

Effect of Some Environmental Factors on the Predation Efficiency of the Mosquito; *Toxorhynchites splendens* (Diptera: Culicidae)

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Abstract

The present work investigates the optimum conditions for the application of the predaceous mosquito, *Toxorhynchites splendens* as a biological control agent for other vector mosquito species in Egypt such as, *Culex pipiens*, *Anopheles pharoensis* and *Aedes caspius* under field conditions.

The predation efficiency of *T. splendens* larvae was found to increase as the temperature increased. At 30-35 C the predaceous larvae consumed greater numbers of both *C. pipiens* and *Ae. Caspius* than that of *A. pharoensis*. The predation of *T. splen – dens* occurred almost similarly in all degree of salinity. On the other hand, light was found to have little effect on *Toxorhynchites* larvae where predation efficiency was decreased in the dark. The crowding had no effect on the predation. Also no mortality or natural diseases were observed among the tested insect larvae during the experimental period, whereas the insect preferred to live in highly concentrated population.

Toxorhynchites splendens larvae could live in turbid and distilled water. The maximum number of prey larvae consumed by all instars of *T. splendens* was at PH (8) followed by (7) and (9). However, pH, below (6) and above 11 had a bad effect on the predaceous activity. The predation efficiency of *T. splendens* larvae increased gradually as the depth of water decreased. However captures of prey occurred either on the surface or at the bottom of the container.

Introduction

Although insecticides continue to play a significant role in the control of mosquitoes involved in disease transmission, biological control of mosquito with predatory organisms in most instances is more acceptable from an environmental stand point and has been applied in many situations with reasonable success (WHO, 1995).

Biological control with indigenous predators/parasites may be more available, comparatively less expensive, and more compatible with the economic realities of many countries of sub-Saharan Africa. Predatory organisms are particularly suitable for control of

aquatic stages of mosquitoes. Among the organisms most extensively studied in this regard are the larvae of the mosquito, *Toxorhynchites splendens* (Mkoji *et al.*, 1999). This species is considered to be one of the most useful agents for the biological control of other vector and nuisance mosquitoes (Cheong and Ganapathipillai, 1964, Chan 1968; Yasuno and Tonn, 1970) and also for aedine vector of various diseases such as filariasis, dengue and yellow fever (Horio *et al.*, 1990).

Recently, *T. splendens* mosquito has been also recognized for use in the isolation of arboviruses and filariae.

Advantages of *Toxorhynchites* adults lie in their safety (nonbiting) and large size (Eshita *et al.*, 1982).

Unlike many other insect predators *T.splendens* can not search for a suitable prey patch, but is committed for pre adult life to the oviposition site of the female (Lounibos 1979). The depth preference for the oviposition site of *Toxorhynchites* species need to be investigated in both laboratory and field. Successful biological control programs requires finding the proper match between the preferred oviposition sites of the most suitable species of *Toxorhynchites* and the intended mosquito prey.

The capacity of *Toxorhynchites* larvae as a predators on some mosquito species larvae had been observed by a number of authors (e.g. Paiva ,1910; Jenkins, 1964; Focks, 1985; Annis *et al.* 1989 and Rawlins *et al.*1991).

The present study was carried out to determine the effect of some environmental factors on the predation efficiency of *T. splendens* larvae on *C. pipiens*, *Ae. caspius* and *A. pharoensis* larvae .

Materials and Methods

Maintenance of mosquitoes

a-*Toxorhynchites splendens* :

Immature and adult mosquitoes were maintained under insectary conditions of $26\pm 1^{\circ}\text{C}$ temperature, $75\pm 5\%$ RH and light : dark regime of 14: 10h at the department of Zoology, Faculty of Science (for girls), Al-Azhar University. About 50 adults were kept in each cage ($20\times 20\times 30\text{cm}$) with a stainless wire frame enclosed with white nylon mesh with a sleeve at a lateral end. Adults were fed on an 8% sucrose solution in a 150-ml Ehrlemeyer flask with a cellulose wick. For oviposition a black plastic cup containing distilled water was

provided. For crossing experiments, larvae were reared individually in 100ml. plastic vials each containing 50ml. of distilled water. For routine maintenance, however, larvae were reared in masses in plastic tray ($20\times 30\times 6.5\text{ cm}$) containing 2 liters of distilled water. They were fed on larvae of *Aedes aegypti* (Linn) with a prey/predator ratio of 15:1. Pupae were sexed and placed individually into small capsules.

b- *Culex pipiens*, *Anopheles pharoensis* and *Aedes caspius*

Colonies of *Anopheles pharoensis*, *Culex pipiens* and *Aedes caspius* were raised in the laboratory. The insects were obtained as larvae or pupae from Ain Shams University and Faiyum governorate. The cultures of these insect species were reared in the laboratory as described by Geberg (1970).

