

EFFECT OF THREE INSECTICIDES ON SOME BIOLOGICAL AND HISTOLOGICAL ASPECTS OF THE SPIDER *ANELOSIMUS AULICUS* (KOCH)

El-Khouly¹, N. M., Rahil¹, A. A. and Dwidar², E. F

Plant Protec. Depart. Fac. of Agric., Fayoum Univ.¹

Botany Depart. Fac., of Agric., Fayoum Univ.²

ABSTRACT

The comb-footed spider, *Anelosimus aulicus*, has great potential to suppress insect pests of agricultural crops. The effect of some insecticides used against these pests on survival of this spider was investigated under laboratory conditions. Mortality and changes in the fine structure of the midgut 48h after treatment with Lannate[®], Reldan[®] and Match[®] were examined. The LC₅₀'s, LC₉₀'s and slope of these insecticides were given. Lannate[®] was the most effective due to high acute toxicity, causing 66.7% mortality in females and 91.6% in males. It was also the most drastically effective on fecundity of females where no egg sacs were laid under the recommended field concentration (0.3gm/ 200 ml water). Reldan[®] caused 58.3% mortality in females and 83.33% in males. The least effective was in Match[®] causing 50% mortality in females and 58.33% in males. Obviously, males were more affected than females. The effect of insecticides on longevity periods and prey consumption of adults and, fecundity of females, at different concentrations of insecticides, were investigated. On the other hand, the histological study on the midgut glands of adults using Transmission Electron Microscope revealed similarity in structure of females and males of untreated spiders, whereas, treated spiders showed different changes indicated by the absence of some cells in the tissues in this organ, which might affect its digestive function and therefore ability for survival.

Keywords: Spiders- *Anelosimus aulicus*- Insecticides- Mortality- Transmission Electron Microscope (TEM) - Histology.

INTRODUCTION

Spiders are known as important predators and one of the most diverse and abundant groups that prevail under different environments with almost 42.473 species described in Phylum Arthropoda (Platnick 2011). Due to their presence in large numbers and capabilities to capture the prey, these arthropods in some crops are considered potentially important bio-control agent. Although spiders are more sensitive to insecticides than insects (Ravi *et al.*, 2008), some spiders not only tolerate the harmful effects of pesticides but also show some resistance as reported by Tanaka *et al.*, 2000., thus require selective pesticides for successful Integrated Pest Management programs, that efficiently affect target organisms but cause minimum damage to non target

organisms and environment. Therefore, the present study was designed to achieve the following objectives; a) assessment of the toxic effect of insecticides commonly used, Lannate[®], Reldan[®] and Match[®] on the most abundant spider *A. aulicus*. Mortality and biology and b) histological changes in the midgut were investigated in the laboratory.

MATERIALS AND METHODS

1. Rearing of the spider *A. aulicus*:

Adults collected from nearby tomato plantations were cultured in the Lab. conditions, $25 \pm 2^\circ\text{C}$ and $65 \pm 5\%$ RH. Females were held in small plastic containers (5.5cm dia. x 4.5cm ht.) for deposition egg sacs. Each of one emerged juveniles were transferred to new containers and from this test spiders were taken. Juveniles and adults reared were fed on 2nd and 3rd larval instars of cotton leaf worm, *Spodoptera littoralis* Boisduva (Lepidoptera, Noctuidae). Adult spiders were allowed copulation, introducing one male to each female immediately after the last molt. Males were separated after mating. After starvation for one week, according to (Benamu et al., 2013) adults were placed each in a Petri dish (9 cm dia.) for experimentation.

2. Rearing of prey; *S. littoralis*:

Egg batches of this cotton leaf worm were collected from nearby cotton field, sterilized with 5 % formalin and kept in glass jars (20 cm dia. x 15cm ht.), covered with muslin cloth held tight by rubber bands until egg hatched. The jars were provided with clean castor oil leaves, replaced daily with fresh food for the emerged larvae. Pupae were later collected and placed in glass chimnies until moth's emergence. Each chimney was provided with a small cotton pad soaked in 10 % sugar solution for adult feeding. Leaves of diafla, *Nerium oleander* Poir, were also provided as oviposition sites. From this culture, 2nd and 3rd larva was obtained as prey for the studied spider.

3. Insecticides tested:

As presented in Table (1), three insecticides; representing three different groups, were chosen for this study namely; Lannate[®] 90% SP, Reldan[®] 50% EC, and Match[®] 5% EC. For each insecticide three concentrations were used, and each was replicated three times and compared with untreated control.

Table (1): Insecticides, active ingredients and concentrations used.

Trade Name	Active Ingredient	Chemical Class	Concentration/ 200ml water		
			1 st conc.	2 nd conc.	3 rd conc.
Lannate [®] 90% SP	Methomyl	Oxime carbamate	0.3 gm	0.15 gm	0.075gm
Reldan [®] 50% EC	Chlorpyrifos methyl	Organophosphate	1ml	0.5ml	0.25ml
Match [®] 5% EC	Lufenuron	Benzoyl urea	0.16 ml	0.080 ml	0.040ml

4. Toxicity tests:

The leaf-dip technique developed by Amalin *et al.*, 2000 was followed. In this method clean castor oil plant leaves were dipping in the desired concentration of insecticide for 60 second and dried for 30 minutes. The test 2nd and 3rd instars larvae of *S. littoralis*, were kept on such treated leaves, for one hour, then four of these larvae were introduced to each one adult spiders. Mortality was recorded 48h post treatment. Rearing of survived spiders continued by feeding on untreated prey to observe adult longevity periods, prey consumption and female fecundity.

5. Histological observations:

This work was performed in the Transmission Electron Microscope (TEM) lab, Fac. of Agric., Cairo University Research Park (FA-CURP) on female and male of *A. aulicus* (Koch) following the technique described by Rocchetta *et al.*, 2007.

