Department of Zoology;
Faculty of Science; Assiut University. Assiut; Egypt .

LABORATORY OBSERVATIONS ON THE EFFICIENCY OF SOME NATURAL ENEMIES OF CULICINE MOSQUITO LARVAE IN ASSIUT - EGYPT

(With 5 Tables and 1 Figure)

By

A. A. ABDEL-AAL

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ملاحظات معملية على كفاءة بعض الأعداء الطبيعية ليرقات بعوض الكيولكس في أسيوط – مصر

عبدالعال عبدالمجيد عبدالعال

لوحظ فى يرقات البعوض المصابة بفطر السيلوميسس وطفيل الفورتيسيلا والتى جمعت في شهر يوليه ١٩٩٨ بمحافظة أسيوط تفاعل اليرقات مع مسببات العدوى أدت إلى تحلل متتابع لجميع الأنسجة. التجويف الهيموسيلى ليرقات بعوض الكيولكس أنتيناتس المعديه بهذا الفطر يظهر ككتلة صلبة نتيجة لتكاثر الفطر وتأخذ لون بنى داكن مائل للاحمرار والفطر يغطى الجسم كله. كما لوحظ أن ذكر واحد من سمك الجامبوزيا بطول ٢ سم يلتهم الطور الرابع ليرقات البعوض بمتوسط ٢١.٤ يرقة في اليوم بينما أنثى واحدة لها نفس الحجم التهمت بمتوسط ٤٩.١ يرقة في اليوم. اليرقة اليافعة من خنفساء السيبستر التهمت بمتوسط ٢٦.٦ يرقة فى اليوم . الحورية اليافعة للرعاش التهمت بمتوسط ٨٢.٩ يرقة في اليوم .

SUMMARY

The infected mosquito larvae with *Coelomomyces* fungus and *Vorticella sp.* were collected in July 1998 from Assiut governorate . The reaction of the host to its pathogen causes progressive lysis of all tissues. The haemocoel of *Culex antennatus* larvae infected with fungus appears as a solid mass of sporangia. The color of larvae may be dull reddish-brown and fungus covered all the body . One *Gambusia affinis* male of 2.0 cm long consumed an average of 21.4 4th instar larva/day , wherease one female of the same size can devour an average of 49.1 larva/day.A full

grown larva of *Cybister* beetle consumed an average of 26.6 larva/day . The consumption of full grown nymph of dragonfly averaged 82.9larva/day.

Key words: *Coelomomyces* fungus, *Vorticella sp.*, *Gambusia affinis*, *Cybister*.

INTRODUCTION

The role of *Gambusia affinis* fish as a biocontrol agent of mosquito larvae in many countries is well documented by several authors. Hildbrand (1921) reported success with *Gambusia affinis* in providing rather permanent anopheline control at reduced cost in several breeding places of the South United States; Kulanin (1975) reported complete absence of culicine larvae in standing water (pits, bogs and catchments) which had been stocked with *Gambusia* at about 0.5 - 0.7 fish/m.

Sato (1989); Walton and Mulla (1991); Blaustein (1993); Prasad et al. (1993); Lounibos et al., (1994); Eric (1994) and Bellini et al. (1994) found mosquito breeding through *Gambusia affinis* in rice fields with fluctuations in their percentage composition, exhibiting species succession in different months .Among the natural enemies of mosquitoes are dragonflies; aquatic beetles and the cyclopoid copepods . Farghal (1974, 1979) observed Odonata nymphs and aquatic beetle predating on mosquito larvae in Assiut.

Also the fungus *Coelomomyces stegomyiae* may infect mosquito larvae as reported by Lucarotti (1995) for *Aedes aegypti* larvae.

The aim of the present study is to examine the efficiency of some predators (*Gambusia affinis, Cybister tripunctatus africanus* and *Hemianax sp.)* and of parasitic fungus (*Coelomomyces sp.*) and protozoan (*Vorticella sp.*) as a biological control agents of mosquitoes in Assiut.

MATERIAL and METHODES

To determine the efficiency of some predators to devour mosquito larvae and pupae, about 200 mixed larvae or pupae associated with a single individual of one predator agent were placed in a porcelain pot filled with 200 ml. of water. After 24 hr the number of larvae consumed by the predator were recorded. The experiments were

conducted with *Gambusia affinis* fish; *Coelomomyces sp.* fungus; *Vorticella sp.* protozoa; *Cybister* beetle larvae and *Hemianax sp.* nymphs). Monthly larval collections were made from different breeding habitats (cesspits and pools) using standard triangular net with tapering end, made of fine muslin (30cm. long). The source of *Gambusia* fish was the farm of the Faculty of Agriculture in Assiut. The collected mosquito larvae were identified according to Kirkpatrick (1925); Natvig (1948)and Abdel-Maleck (1956). To estimate the mortality rate among culicine larvae caused by the flagellate protozoa *Vorticella sp.* and the fungus *Coelomomyces sp.*, 30-50 culicine larvae infected with protozoa or fungus were collected from the field. Larvae were kept in porcelain pots (15 cm. diameter) containing 200 ml.tap water until pupation, larvae were fed on a small amount of dried yeast and fine dried bred. The number of larvae failing to pupate were counted. On the other hand, normal larvae collected from the field were maintained for comparison.