Number of prey larvae consumed by *Toxorhynchites splendens* larvae.

A colony of *T. splendens* was maintained as described before. Eggs for experimentation were removed from the container in the colony cages as soon as possible after oviposition and placed in separate vials ($2\times 3\text{ cm}$) containing distilled water. All materials was kept at $30^{\circ}\text{C}\pm 1$ and monitored for eclosion. Prey consumption were counted in all instars. Each freshly hatched *T. splendens* larvae were offered 100 1st instar *C. pipiens*, *Ae. Caspinus* and *A. pharoensis* larvae as prey separately before the head capsule hardened. When the 1st moult occurred, the *Toxorhynchites* larvae were transferred to clean vials and the remaining prey were counted. The procedure was repeated supplying the newly moulted 2nd instar *Toxorhynchites* with 100 2nd instar *C. pipiens*, *A. pharoensis* and *Ae caspius* (each in a separate vial). When the *Toxorhynchites* larvae moulted to the 3rd

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instar, they were transferred to larger jars (5 x 7 cm) and offered 100 third instar *C. pipiens*, *A. pharoensis* and *Ae. caspius* daily until pupation occurred. All jars were cleaned every 24 hrs to minimize microbial contamination.

Five replicates of hundred individuals were used. The vials were examined daily and the number of prey consumed by *T. splendens* larvae were calculated.

Effect of certain factors affecting the predation efficiency:

I- Temperature :

The predation efficiency of *T. splendens* was tested under different temperature degrees. Seven temperature degrees were tested ranging between 15°C up to 45°C. Temperature degrees below and above this range level was found to have an undesirable effect on the activity of the insects. The tested larvae were reared in the laboratory at 30°C ± 1 in distilled water.

Groups of thirty to fifty larvae were used in these experiments and were divided into 5 replicates, at each temperature degree. The predation efficiency of *T. splendens* from 1st instars to 4th instars was determined. The tested larvae were incubated at 45, °C, 40°C, 35°C, 30°C, 25°C, 20°C and 15°C ± 1. Examination for larval predation were continued daily for 3 days.

II – Salinity

In order to trace the effect of different salinity degrees on the predation of *T. splendens* larval instars they were tested against, *Ae. caspius*, *C. pipiens*, and *A. pharoensis* at salinity degrees of 0.39 ‰, 0.12 ‰, 0.16 ‰ and 0.675 ‰.

This salinity was prepared according to a procedure suggested by Ward (1962). Fifty larvae from each instar in two replicates were tested at each

salinity. The larvae were put in plastic boxes filled with water of different salinities. The numbers of prey larvae consumed from the three mosquitoes species at the different salinities were calculated.

III- Dark and light

In order to evaluate the response and the predation efficiency of *T. splendens* larvae under daylight and complete darkness, fifty 1st instar larvae of *T. splendens* were divided into two groups with *Culex* larvae as host. In the 1st group, the larvae were transferred into transparent plastic boxes. These boxes were exposed to diffused sunlight in the laboratory. Larvae in the second group were kept in the same plastic boxes but incubated inside a tight wooden cage which didn't permit any day light. Numbers of *Culex* larvae consumed were recorded in each group. The experiments were carried out using the 1st, 2nd, 3rd and 4th instar larvae. The number of prey larvae consumed by *T. splendens* was calculated. Also, 50 *T. splendens* larvae (in two groups) from each of 1st, 2nd, 3rd and 4th instars were tested against *Ae. caspius* and *A. pharoensis* larvae.

IV- Crowding :

To detect the effect of crowding on the predation of *Toxorhynchites* larvae, the fourth instar larvae were transferred to plastic boxes. The larvae were divided into groups of different numbers ranging from one to twenty in each. Five replicates for each group was tested. Larvae were given different numbers of their prey mosquito larvae from *C. pipiens*, *A. pharoensis*, and *Ae. caspius*. The number of consumed larvae were counted in each group.

V- Water – type preference

These experiments were carried out to investigate the preferred type of

water for the predation of *T. splendens* larvae on the other mosquito larvae tested.

Eggs were removed from the container in the colony cage as soon as possible after oviposition and placed in separated vials (2 x 3 cm) containing distilled water. Another vials containing polluted water (Sewage) and turbid water with some of the eggs were tested. All materials were kept at $30^{\circ}\text{C} \pm 1$ and monitored for eclosion. The Freshly hatched *T.splendens* larvae were offered hundred 1st instar *C.pipiens* as a prey in 3 vials. The 3 vials with the different types of water were examined daily and the number of prey consumed by *T. splendens* were calculated. The same experiment were carried out using the 2nd, 3rd, and 4th instar larvae against *A.caspius*, and *A.pharoensis* larvae. Five replicate were tested for each instar.