Spiders were decapitated and dissected to separate the alimentary canal, which was then fixed in 2.5% glutaraldehyde in 0.1 M sodium phosphate buffer (PH 7.4, 4°C, 2h). Subsequently, the material was post-fixed in 2% osmium tetroxide in 0.1 M phosphate buffer (4°C, 1.5h) and dehydrated through a graded series of ethanol concentrations (50, 70, 90, 95 and 100 percent, each for 15min) and acetone (15min), and then embedded in epoxy resin. Semi- and ultra-thin sections were cut on a Leica Ultra cut UCT25 ultra microtome. Thin sections were stained on a hot plate with Toluidine blue (1x) then examined by camera Leica ICC50 HD. Ultrathin sections, prepared at approximately 75-90 µm thickness, were stained with uranyl acetate and lead citrate, and then examined by transmission electron microscope JEOL (JEM-1400 TEM).

RESULTS AND DISCUSSION

A) Toxicity of insecticide-treated *S. littoralis* larvae to adult spiders *A. aulicus*:

Evaluation of the effect of the use of recommended insecticides, Lannate[®], Reldan[®], and Match[®] against *A. aulicus* revealed the following as shown in Table (2)

1- The toxic effect of Lannate[®]:

The LC₅₀ was 1083.32 ppm for females and 668.43 for males, with LC₉₀ values 2684.69 ppm and 1444.45 and the slope was 3.25 and 3.83, respectively. Mortality in females reached 66.66, 16.67 and 8.33% and 91.67, 41.33 and 16.66% in males at 1350, 675 and 338 ppm concentrations, respectively 48h post treatment. Males were more sensitive to Lannate than females.

2- The toxic effect of Reldan[®]:

The LC₅₀ was 2265.84 ppm for females and 1414.98 ppm for males, with LC₉₀ values 6433.11 and 3518.76 and the slope was 2.82 and 3.23, respectively. Mortality in females reached 58.33, 16.66 and 8.33% and 83.33,

33.33 and 16.66% in males at 2500, 1250 and 625 ppm concentrations, respectively 48h post treatment.

3- The toxic effect of Match®:

The LC₅₀ was 850.17 ppm for females and 693.26 ppm for males, with LC₉₀ values 2872.54 ppm and 2989.95 and the slope was 2.42 and 2.02, respectively. Mortality in females reached 50.00, 16.66 and 8.33% and in males being, 58.33, 25.00 and 16.66% at 800, 400 and 200 ppm concentrations, respectively 48h post treatment.

Table (2): Mortality, LC₅₀, LC₉₀ and slope values of insecticides on adults of the spider *A. aulicus* (Koch) two days after feeding on treated prey.

Sex	Mortality Percentages% at given conc. (ppm)			LC ₅₀ ppm	LC ₉₀ ppm	Slope± S.E
Lannate®						
	1350	675	338			
♀	66.66	16.67	8.33	1083.32	2684.69	3.25± 1.10
♂	91.67	41.33	16.66	668.43	1440.45	3.83±1.09
Reldan®						
	2500	1250	625			
♀	58.33	16.67	8.33	2265.84	6433.11	2.82±1.08
♂	83.33	33.33	16.66	1414.98	3518.76	3.23±1.02
Match®						
	800	400	200			
♀	50	16.66	8.33	850.17	2872.54	2.42±1.06
♂	58.33	25	16.66	693.26	2989.95	2.02±0.95

B) Effect of toxicated feeding on some biological aspects of *A. aulicus*:

Adults of the spider *A. aulicus* that survived feeding previously on insecticide-treated larvae of *S. littoralis* were reared on untreated ones under incubator condition, 25± 2°C and 65± 5% R.H., and observed daily. The longevity periods and prey consumption, and female fecundity data were recorded.

The results for untreated spiders (control) revealed the following:

1. Longevity and prey consumption of adults:

As shown in Table (3), female longevity averaged 167.25 days. The pre-oviposition, oviposition and post-oviposition periods averaged 13.67, 42.33 and 111.25 days, respectively. Male longevity was shorter and averaged 143.92 days. The total number of prey consumed per female ranged between 156 and 317 larvae. The total number of prey consumed per male ranged 132-234 larvae. In general, adult females consumed more larvae than adult males.

2. Egg incubation period, % hatch and fecundity of females:

As shown in Table (4), females laid an average of 3.50 egg sacs/female, which contained 13.00- 45.00 spiderlings. Eggs hatched after 16

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 days, and hatching percentage reached 94.5%. The average number of spiderlings per female was 107.25.

Table (3): Longevity (L) in days and prey consumption (C) of the untreated *A. aulicus* adults (Koch).

Parameters	Females								Males	
	Preoviposition		Oviposition		Postoviposition		Total		Total	
	(L)	(C)	(L)	(C)	(L)	(C)	(L)	(C)	(L)	(C)
Range	10.00-17.00	28.00-50.00	23.00-54.00	83.00-183.00	53.00-133.00	45.00-108.00	86.00-183.00	156.00-317.00	75.00-156.00	132.00-234.00
Mean± S.E	13.67±0.68	39.00±1.97	42.33±2.61	146.41±8.42	111.25±6.13	92.58±4.89	167.25±7.65	278.00±12.38	143.92±6.35	212.75±7.93

Table (4): Egg incubation period, % hatch and fecundity of untreated *A. aulicus* (Koch) females (control).

Parameters	Egg incubation period (days)	Fecundity of Females			
		No. egg sac/ female	No. spiderlings/ egg sac	No. spiderlings/ female	% hatch
Range	10.00-20.00	2.00-4.00	13.00-45.00	42.00-135	94.5
Mean± S.E	16.50±0.37	3.50±0.19	30.64±1.41	107.25±7.08	

A) Effect of Lannate® at field recommended lethal and sublethal doses on *A. aulicus* biology:

The effect of Lannate® at lethal and sublethal concentrations on longevity in days (L), prey consumption (C), egg incubation period, % hatch and fecundity of *A. aulicus* Koch adults were showed in Tables 5 & 6.

1- Effect of lethal concentration (0.3gm):

a. Longevity and prey consumption of adults:

Female longevity averaged was 11.50 days. The number of prey consumed per female ranged between 5 and 8 larvae. Male longevity was shorter and averaged 3.50 days. The total numbers of prey consumed per male being 2.20 larvae.

b. Fecundity of females:

Females were incapable of reproduction since no egg sacs were observed.