Statistical analysis and interpretation of results were performed using the F-test to evaluated the effect of some predators on mixed culicine mosquito larvae (*Culex pipiens molestus; Culex antennatus; Culex univittatus* and *Aedes caspius*). Simple correlation coefficients and regression coefficients between the predators and culicine larvae and pupae were estimated. The abbreviation (ns) indicates that the difference between means was not significant at the 5 percent level. The single asterisk* and double asterisk** indicate significance at the 5 percent and 1percent levels respectively.

RESULTS

Data represented in Table (1) show the simple correlation coefficients between water temperature and numbers of culicine larvae, pupae and their predators as computed separately. The corresponding regression coefficients were also determined for each case. Generally, water temperature was found to affect some mosquito predators than mosquito larvae and pupae. *Culex antennatus* larvae seem to be the more sensitive species of mosquito to water temperature *Aedes caspius* larvae were found to be negatively correlated with water temperature.

A-a preliminary results on the efficiency of *Vorticella sp.*and *Coelomomyces sp.* against culicine mosquito larvae:

The efficiency of the flagellate protozoa (*Vorticella sp.*) and fungus (*Coelomomyces sp.*) to induce mortality in culicine larvae were

studies died. Data represented in Table (2) show that the percentages of mortality in parasitized larvae with *Vorticella sp.* and *Coelomomyces sp.*were 20% and 38% in *Culex univittatus;* 22% and 48% in *Culex antennatus;* 16%and 24% in *Aedes caspius* and 12% and 32% in *Culex pipiens molestus* respectively.

The infected mosquito larvae of *Culex antennatus* with *Coelomomyces sp.* fungus (Fig.1) collected during July 1998 in Assiut were examined in the laboratory to investigate the symptoms of such infection. The larva was filled with mature and immature sporangia however, hyphae were not present. Resting sporangia were of three types;(1) thin walled, nonstained and smooth oval bodies; (2) more mature thin walled, transparent bodies, with surface ridges; and (3) mature yellow to brownish sporangia with surface ridges in continuous depressed areas . The haemocoel in the fourth instar larvae may appear as a solid mass of sporangia, which gives the larva an opaque appearance. The color of such larvae may be dull or yellowish-white, bright yellow, orange or even dull reddish-brown. Frequently the mycelia and spores of the fungus may occur even within the head of the larva (Fig. 1).No mortality was recorded in unparasitized larvae under similar conditions . Mortality was achieved only in heavy infestations in which the protozoa and fungus covered all the body of mosquito larvae.

B- The efficiency of some predators against culicine mosquitoes:

1- *Gambusia affinis:*

The daily consumption of *Gambusia affinis* (male and female) from the fourth instar larvae of mixed culicine mosquitoes are represented in Table (3). Data show that one male of fish measuring 2.0 cm.long consumed from 18 to 25 fourth instar larvae with an average of 21.4 larva/day, whereas one female of the same size can devour from 35 to 58 with an average of 49.1. Statistical analysis show that a highly significant rate of larval consumption was obtained with female fish than with male. Moreover, a highly significant consumption rate was observed in case of large fishes.

2-Larvae of *Cybister tripunctatus africanus* beetle :

Data in Table (4) show the number of fourth instar larvae of mixed culicine mosquitoes consumed by one larva of *Cybister* /day as observed from July to September 1998. It was found that the second instar larva of *Cybister* beetle could predate on 20 to 32 larvae of mixed culicine mosquitoes/day with an average of 26.6 during July, whereas

full grown larva could predate on 25 to 58 with an average of 39.0. The corresponding minimum, maximum and mean number of 4th instar larvae of culicine mosquitoes which were consumed by the 2nd instar larva of *Cybister* /day during August and September were 18 to 30 with an average of 23.8 and 10 to 20 with an average of 14.0 respectively. While full grown larva consume 22 to 56 with an average of 41.8 and 20 to 43 with an average of 28.6 respectively. Data reveal that there were insignificant differences in the consumption rate between different larval instars of *Cybister* beetle also predation of *Cybister* beetle larvae was not affected by water temperature.

Cybister beetle larvae were observed to predate on mosquito larvae by introducing their mandibles into the larval body to suck the contents of larvae till death.

