Balanites plants grow naturally. The idea is to allow for easy adoption of this methodology by the local communities. The outer cover (epicarp) of the fruit was removed by hand and the pulp was scraped manually. The endocarp (stone) of the fruits was broken manually and the seed kernel was then collected, dried, and pulverized. To prepare the extracts from leaf, bark, and root, fresh leaves, bark, and roots were collected, washed, chopped, dried, and ground to powder. Similarly, the fruit pulp was dried and powdered. Twenty grams of each ground/pulverized part were placed in separate Erlenmeyer flasks, 100 ml of tap water was added, and mixed vigorously. The mixture was kept 24 h with occasional shaking. After twenty-four hours, the mixture was filtered using a very fine muslin cloth and the final volume adjusted to 100 ml. A series of dilutions (0, 0.1, 0.2, 0.5, 1.0, and 2.0 %) was prepared using this stock solution using tap water.

Eggs of the *C. pipens* mosquito were obtained from the Entomology Laboratory of the Israel Ministry of Health, Jerusalem, and necessary larvae were prepared in the laboratory of the Institutes of Applied Research as described by the standard WHO protocol (1973). Twenty larvae of the early fourth instars were placed in a 250 ml disposable plastic cup containing 100 ml of treatment solution. After adding the larvae, the plastic cups containing the larvae were kept in the growth room maintained at room temperature. The effects of the extracts were monitored by counting the number of dead larvae each day up to three days. No adult emergence was observed, including in the control (0 %), during the three days.

The data obtained were statistically assessed for mean and standard error of the mean (±SE) using JMP software (SAS, 2000). Each treatment was conducted twice with similar results; data here represent a single independent experiment.

Table 1. Mortality of *Culex pipiens* mosquito larvae from different concentrations of aqueous extracts of Balanites kernel.

Kernel extract concentration (%)	Mortality (%)		
	1 day	2 days	3 days
0.0	0 ± 0	0 ± 0	0 ± 0
0.1	7 ± 2	10 ±3	27 ± 2
0.2	10 ± 3	27 ± 2	43 ± 2
0.5	25 ± 3	40 ± 2	68 ± 2
1.0	30 ± 3	70 ± 2	92 ± 2
2.0	38 ± 2	73 ± 2	95 ± 2

Values are the mean of 3 (n=3 ± SE).

Table 2. Mortality of *Culex pipiens* mosquito larvae from different concentrations of aqueous extracts of Balanites root.

Root extract concentration (%)	Mortality (%)		
	1 day	2 days	3 days
0.0	0 ± 0	0 ± 0	0 ± 0
0.1	33 ± 2	70 ± 3	100 ± 0
0.2	52 ± 2	90 ± 0	100 ± 0
0.5	55 ± 0	92 ±2	100 ± 0
1.0	78 ± 2	93 ± 2	100 ± 0
2.0	83 ± 2	95 ± 0	100 ± 0

Values are the mean of 3 (n=3 ± SE).

Table 3. Mortality of *Culex pipinens* mosquito larvae from different concentrations of aqueous extracts of Balanites bark.

Bark extract concentration (%)	Mortality (%)		
	1 day	2 days	3 days
0	0 ± 0	0 ± 0	0 ± 0
0.1	48 ± 2	75 ± 2	80 ± 2
0.2	63 ± 2	88 ± 2	93 ± 2
0.5	72 ± 2	92 ± 2	100 ± 0
1.0	80 ± 2	95 ± 0	100 ± 0
2.0	100 ± 0	100 ± 0	100 ± 0

Values are the mean of 3 (n=3 ± SE).

RESULTS AND DISCUSSION

The effects of the various aqueous extracts of *B. aegyptiaca* on the mortality of the *C. pipiens* mosquito larvae are presented in Tables 1 to 5. With kernel extract treatment there was less than 50% mortality of larvae on

Table 4. Mortality of *Culex pipiens* mosquito larvae from different concentrations of aqueous extracts of Balanites fruit pulp.

Fruit pulp extractconcentration (%)	Mortality (%)		
	1 day	2 days	3 days
0	0 ± 0	0 ± 0	0 ± 0
0.1	0 ± 0	0 ± 0	28 ± 2
0.2	0 ± 0	5 ± 3	32 ± 3
0.5	0 ± 0	7 ± 2	60 ± 3
1.0	5 ± 3	20 ± 3	78 ± 2
2.0	20 ± 3	35 ± 2	88 ± 2

Values are the mean of 3 (n=3 ± SE).

Table 5. Mortality rate of *Culex pipiens* mosquito larvae from different concentrations of aqueous extracts of Balanites leaf.

Leaf extract concentration (%)	Mortality (%)		
	1 day	2 days	3 days
0.0	0 ± 0	0 ± 0	0 ± 0
0.1	0 ± 0	0 ± 0	12 ± 3
0.2	0 ± 0	0 ± 0	23 ± 3
0.5	7 ± 2	12 ± 2	30 ± 5
1.0	8 ± 2	25 ± 3	70 ± 5
2.0	20 ± 3	75 ± 3	92 ± 3

Values are the mean of 3 (n=3 ± SE).

the first day, even at the highest concentration. The second day, both 1 and 2% concentrations killed more than 50% of the larvae. The third day, both 1 and 2% concentrations killed more than 90% of the larvae but neither treatment killed 100%. A weaker pattern was shown in fruit pulp and leaf extract treatments than the kernel extract treatment. The highest concentration (2%) of both the pulp extract and leaf extract showed only 20% larval mortality the first day. Only the highest concentration of both these extracts killed approximately 90% of the larvae after 3 days. As with the kernel extract treatment, none of these treatments killed 100% of the larvae within three days. Both bark and root extract showed a very high mortality. Root extract showed the highest rate of larval mortality compared to all treatments. More than 30% of the larvae were found dead in the first day, even at the lowest concentration (0.1%) of root extract. The third day, all tested concentrations of root extract killed 100% of the tested larvae population. The first day, the bark extract was found to be even more effective than root extracts. However, at the end of the experiment (3 days), only more than 0.5% concentration of the bark extract killed 100% of the larvae. The control (0%) showed no larval mortality on any day. A gradient of increasing mortality with increasing concentration was observed in all treatments

This work demonstrates the potency of *B. aegyptiaca* extract in the control of *C. pipiens* mosquito larvae. Root extract seemed the most lethal, followed by bark, among the various parts tested. Earlier studies have shown these tissues of *B. aegyptiaca* plants contain high amounts of saponins (Liu and Nakanishi, 1982; Jain and Tripathi, 1991; Kamel et al., 1991; Farid et al., 2002), so the high mortality of various parts of the extract might be attributed to the presence of saponin compounds in the Balanites tissues. Interaction of saponin molecules with the cuticle membrane of the larvae, ultimately disarranging this membrane by the association of the saponin molecule with these membranes (Morrissey and Osbourn, 1999), could be the most probable reason for the larvae death. The deficiency of dissolved oxygen in the water due to the active presence of the antioxidant saponin molecule could not be ignored. However, the mechanism by which saponin kills the larvae is the subject of research currently under way by our team. Previous studies have shown that many plant extracts do possess insecticidal properties (Arnason et al., 1989). Our results are in line with these results. This study shows that aqueous extracts of the Balanites plant can be used as environmentally-friendly and sustainable insecticides to control mosquitoes.

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