2- Effect of 1/2 lethal concentration (0.15 gm):

a. Longevity and prey consumption of adults:

Female longevity averaged was 86.30 days. The pre-oviposition, oviposition and post-oviposition periods averaged 18.50, 21.50 and 46.30 days, respectively. Male longevity averaged 70.57 days.

The total number of prey consumed per female ranged between 44.0 and 121.0 larvae. Predation during the pre-oviposition, oviposition and post-oviposition periods averaged 20.20, 43.00 and 28.60 larvae, respectively. The total number of prey consumed per male ranged between 32-91 larvae.

b. Egg incubation period, % hatch and fecundity of females:

Females laid 1-2 egg sacs each contained 13-33 spiderlings. Eggs hatched after 15.0-22.0 days, with % hatch of 78.3%. The average of spiderlings produced per female was 40.20.

3. Effect of 1/4 lethal concentration (0.75gm):

a. Longevity and prey consumption of adults:

Female longevity averaged was 117.50 days. The pre-oviposition, oviposition and post-oviposition periods averaged were 17.25, 37.33 and 62.92 days, respectively. Male longevity averaged was 95.70. The total number of prey consumed per female ranged between 67 and 190 of larvae. The total number of prey consumed per male ranged between 79-172 larvae.

b. Egg incubation period, % hatch and fecundity of females:

Females laid 1-3 egg sacs; one contained 14-39 spiderlings. Eggs hatched 15.0-22.0 days (average 18.62), with % hatch of 89 %. The average of spiderlings produced per female was 68.17.

Table(5):Effect of Lannate® at lethal and sublethal concentrations on longevity in days (L) and prey consumption (C) of *A. aulicus* Koch adults.

Parameters	Females						Males			
	Lethal conc. (0.3gm)									
	Pre-oviposition		Oviposition		Post-oviposition		Total	Total	Total	Total
	(L)	(C)	(L)	(C)	(L)	(C)	(L)	(C)	(L)	(C)
Range	-	-	-	-	-	-	10.00-13.00	5.00-8.00	0.00-7.00	0.00-4.00
Mean± S.E	-	-	-	-	-	-	11.50±0.65	6.50±0.64	3.50±3.49	2.20±2.19
Parameters	Sub-lethal conc. (0.15gm)									
	Pre-oviposition		Oviposition		Post-oviposition		Total	Total	Total	Total
	(L)	(C)	(L)	(C)	(L)	(C)	(L)	(C)	(L)	(C)
	Range	15.00-21.00	15.00-23.00	1.00-32.00	0.00-67.00	32.00-60.00	15.00-36.00	48.00-106.00	44.00-121.00	45.00-82.00
Mean± S.E	18.50±0.65	20.20±0.71	21.50±4.50	43.00±9.30	46.30±2.89	28.60±1.88	86.30±5.69	91.80±9.74	70.57±4.83	75.57±7.43
Parameters	Sub-lethal conc. (0.75gm)									
	Pre-oviposition		Oviposition		Post-oviposition		Total	Total	Total	Total
	(L)	(C)	(L)	(C)	(L)	(C)	(L)	(C)	(L)	(C)
	Range	13.00-20.00	17.00-35.00	1.00-53.00	2.00-118.00	44.00-85.00	30.00-74.00	65.00-133.00	67.00-190.00	52.00-113.00
Mean± S.E	17.25±0.64	22.33±1.53	37.33±4.48	89.50±9.52	62.92±3.98	45.67±3.84	117.50±5.19	157.50±9.62	95.70±5.97	125.80±9.64

Table (6): Effect of Lannate® at lethal and sublethal concentrations on egg incubation period, % hatch and fecundity of *A. aulicus* Koch adults.

Lethal conc. (0.3gm)					
Parameters	Egg incubation period (days)	No. egg sac/ female	No. spiderlings/ egg sac	No. spiderlings/ female	hatch %
Range	-	-	-	-	-
Mean± S.E	-	-	-	-	-
Sublethal conc. (0.15gm)					
Range	15.00- 22.00	1.00- 2.00	13.00- 33.00	20.00- 52.00	78.3
Mean± S.E	18.59± 0.44	1.70± 0.15	23.65± 1.67	40.20± 3.68	
Sublethal conc. (0.75gm)					
Range	15.00- 21.00	1.00- 3.00	14.00- 39.00	30.00- 94.00	89
Mean± S.E	18.62± 0.27	2.41± 0.19	28.21± 1.26	68.17± 4.77	

A) Effect of Reldan® at field recommended lethal and sublethal doses on *A. aulicus* biology: Effect of Reldan at lethal and sub-lethal concentrations on longevity in days (L), prey consumption (C), egg incubation period, % hatch and fecundity of *A. aulicus* Koch adults were showed in Tables 7 & 8

1- Effect of lethal concentration (1 ml):

a. Longevity and prey consumption of adults:

Female longevity averaged was 17.40 days. Pre-oviposition, oviposition and post-oviposition periods averaged 6.00, 0.40 and 1.80 days, respectively. Male longevity averaged was 5.33 days. The total number of prey consumed per female ranged between 11.0 and 17.0 larvae. Predation during the pre-oviposition, oviposition and post- oviposition periods averaged 5.00, 0.40 and 1.00 larvae, respectively. The total number of prey consumed per male ranged between 0-6 larvae.

b. Egg incubation period, % hatch and fecundity of females:

Females laid 0-1 egg sac each contained 0-18. Eggs hatched after 0-22 days, with % hatch of 63.2 %. The average of spiderlings produced per female was 6.60.

2- Effect of 1/2 lethal concentration (0.5 ml):

a. Longevity and prey consumption of adults:

Female longevity averaged was 95.80 days. Pre-oviposition, oviposition and post-oviposition periods averaged 16.70, 21.30 and 57.80 days, respectively. Male longevity averaged was 75.00 days. The total number of prey consumed per female ranged between 45 and 130 larvae. Predation during the pre-oviposition, oviposition and post-oviposition periods averaged 21.30, 48.30 and 29.60 larvae, respectively. The total number of prey consumed per male ranged between 39-100 larvae.

b. Egg incubation period, % hatch and fecundity of females:

Females laid 1-2 egg sacs each contained 14-35 spiderlings. Eggs hatched after 13.0-22.0, with % hatch of 79 %. The average of spiderlings per female was 44.10.