3- Dragonfly nymphs (Fam. Aeschnidae- *Hemianax sp.*):

As shown in Table (5), the number of fourth instar larvae of mixed culicine mosquitoes consumed weekly by one mature nymph of dragonfly ranged between 20 and 100 larva/day with an average of 72.9/day. While in case of second instar larvae of mixed culicine mosquitoes, one mature nymph predates on 50 to 100 larva/day with an average of 79.1. The daily consumption of culicine pupae by one nymph ranged between 40 and 100 larva with an average of 82.9.

DISCUSSION

Fungal pathogens of the genus *Coelomomyces* (Keilin, 1921) have been reported for many species of mosquitoes and certain other hosts in several countries. Despite the progressively cumulative records of this genus from Asia, Laird (1959a, 1959b) and Fedder et al..(1971). Laird et al.. (1975) described the organism as *Coelomomyces*, as well as the incidence of which in third and fourth instar of mosquito larvae from Japan. Lucarotti and Andreadis (1995) observed *Aedes aegypti* larvae infected with *Coelomomyces stegomyiae,* can pupate and eclose to produce adult that carry the fungal infection.Such preleminary observations may encourage further studies on Egyptian flora of *Coelomomyces* as one of the possible biological control agents against mosquitoes. *Gambusia affinis* as an efficient tool for mosquito control was reported by many authors in different countries of the world

(Kulanin, 1975; John, 1977; Blaustein, 1992; Prasad, et al. 1993; Bellini et al., 1994 and Lounibos, 1994).

The high degree of predation of *Gambusia affinis* recorded in the present work agrees with that of Sugur et al. (1974) who found that one fish (female) of 2-6 cm. long can devour 100 of the fourth instar mosquito larvae in 24hr. Farghal (1979) observed one fish male of 2.0cm. long predating from 20-24 4th instar larvae of mixed culicine mosquitoes with an average of 21.5/day, whereas one female of the same size can consume from 25-55 larva/day with an average of 40.

Jenkins (1964) stated that, larvivorus fishes are considered as the most natural enemies of mosquitoes for practical value. However, Notestine (1972) found that potential invertebrate predators of mosquito larvae including, nymphs of dragonfly and *Cybister* as mentioned also mentioned in this paper, were not effective for controlling mosquito larvae in the absence of other control measures. It is worth mentioning that *Cybister* beetle larvae were found to be nonspecific in predation, besides the canibalism phenomenon which might restrict their predatory efficiency against mosquito larvae .

The foregoing results indicate that full grown nymphs of dragonfly (Fam.Aeschnidae) showed intense predation against culicine mosquito larvae.This is in agreement with El-Rayah (1976) who reported that nymphs of dragonflies may have important role in the natural control of mosquito larvae.

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Table (1): Monthly average of water temperatures; Simple correlation coefficient and regression coefficient between water temperature; numbers of culicine larvae ,pupae and their predators in Assiut (1998) .

Months	Average of water temperature	Criteria	Simple correlation coefficients	Regression coefficients
January	12.3	*Cybister beetle*	-	-
February	12.5	Dragonfly nymphs	0.407	0.624
March	20.0	Total culicine larvae	0.334	9.185
April	21.0	Culicine pupae	0.306	1.148
May	21.5	*Culex pipiens molestus*	0.022	1.184
June	24.5	*Culex antennatus*	0.741 **	6.470
July	27.5	*Culex univittatus*	0.336	2.024
August	25.0	*Aedes caspius*	-	-
September	26.0			
October	24.0			
November	18.6			
December	14.0			

Table (2): The efficiency of *Vorticella sp.* and *Coelomomyces sp.* Aginst four of culicine mosquito larvae .

Species	Infected larvae by *Vorticella sp.*			Infected larvae by *Coelomomyces sp.*			Normal larvae % mortality
	No.of larvae examined	No.dead larvae	Mortality %	No.of larvae examined	No.dead larvae	Mortality %	
Culex univittatus	50	10	20	50	18	38	0
Culex antennatus	50	11	22	50	24	48	0
Aedes caspius	50	8	16	50	12	24	0
Culex pipiens molestus	50	6	12	50	16	32	0

Table (3): Daily consumption of *Gambusia affinis* fish (Male and Female) of fourth instar larvae of culicine mosquitoes .