VI- Water PH :

In order to trace the effect of different water PH on the predation of out to investigate the preferred type of *Toxorhynchites* larval instars against tested *Ae.caspius*, *C.pipiens* and *A.pharoensis* larvae. 4,5,6,7,8,9,10,11PH were used . These PH were prepared by potentiometric instrument. The range of tested PH almost brackets the PH in the field and that of the tap water. Fifty larvae from each instar of *T. splendens* were tested in five replicates at each PH. The larvae were put in plastic boxes filled with water of different PH, and provided with their host. The number of prey larvae consumed from the three mosquito species at different PH were recorded.

VII- Water depth:

The effect of water depth on the predation of *T. splendens* larval instars were tested.

The first instar larvae were put in 3 plastic boxes filled with water of different depths 40, 20 and 15cm. Fifty larvae were used in five replicates for each depth. The same experiments were carried out on the 2nd, 3rd and 4th instar larvae. The number of prey larvae consumed by all instars and at different depth were calculated.

Results and Discussion

Number of prey larvae consumed by *Toxorhynchites splendens* larvae:

These experiments were carried out to test the number of prey larvae consumed by *Toxorhynchites splendens* from the three mosquito species *Culex pipiens*, *Anopheles pharoensis* and *Aedes caspius*. To confirm the results obtained from the experiments, they were repeated 3 times. Results obtained on the response of *Toxorhynchites splendens* to the 3 species of mosquitoes are presented in the (Table, 1).

The data in table (1), show that the average number of preys consumed the 1st larval instar of *Toxorhynchites splendens* from their prey *Culex pipiens*, *Anopheles pharoensis* and *Aedes caspius* are 6.5 ± 0.557 , 2.75 ± 0.5 and 4.5 ± 0.33 larvae per larva respectively. In case of the 2nd instar larvae, the number of prey consumed are 9.5 ± 0.577 , 3.5 ± 0.57 and 9 ± 1.154 larvae per larva respectively. Also, it is clear that the number of prey larvae consumed by the 3rd and 4th instars were 8.25 ± 0.957 , 3.75 ± 0.5 and 9 ± 1.154 in case of the former; 6.5 ± 0.577 , 2.5 ± 0.57 and 5.5 ± 1.29 respectively in case of the latter instar larvae.

From these data it is clear that *Toxorhynchites splendens* larvae responded similarly to *Culex pipiens* and *Aedes caspius* larvae. The feeding capacity of the 2nd and 3rd instar is greater than that in the 1st and 4th larval instar. From the

statistical analysis of the data, it is clear that: (i) There are significant differences between the number of both *Culex pipiens* and *Aedes caspius* larvae and those of *Anopheles pharoensis* consumed by *Toxorhynchites splendens* larvae in all instars. (ii) There is no significant difference between the number of *Culex pipiens* larvae and those of *Aedes caspius* consumed by *Toxorhynchites splendens* larvae.

The effect of different temperature degrees on the predation efficiency of *T. splendens* larvae is shown in Table (2), the results indicated that the predation efficiency of *T. splendens* larvae was increased as the temperature increased, where more prey of *C. pipiens* and *Ae. caspius* were consumed at degree from 30-35°C than that of *A. pharoensis*.

The analysis of the data (Table 2) showed that, in case of the 1st instar, there were significant differences between the number of prey larvae of *C. pipiens*, *A. pharoensis* and *A. caspius* consumed by *Toxorhynchites* larvae incubated at 30°C ± 1 and those incubated at 15, 20, 40 and 45°C.

In case of the 2nd, 3rd and 4th instars, there were significant differences between the number of prey larvae consumed by *Toxorhynchites* larvae incubated at 30°C ± 1 and those incubated at 15, 20, 25, 40 and 45°C. At 30°C there were significant differences between the number of both *C. pipiens* and *Ae. Caspius* larvae consumed by *Toxorhynchites* larvae and those of *A. pharoensis* larvae. Also the results showed that at the degrees from 30-35°C the predator larvae consumed greater numbers of both *C. pipiens*, *Ae. caspius* than that of *A. pharoensis*. These results agree with the findings of Trips (1972) who tested the effect of temperature on the predation of *Toxorhynchites brevipalpis*. This data indicated that predation in *T. brevipalpis* larvae was greater at high temperature degrees with more prey was consumed at 30-

35°C than at 20-25°C. It may be pointed out that the temperature surrounding the larvae affect their feeding capacity.