3- Effect of 1/4 lethal concentration (0.25 ml):

a. Longevity and prey consumption of adults:

Female longevity averaged was 122.42 days. Pre-oviposition, oviposition and post-oviposition periods averaged were 14.67, 33.42 and 74.33 days, respectively. Male longevity averaged 97.64 days. The total number of prey consumed per female ranged between 68 and 221 larvae. Predation during the pre-oviposition, oviposition and post-oviposition periods averaged were 26.33, 97.08 and 50.83 larvae, respectively. The total number of prey consumed per male ranged between 86-183 larvae.

b. Egg incubation period, % hatch and fecundity of females:

Females laid 1-3 egg sacs, each contained 15-40 spiderlings. Eggs hatched after 14.0-20.0 days, with % hatch of 90 %. The average of spiderlings produced per female was 72.67.

Table (7): Effect of Reldan® at lethal and sublethal concentrations on longevity in days (L) and prey consumption (C) of *A. aulicus* Koch adults.

Parameters	Females						Males			
	Lethal conc. (1 ml)									
	Pre-oviposition		Oviposition		Post-oviposition		Total	Total	Total	Total
	(L)	(C)	(L)	(C)	(L)	(C)	(L)	(C)	(L)	(C)
Range	0.00-15.00	0.00-13.00	0.00-1.00	0.00-1.00	0.00-5.00	0.00-3.00	14.00-21.0	11.00-17.00	0.00-9.00	0.00-6.00
Mean±S.E	6.00±3.67	5.00±3.07	0.40±0.24	0.40±0.24	1.80±1.11	1.00±0.63	17.40±1.36	14.40±1.08	5.33±2.37	3.33±1.76
1/2 lethal conc. (0.5 ml)										
Parameters	Pre-oviposition		Oviposition		Post-oviposition		Total	Total	Total	Total
	(L)	(C)	(L)	(C)	(L)	(C)	(L)	(C)	(L)	(C)
Range	13.00-20.00	17.00-29.00	1.00-35.00	1.00-71.00	35.00-71.00	19.00-40.00	54.00-115.00	45.00-130.00	49.00-85.00	39.00-100.00
Mean±S.E	16.70±0.79	21.30±1.11	21.30±3.63	48.30±7.99	57.80±3.99	29.60±2.23	95.80±6.89	98.60±9.01	75.00±3.95	86.50±7.14
1/4 lethal conc. (0.25 ml)										
Parameters	Pre-oviposition		Oviposition		Post-oviposition		Total	Total	Total	Total
	(L)	(C)	(L)	(C)	(L)	(C)	(L)	(C)	(L)	(C)
Range	11.00-18.00	19.00-38.00	1.00-46.00	2.00-125.00	48.00-96.00	34.00-85.00	66.00-136.00	68.00-221.00	61.00-116.00	86.00-183.00
Mean±S.E	14.67±0.66	26.33±1.58	33.42±3.59	97.08±9.82	74.33±4.26	50.83±3.77	122.42±6.21	174.25±11.39	97.64±4.40	126.64±7.54

Table (8): Effect of Reldan® at lethal and sublethal concentrations on egg incubation period, % hatch and fecundity of *A. aulicus* Koch adults.

Lethal conc. (1 ml)					
Parameters	Egg incubation period (days)	No. egg sac/ female	No. spiderlings/ egg sac	No. spiderlings/ female	hatch %
Range	0.00- 22.00	0.00- 1.00	0.00- 18.00	0.00- 18.00	63.2
Mean±S.E	8.40± 5.15	0.40± 0.24	6.60± 4.07	6.60± 4.07	
1/2 lethal conc. (0.5ml)					
Range	13.00-22.00	1.00- 2.00	14.00- 35.00	18.00- 63.00	79
Mean±S.E	18.22± 0.51	1.80± 0.13	24.50± 1.48	44.10± 4.04	
1/4 lethal conc. (0.25ml)					
Range	14.00- 20.00	1.00-3.00	15.00- 40.00	33.00- 96.00	90
Mean±S.E	17.03± 0.29	2.50± 0.19	29.07± 1.43	72.67± 4.93	

A) Effect of Match® at field recommended lethal and sublethal doses on *A. aulicus* biology:

Effect of Match at lethal and sub-lethal concentrations on longevity in days (L), prey consumption (C), egg incubation period, % hatch and fecundity of *A. aulicus* Koch adults, were showed in **Tables 9& 10**

1. Effect of lethal concentration (0.16 ml):

a. longevity and prey consumption of adults:

Female longevity averaged was 76.00 days. The pre-oviposition, oviposition and post-oviposition periods averaged were 13.86, 15.00 and 47.14 days, respectively. Male longevity averaged was 51.83 days. The total number of prey consumed per female ranged between 36 and 116 of larvae. Predation during the pre-oviposition, oviposition and post- oviposition periods averaged 19.57, 43.71 and 26.29 larvae, respectively. The total number of prey consumed per male ranged between 29-58 larvae.

b. Egg incubation period, % hatch and fecundity of females:

Females laid 1-2 egg sacs, each one contained 14-29 spiderlings. Eggs hatched after 15.0-20.0 days, with % hatch of 81 %. The average of spiderlings per female was 39.00.

2. Effect of 1/2 lethal concentration (0.080 ml):

a. longevity and prey consumption of adults:

Female longevity averaged was 127.18 days. The pre-oviposition, oviposition and post-oviposition periods averaged 14.90, 35.73 and 76.55 days, respectively. Male longevity averaged 98.00 days. The total number of prey consumed per female ranged between 55 and 201 larvae. Predation during the pre-oviposition, oviposition and post-oviposition periods averaged 24.82, 92.18 and 43.45 larvae, respectively. The total number of prey consumed per male ranged between 80-190 larvae.

b. Egg incubation period, % hatch and fecundity of females:

Females laid 1-3 egg sacs, each contained 15-40 spiderlings. Eggs hatched after 14.0-20.0, with % hatch of 90 %. The average of spiderlings per female was 76.45.