Sex	Size of fish(cm)	No. of mosquito larvae consumed / fish / day Replicates 1	2	3	4	5	6	7	8	9	10	Mean	S.E ±
Male	2.0	23	20	21	18	20	22	23	20	25	22	21.4	0.36
	2.5	25	30	28	25	26	21	31	37	35	32	29.0	1.56
	2.9	28	26	33	35	30	34	50	43	38	39	35.6	2.28
	3.2	32	28	42	29	38	52	48	42	43	43	39.7	2.50
	3.5	37	30	41	51	50	49	40	32	29	53	41.2	2.88
Female	2.0	38	53	58	49	44	60	57	51	35	46	49.1	2.66
	2.5	40	51	55	53	60	49	48	53	48	50	50.7	1.66
	2.8	39	48	54	48	58	63	49	49	58	54	52.0	1.61
	3.6	43	58	50	52	50	55	51	48	50	63	52.0	1.75
	3.8	40	48	38	60	47	68	61	70	52	51	53.5	3.47

Statistical analysis : Criterion Comparison F- Value

Consumption Between sex **

Between size **

Between sex&size ns

Table (4): Daily consumption of culicine larvae by *Cybister tripunctatus africanus larvae* (From July- September 1998).

Months	Number of fourth instar larvae consumed / day											
	Second instar larvae of *Cybister*							Full grown larvae of *Cybister*				
	Replicates					Mean	S.E ±	Replicates					Mean	S.E ±
	1	2	3	4	5			1	2	3	4	5		
July	27	32	28	20	26	26.6	1.73	25	32	58	50	30	39.0	5.69
August	18	26	30	20	25	23.8	1.92	22	39	50	56	42	41.8	5.18
September	10	20	13	12	15	14.0	1.52	20	26	23	43	31	28.6	3.61

Table(5):The efficiency of dragonfly nymphs (*Hemianax sp.*) against culicine larvae and pupae (from 1 - 10 September, 1998).

Date	Stage consumed	Number of culicine larvae and pupae consumed / day Replicates											
		1	2	3	4	5	6	7	8	9	10	Mean	S.E ±
1/9	Fourth instar	63	42	38	46	58	60	70	62	83	94	61.6	5.56
2/9	larvae	58	20	45	65	76	71	83	94	90	98	70.0	7.64
3/9		80	70	82	80	73	68	73	91	83	100	80.0	3.12
4/9		42	58	66	58	61	63	72	76	84	98	67.8	4.93
5/9		75	70	52	67	70	60	83	90	96	98	76.1	4.84
6/9		76	56	90	78	83	69	72	84	97	100	80.5	4.20
7/9		76	55	39	59	83	92	69	84	92	99	74.8	6.05
											Mean	72.9	
8/9	Second instar	70	60	58	79	83	79	88	69	89	99	77.4	4.15
9/9	larvae	78	80	50	67	80	83	82	90	98	100	80.8	4.60
											Mean	79.1	
10/9	Pupae	68	76	40	83	78	88	100	98	98	100	82.9	5.96

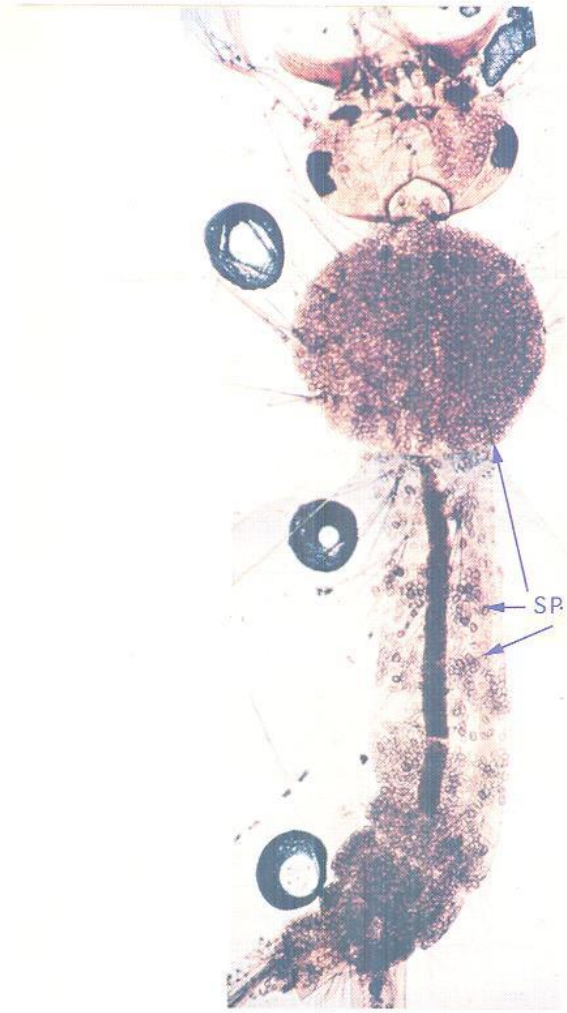

Fig. (1): Head, thorax and abdomen of *Culex antennatus* larva, containing oval resting sporangia of Coelomomyces sp. fungus (Sp.).