The data shown in Table (3) represent the effect of salinity on the predation efficiency of *T. splendens* larvae. The response of the insect larvae in all salinities studied are nearly equal in their predation to *C. pipiens* and *Ae. caspius*. Also it was clear that the number of *Anopheles* larvae consumed by all instars of *Toxorhynchites* larvae was less than in case of the former two mosquito species. Moreover the data (Table 3) indicated that (i) In all instars there was significant difference between the number of prey larvae (*C. pipiens*, *A. pharoensis* and *Ae. caspius*) consumed by *Toxorhynchites* larvae reared in water at salinity of 0.16‰ and those reared in water at the salinity of 0.39‰ except that in case of the 3rd instar. There was no significant difference between the number of *Anopheles* larvae consumed by *Toxorhynchites* larvae at the salinity of 0.16‰ and those at 0.675‰.

(ii) There was no significant difference between the number of prey larvae (*C. pipiens*, *A. pharoensis* and *Ae. caspius*) consumed by 1st, 2nd, 3rd and 4th instars of *T. splendens* reared in water at a salinity of 0.16 and those at the salinity 0.12.

(iii) In all instars, there was a significant difference between the number of host larvae (*C. pipiens*, *A. pharoensis* and *Ae. caspius*) consumed by *Toxorhynchites* larvae reared in water at the salinity 0.16‰ and those reared at 0.675‰ except in case of the 2nd and 3rd instars where there was no significant difference between the number of *Ae. caspius* larvae consumed by *Toxorhynchites* larvae.

(iv) There was a significant difference between the number of larvae consumed from *C. pipiens*, *Ae. caspius*, and those

consumed from *A. pharoensis* in all salinities.

These results agree well with that obtained by Richards (1938) who found that *Ae. stimulans* (Walker) and *Ae. vexans* (Meigen), (the two flood water mosquitoes usually found in water low in salts) respond differently when reared in a graded series of saline solution. But Kardatzk & Lien (1972) reported that the two coexisting mosquitoes *Ae. vexans* and *Ae. stimulans* have different abilities to tolerate varied salinities, where *Ae. vexans* larvae were able to tolerate higher saline conditions than those of *Ae. stimulans*.

Results in Table (4) revealed that light seemed to have little effect on *Toxorhynchites* larvae but it increased predation than that in the dark due to the greater activity of the prey in the light.

Also the results cleared that the response of all larval instar of *T. splendens* to their host *C. pipiens* and *Ae. caspius* in both light and dark was nearly equal. It was 6.5 ± 0.5 , 8.75 ± 0.5 , 8.75 ± 0.5 , & 5.5 ± 0.57 and 4.5 ± 0.57 , 8.75 ± 0.5 , 8.78 ± 0.5 & 5.5 ± 0.5 prey for 1st, 2nd, 3rd and 4th instars, respectively. The number of Anopheles larvae consumed by the *Toxorhynchites* larvae in both dark and light was decrease it was 1.5 ± 0.57 , 1.5 ± 0.5 , 2.75 ± 0.5 & 3.25 ± 0.5 and 2.5 ± 0.57 , 3.75 ± 0.5 , 3.75 ± 0.577 & 4.5 ± 0.54 prey in case of 1st, 2nd, 3rd and 4th instars, respectively. So, light is one of the important ecological factors that affect predation.

The statistical analyses of the Table (4) cleared that in general there are significant differences between the number of host larvae *C. pipiens*, *A. pharoensis* and those of *Ae. caspius*, consumed by all instars of *T. splendens* larvae which kept in light and those kept in dark. These finding support those obtained by Goma (1964) who reported that light seemed to has a little effect on

predation of *T. brevipalpis condradi*, but it greatly increased predation in *T. raimosi* larvae.

The results of the crowding experiment (table 5) revealed that in all groups used the crowding had no effect on the predation. Also no mortality or natural diseases were observed among the tested insects larvae during the experimental periods. This phenomenon may be return to that these insect larvae prefer always to live in highly concentrated population, which doesn't permit any pathological effects and doesn't affect their predation.

These results are on contrarily with some others as those of Terzian and Stahler (1949) who found that in the case of mosquito larvae living in over crowding condition generally resulted in retarded growth, high mortality among the small larvae, adults of non-uniform size and a decreased fecundity. Meanwhile Andrewartha and Birch (1954) reported that over crowding of organisms produced adverse effects on their survival, rate of development, and population growth. These findings contradict with the present results, since the crowding has no effect on the predation efficiency of *T. splendens* larvae. Also no mortality or natural diseases was observed among the tested insects larvae during the experimental periods.