1. Effect of 1/4 lethal concentration (0.040 ml):

a. Longevity and prey consumption of adults:

Female longevity averaged was 451.92 days. The pre-oviposition, oviposition and post-oviposition periods averaged 14.67, 39.75 and 97.50 days, respectively. Male longevity averaged 127.00 days.

The number of prey consumed per female ranged between 80 and 290 of larvae. Predation during the pre-oviposition, oviposition and post-oviposition periods averaged 31.92, 139.50 and 72.42 larvae. The total number of prey consumed per male ranged between 115-218 larvae.

b. Egg incubation period, % hatch and fecundity of females:

Females laid 1-4 egg sacs, each contained 13-42 spiderlings. Eggs hatched after 10.0-21.0 days, with % hatch of 93 %. The average of spiderlings per female was 92.83.

Table (9): Effect of Match[®] at lethal and sub-lethal concentrations on longevity in days (L) and prey consumption (C) of *A. aulicus* Koch adults.

Parameters	Females								Males	
	Lethal conc. (1 ml)									
	Pre-oviposition		Oviposition		Post-oviposition		Total	Total	Total	Total
	(L)	(C)	(L)	(C)	(L)	(C)	(L)	(C)	(L)	(C)
Range	12.00-18.00	16.00-25.00	1.00-26.00	1.00-65.00	30.00-59.00	19.00-30.00	43.00-90.00	36.00-116.00	31.00-65.00	29.00-58.00
Mean± S.E	13.86±0.74	19.57±1.09	15.00±3.74	43.71±11.04	47.14±3.73	26.29±1.80	76.00±7.27	89.71±13.35	51.83±4.59	51.33±4.52
1/2 lethal conc. (0.5ml)										
Parameters	Pre-oviposition		Oviposition		Post-oviposition		Total	Total	Total	Total
	(L)	(C)	(L)	(C)	(L)	(C)	(L)	(C)	(L)	(C)
Range	12.00-19.00	15.00-30.00	1.00-46.00	2.00-120.00	45.00-90.00	26.00-63.00	59.00-145.00	55.00-201.00	56.00-121.00	80.00-190.00
Mean± S.E	14.90±0.72	24.82±1.31	35.73±4.00	92.18±11.15	76.55±4.26	43.45±3.48	127.18±7.69	160.45±13.12	98.00±5.68	120.70±9.08
1/4 lethal conc. (0.25ml)										
Parameters	Pre-oviposition		Oviposition		Post-oviposition		Total	Total	Total	Total
	(L)	(C)	(L)	(C)	(L)	(C)	(L)	(C)	(L)	(C)
Range	11.00-18.00	25.00-44.00	1.00-55.00	3.00-178.00	58.00-124.00	43.00-94.00	75.00-174.00	80.00-290.00	70.00-149.00	115.00-218.00
Mean± S.E	14.67±0.61	31.92±1.41	39.75±4.08	139.50±13.20	97.50±4.51	72.42±3.67	151.92±7.30	243.83±15.92	127.00±6.94	178.27±9.01

Table (8): Effect of Reldan® at lethal and sublethal concentrations on egg incubation period, % hatch and fecundity of *A. aulicus* Koch adults.

Lethal conc. (1 ml)					
Parameters	Egg incubation period (days)	No. egg sac/ female	No. spiderlings/ egg sac	No. spiderlings/ female	hatch %
Range	0.00- 22.00	0.00- 1.00	0.00- 18.00	0.00- 18.00	63.2
Mean±S.E	8.40± 5.15	0.40± 0.24	6.60± 4.07	6.60± 4.07	
1/2 lethal conc. (0.5ml)					
Range	13.00-22.00	1.00- 2.00	14.00- 35.00	18.00- 63.00	79
Mean±S.E	18.22± 0.51	1.80± 0.13	24.50± 1.48	44.10± 4.04	
1/4 lethal conc. (0.25ml)					
Range	14.00- 20.00	1.00-3.00	15.00- 40.00	33.00- 96.00	90
Mean±S.E	17.03± 0.29	2.50± 0.19	29.07± 1.43	72.67± 4.93	

A) Effect of Match® at field recommended lethal and sublethal doses on *A. aulicus* biology:

Effect of Match at lethal and sub-lethal concentrations on longevity in days (L), prey consumption (C), egg incubation period, % hatch and fecundity of *A. aulicus* Koch adults, were showed in Tables 9& 10

1. Effect of lethal concentration (0.16 ml):

a. longevity and prey consumption of adults:

Female longevity averaged was 76.00 days. The pre-oviposition, oviposition and post-oviposition periods averaged were 13.86, 15.00 and 47.14 days, respectively. Male longevity averaged was 51.83 days. The total number of prey consumed per female ranged between 36 and 116 of larvae. Predation during the pre-oviposition, oviposition and post- oviposition periods averaged 19.57, 43.71 and 26.29 larvae, respectively. The total number of prey consumed per male ranged between 29-58 larvae.

b. Egg incubation period, % hatch and fecundity of females:

Females laid 1-2 egg sacs, each one contained 14-29 spiderlings. Eggs hatched after 15.0-20.0 days, with % hatch of 81 %. The average of spiderlings per female was 39.00.

2. Effect of 1/2 lethal concentration (0.080 ml):

a. longevity and prey consumption of adults:

Female longevity averaged was 127.18 days. The pre-oviposition, oviposition and post-oviposition periods averaged 14.90, 35.73 and 76.55 days, respectively. Male longevity averaged 98.00 days. The total number of prey consumed per female ranged between 55 and 201 larvae. Predation during the pre-oviposition, oviposition and post-oviposition periods averaged 24.82, 92.18 and 43.45 larvae, respectively. The total number of prey consumed per male ranged between 80-190 larvae.

b. Egg incubation period, % hatch and fecundityof females:

Females laid 1-3 egg sacs, each contained 15-40 spiderlings. Eggs hatched after 14.0-20.0, with % hatch of 90 %. The average of spiderlings per female was 76.45.

Table (10): Effect of Match® at lethal and sublethal concentrations on egg incubation period, % hatch and fecundity of *A. aulicus* Koch adults.

Lethal conc. (1 ml)					
Parameters	Egg incubation period (days)	No. egg sac/ female	No. spiderlings/ egg sac	No. spiderlings/ female	hatch %
Range	15.00- 20.00	1.00- 2.00	14.00- 29.00	25.00- 48.00	81
Mean± S.E	17.33± 0.43	1.71± 0.18	22.75± 1.29	39.00± 3.50	
1/2 lethal conc. (0. 5ml)					
Range	14.00- 20.00	1.00-3.00	15.00- 40.00	28.00- 95.00	90
Mean± S.E	17.57± 0.30	2.55± 0.21	30.04± 1.37	76.45±5.90	
1/4 lethal conc. (0.25ml)					
Range	10.00- 21.00	1.00- 4.00	13.00- 42.00	29.00- 133.00	93
Mean± S.E	16.26± 0.39	2.92± 0.26	31.80± 1.25	92.83± 8.22	

Discussion:-

Spiders are important predators of insect pests in agro-ecosystems throughout the world. They are highly sensitive to insecticides (Rezac *et al.*, 2010).

In the present study Lannate®, Reldan® and Match® insecticides for use in tomato fields against the cotton leaf worm, in Fayoum Governorate were evaluated for their effect on the biology of *A. aulicus*. Mortality of adult spiders and some biological aspects as prey consumption, longevity periods, number of eggs laid, incubation period, and % hatch were investigated in the laboratory. Daane *et al.*, 2004, proved that Lorsban® and Lannate® caused 100% mortality in black widow spiders after 1 day exposure. Armenta *et al.*, (2003) claimed that Chlorpyrifos® exposure produced up to 73% reduction in abundance of natural enemies in the agricultural fields. Pekar (2002) proved that Alpha cypermethrin® and Fluvalinate + Thiometon® produced the highest mortality in spiders two days after application while Bifenthrin® and Deltamethrin® showed highest mortality after three days after the application. Decis® caused 80% mortality in *Philodromus cespitum* (Rezac *et al.*, 2007). Bajwa and Aliniabee (2001) also, claimed that organophosphate Azinphosmethyl® (25g/100 liters) and carbamate Carbaryl® (60g/100L) produced 25-75% mortality of spiders while pyrethroids, esfenvalerate® (2.5g/100L) and Permethrin® (4g/100 liters) produced 50-75% mortality.

Tahir *et al.*, (2014) demonstrated the effects of Thiodan® (insecticide) on the survival, locomotion, behaviour and predation rate of the jumping spider *Plexippus paykulli*. Thiodan® caused 40% mortality at the recommended field rate concentration of *P. paykulli* did not avoid the surface treated with the Thiodan® (1/4 recommended field rate).

A) The ultrastructure of the midgut of toxicated and healthy adults of *A. aulicus*:

1- Spiders untreated (control):

Using the transmission electron microscope (TEM), the ultrastructure of the midgut of *A. aulicus* adults is shown in (Figs. 1 and 2); female and male spiders are similar in this respect. The midgut examination of untreated spiders (control) of *A.*

aulicus, revealed the presence of two types of cells; secretory cells (Sc) which are characterized by densely stained enzyme granules, and resorptive cells (R) which possess irregular food vacuoles (Vc). Also, shown the lumen gut (Lu), interstitial tissue (IS), bar = 12,000x. The secretory cell with Zymogene granules (Z) and extensive endoplasmic reticulum; the light resorptive cell (R) with large food vacuoles (Vc), are further shown in Fig. 2, bar = 16,000 x.

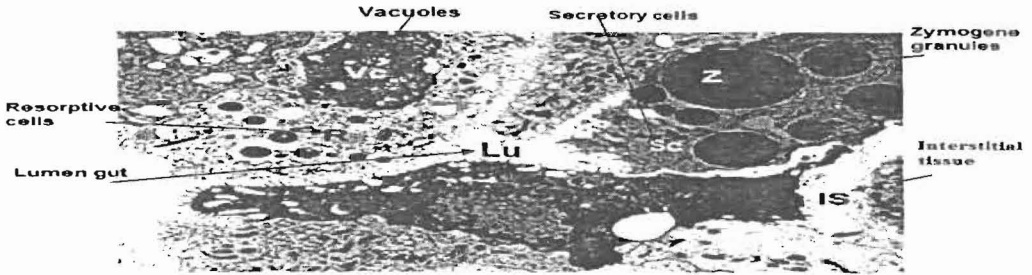


Fig (1): Ultrastructure of the midgut of untreated female spider *A. aulicus* bar= 12000x.

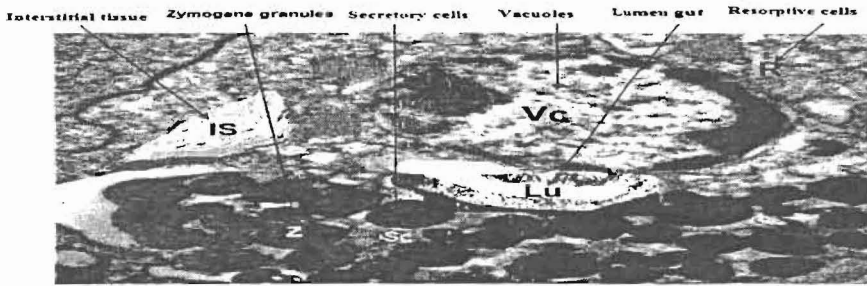


Fig (2): Ultrastructure of the midgut of untreated male spider *A. aulicus* bar= 16000x.

B) Changes in ultrasructure of the midgut of *A. aulicus* due to insecticides-treated:

1. Spiders treated with Lannate® (0.3gm):

a. Females:

As shown in Fig (3), changes in the secretory cells of the midgut which showed the appearance of zymogene granules and extensive endoplasmic reticulum. Also, changes in the nuclear structure of the cells as lobular nucleus (n) with condensed chromatin accumulated mainly near the nuclear envelope which called "necrotic changes" (ne). A large food vacuoles and muscular cells (mc) were present.