The results of water type preference of *T. splendens* larvae are presented in Table (6). From these data it is clear that *Toxorhynchites* larvae and their prey could not be live in the polluted water. The data indicated that the average number of prey (*C. pipiens*, *A. pharoensis* and *Ae. caspius*) consumed by the 1st instar was 6.5 ± 0.57 , 3.25 ± 0.5 , and 5.5 ± 0.57 prey in distilled water and 4.5 ± 0.57 , 1.5 ± 0.5 and 6.5 ± 0.57 prey in turbid water for the 2nd instar. Meanwhile, this average was 8.75 ± 0.5 , 4.7 ± 0.5 , 8.75 ± 0.5 prey and 9 ± 1.154

prey and 3.25 ± 0.5 and 7.78 ± 0.5 prey for the 3rd instar in distilled and turbid water, respectively. Also the data revealed that the 4th instar consumed 6.5 ± 0.57 , 3.5 ± 0.57 and 5.5 ± 0.57 larvae from *C. pipiens*, *A. pharoensis*, *Ae. caspius* in distilled water and 5.5 ± 0.57 , 3.25 ± 0.5 and 6.5 ± 0.57 in turbid water respectively.

From the statistical analysis of the data (table 6) it is clear that: (i) There are no significant differences between the number of prey larvae (from *C. pipiens*, *A. pharoensis* and *Ae. caspius*) lived in distilled water and those lived in turbid water which consumed by the 2nd and 3rd *Toxorhynchites* larvae.

(ii) There are no significant differences between the number of prey larvae *Toxorhynchites* larvae.

(ii) There are no significant differences between the number of prey larvae (*C. pipiens*, *Ae. caspius*) lived in distilled water and consumed by 1st and 4th *Toxorhynchites* larvae and those lived in turbid water. But in case of *Anopheles* host. There are significant differences between these lived in distilled water and those lived in turbid water which consumed by 1st or 4th *Toxorhynchites* larvae. These findings support those reached by Jenkins (1946) who found that the larvae of *T. rutilus septentrionalis* died when placed in sewage contaminated water or tap water containing approximately 0.3 PPM of chlorine. These results confirm that the insect larvae respond differently to the same host at different type of water.

The effect of PH on the predation efficiency of *Toxorhynchites* larvae is shown in table (7). The results indicated that PH below 6 and above 11 had bad effect on the activity of the 1st instar larvae hence these tests were excluded. Also the results cleared that the number of consumed larvae at the same PH by

2nd, 3rd and 4th instars were fewer in number than at any of the other PH. It is clearly indicated that the maximum number of prey larvae consumed by all instars of *Toxorhynchites* larvae was at PH8 then followed by the PH7 and 9 where the number of consumed larvae were nearly the same. At the PH6 as well as PH10, the *Toxorhynchites* larvae showed a bad appetite where they consumed few number of their prey. So the insect larvae responded differently to the same host at different PH.

The statistical analysis of the data (table 7) indicated there was highly significant difference between the number of prey larvae, from *C. pipiens*, *A. pharoensis* and *Ae. caspius*, consumed by 2nd, 3rd and 4 instars of *Toxorhynchites* larvae which lived at PH8 and those placed at PH (4,5 and 11). Also there was highly significant difference between the number *C. pipiens*, *A. pharoensis* and *Ae. caspius* larvae consumed by 1st, 2nd, 3rd and 4th instars of *Toxorhynchites* larvae which lived at PH8 and those lived at PH (6, 7,9 and 10). It was seemed that the insect larvae responded differently to the same host at different PH. These results are in agreement with that found by Laird (1947).

The effect of water depth on the ability of *Toxorhynchites* to prey is shown in table (8). The data indicated that predation increased as the depth decreased. Also the predator larvae consumed more larvae in case of the *C. pipiens*, *Ae. caspius* in all instars than in case of *A. pharoensis* larvae. The statistical analysis of data (table 8) indicated no significant differences between the number of consumed larvae from *C. pipiens*, *A. pharoensis* and *Ae. caspius*, by 2nd, 3rd and 4th instars of *Toxorhynchites* larvae at the of depth 15cm and 20cm, but there was a significant difference between the

number of consumed hosts of these two depth and the depth of 40 cm in case of the same three instars. In case of 1st *Toxorhynchites* larval instar there was significant difference between the number of consumed larval host at the three depth used. The results are in agreement with that obtained by Breland (1949), Furumizo *et al*(1978) and Crans and Staff (1977), using *T.rutilus Septentrionalis*, where the subsurface predation appeared to be more common.

The present results may be recommended as a useful control measure for mosquitoes.

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