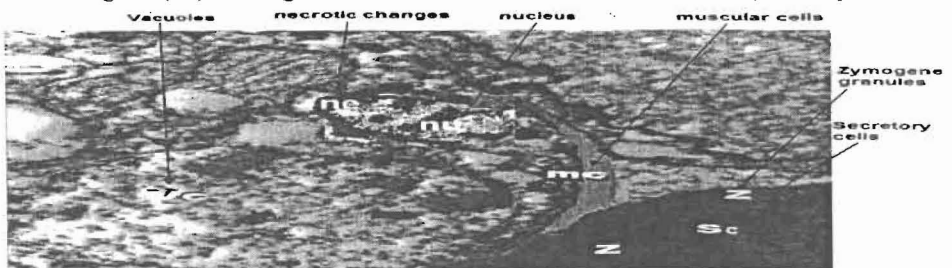


Fig (3): Ultrastructure of the midgut of female spider *A. aulicus* treated with Lannate, bar= 10000x.

b. Males:

As shown in Fig (4), changes in the nuclear structure of the cells as lobular nucleus (n) with condensed chromatin accumulated mainly near the nuclear envelope which called "necrotic changes" (ne). Also, secretory cells were found without zymogene granules but with, large food vacuoles.

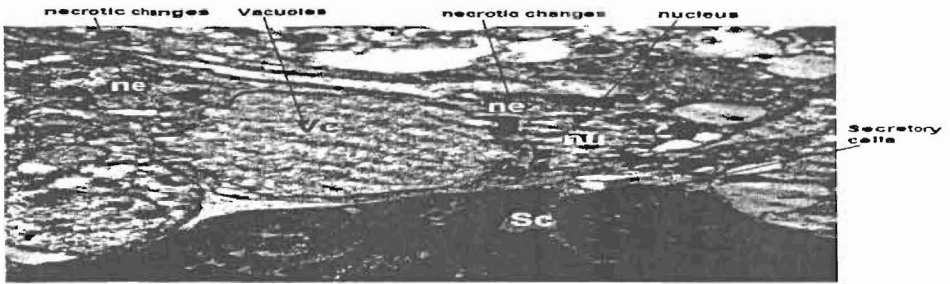


Fig (4): Ultrastructure of the midgut of male spider *A. aulicus* treated with Lannate, bar= 4000x.

2- Spiders treated with Reldan® (1ml):

a. Females:

As shown in Fig (5), changes in the fine structure of the midgut of females which a large food vacuoles and secretory cells with zymogene granules were presence.

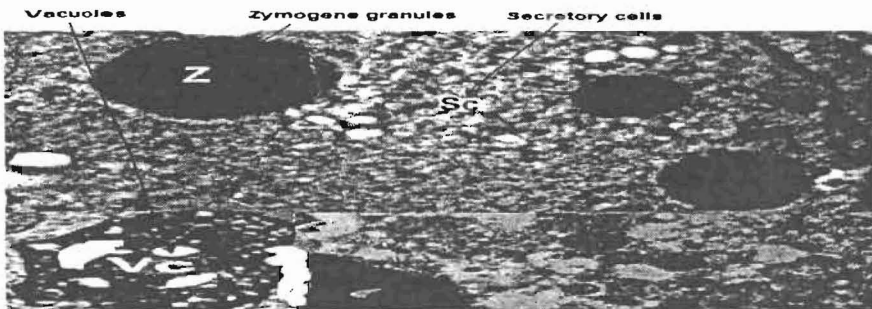


Fig (5): Ultrastructure of the midgut of treated female spider *A. aulicus* with Reldan, bar= 10000x.

b. Males:

As shown in Fig (6), changes in the midgut which appeared two largest food vacuoles and zymogene granules without secretory cells.

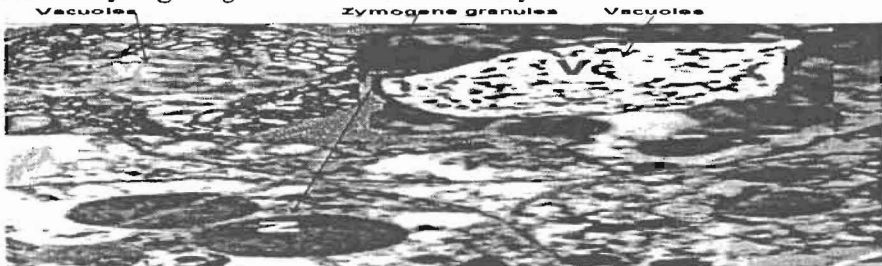


Fig (6): Ultrastructure of the midgut of treated male spider *A. aulicus* with Reldan, bar = 4000x.

3- Spiders treated with Match® (0.16ml):

a. Females:

As shown in Fig (7), the ultrastructure of the midgut revealed the changes in shape of the nucleus which appeared longitudinal shape (malformed nucleus). Also, the secretory cells with zymogene granules and resorptive cells which possess irregular food vacuoles were presence.

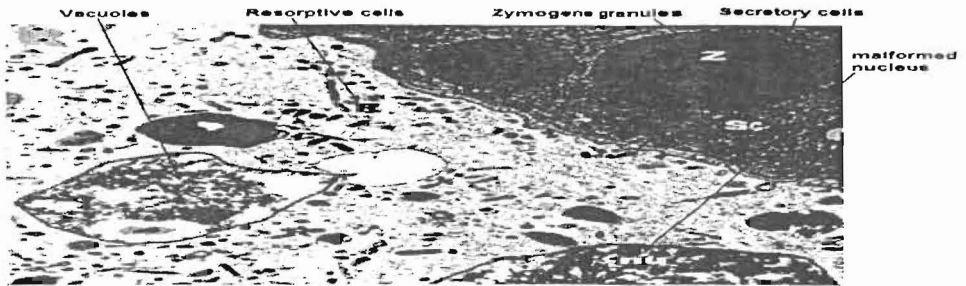


Fig (7): Ultrastructure of the midgut of treated female spider *A. aulicus* with Match, bar= 9000x.

b. Males:

As shown in Fig (8), the ultrastructure of the midgut revealed the changes in shape of the nucleus which appeared longitudinal shape (malformed nucleus). Also largest food vacuoles were presence.

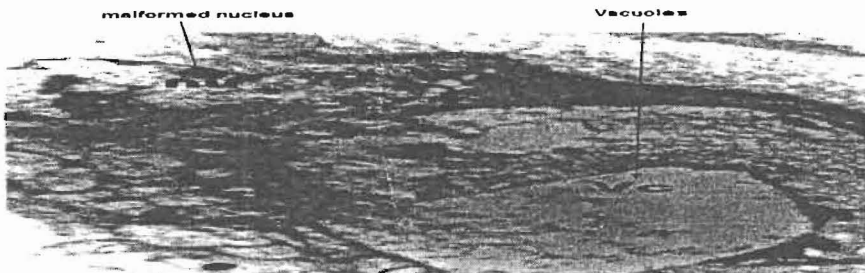


Fig (8): Ultrastructure of the midgut of treated male spider *A. aulicus* with Match, bar= 8000x.

Discussion:-

In these respect, (Bertkau, 1985 and Millot, 1926) mentioned that spiders possess two different kinds of cells in the intestinal epithelium: secretory cells and resorptive cells. The secretory cells contain digestive enzymes, which can be seen as dark cytoplasmic granules under the microscope. Most of the intestinal cells are of the resorptive type. They can be recognized by their many inclusions the food vacuoles. The resorptive cells process the nutrients further and then pass them on to the underlying interstitial tissue or into the hemolymph.

Wilczek *et al.*, (2014) examined the organization of the midgut gland of the wolf spider *Xerolycosa nemoralis* (Lycosidae). The frequency of degenerative changes in starving individuals was twice as high as in the specimens intoxicated

with dimethoate[®], the percentage of apoptotic and necrotic cells was higher in starving males than females.

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تأثير ثلاثة مبيدات حشرية علي بعض المظاهر البيولوجية والهستولوجية للعنكبوت أنوليسماس إيوليكاس
نبيل محمد الخولي¹، أشرف عبد الحفيظ رحيل¹ و عماد فتحي دويدار²
¹ قسم وقاية النبات- كلية الزراعة- جامعة الفيوم
² قسم النبات الزراعي- كلية الزراعة- جامعة الفيوم

تم دراسة تأثير استخدام ثلاث مبيدات حشرية بثلاث تركيزات مختلفة لكل منهم وهم اللانبيث ٩٠% والريلدان ٥٠% ومبيد الماتش ٥% والموصي بهم لمكافحة دودة ورق القطن في حقول الطماطم وذلك علي العنكبوت المفترس أنوليسماس إيوليكاس بغرض التقييم الحيوي لهذه المبيدات ودراسة تأثيراتها المختلفة علي بعض المظاهر البيولوجية والهستولوجية لهذه العنكب.

وقد أسفرت النتائج عن الآتي:

- في حالة العنكب الغير متغذية علي يرقات معاملة بالمبيدات (الكنترول) أسفرت الدراسة البيولوجية للنوع أنوليسماس إيوليكاس عند تغذيته علي يرقات دودة ورق القطن عن الآتي:-
- متوسط فترة حياة الإناث من البلوغ للموت هي ١٦٧,٢٥ يوم بينما متوسط فترة حياة الذكور من البلوغ للموت هي ١٤٣,٩٢ يوم ومعدل استهلاك الغذاء للإناث كان ٢٧٨,٠٠ فريسة بينما معدل استهلاك الغذاء للذكور ٢١٢,٧٥ فريسة، ومتوسط الفترة اللازمة لفقس البيض هي ١٦,٥٠ يوم وكان معدل فقس البيض هو ٩٤% وكان متوسط عدد اكياس البيض لكل انثي ٣,٥٠ بيضة ومتوسط عدد صغار العنكب لكل كيس بيض هو ٣٠,٦٤ فرد ومتوسط عدد صغار العنكب لكل انثي هو ١٠٧,٢٥ فرد. بعكس المعاملة بالمبيدات الثلاثة والتي كانت لها تأثير علي معدل البقاء، معدل استهلاك الغذاء للذكور والإناث، والخصوبة للإناث.
- في حالة العنكب المتغذية علي اليرقات المعاملة بالتركيزات المختلفة أسفرت النتائج ان مبيد اللانبيث عند التركيز الموصي (٠,٣ جرام) كان له تأثير كبير علي بيولوجية العنكب المعاملة يليه مبيد الريلدان تأثير متوسط (١ مللي) ثم تأثير أقل لمبيد الماتش (٠,١٦ مللي) بينما في حالة نصف التركيز الموصي لمبيد اللانبيث (٠,١٥ جرام) كان له تأثير متوسط يليه مبيد الريلدان (٠,٥ مللي) تأثير أقل يليه مبيد الماتش (٠,٠٨٠ مللي) تأثير ضعيف، وفي حالة الربع التركيز الموصي للمبيدات الثلاثة كانت تأثيراتهم ضعيفة جدا علي العنكب المتغذية علي اليرقات المعاملة.
- تم حساب النسب المئوية لهذه المبيدات فكانت عند التركيز الموصي سجل مبيد اللانبيث أعلى نسبة موت للذكور فكانت ٩١,٦٧% وللإناث ٦٦,٦٦% بينما في حالة مبيد الريلدان سجل أيضا أعلى نسبة موت للذكور فكانت ٨٣,٣٣% وللإناث ٥٨,٢٣% وفي حالة مبيد الماتش سجل نسبة متوسطة عند التركيز الموصي ففي حالة الذكور ٥٨,٢٣% بينما في حالة الإناث ٥٠%، وبصفة عامة الذكور أكثر تأثرا وحساسية للمبيدات المستخدمة في هذه الدراسة عن الإناث.
- أسفرت نتائج الهستولوجي باستخدام الميكروسكوب الإلكتروني للقطاعات في القناة الهضمية للعنكب بعد تغذيتها علي اليرقات المعاملة بالمبيدات وجود اختلافات في شكل بعض العضيات الموجودة مثل النواة خاصة في حالة مبيد اللانبيث والماتش ووجود أو غياب بعض الخلايا أو الأنسجة داخل القطاعات في حالة مبيد الريلدان وايضا اللانبيث والماتش مقارنة بالعنكب الغير معاملة وربما يرجع ذلك الي تداخل فعل هذه المبيدات مع بعض وظائف القناة الهضمية.