4. RESULTS AND DISCUSSION

4.1. Testing the Insecticidal Effect of Herbicides Against Mosquitoes :

The present set of laboratory experiments aimed at the detection of whether or not the tested herbicides (Ordram, Ronstar, Machete, Stomb 330 E and Rifit 550 EC) have insecticidal action against the different stages of C. pipiens. This group of herbicides has been chosen because they are already used for weed control in rice fields in Egypt. Therefore, all stages of mosquitoes might be exposed to the herbicides in the field. The present work was designed so as to expose C. pipiens to each of the tested herbicides solely using the recommended field rate as a basal concentration; higher and lower concentrations were tested when necessary. It is estimated that one feddan of rice may contain approximately 800,000 liter of irrigation water. Meanwhile, the rates recommended for field application of Ordram, Ronstar, Machete, Stomb and Rifit are 2500, 750, 2000, 700 and 700 cc/feddan, respectively. These field rated could be transferred in terms of numbers of milliliters/one liter of water to be equivalent to 0.003, 0.0009, 0.003, 0.01 and 0.01 ml/L, respectively. In such a manner, these concentrations represented the basal ones which are referred to as (R) or recommended rates. Egg rafts, 2nd and 4th instar

larvae, pupae and adults of C. pipiens were exposed to the tested herbicides at the above-mentioned rates and their doubles and dilutions as indicated in the tables.

4.1.1. Effect on egg rafts :

All the tested herbicides did not interfere with egg hatching when similar egg rafts of C. pipiens were placed in water containing concentrations equivalent to the recommended rates of the herbicides. There were no noticeable reduction in hatchability in comparison with the egg rafts kept in distilled water as untreated control.

4.1.2. Effect on larvae :

Second and fourth-instar larvae of laboratory-reared colony of C. pipiens were exposed to five different concentrations of each tested herbicide. Results of these tests are summarized in Tables 1, 2 and 3.

Under laboratory temperature of 24°C in average (24-27°C), as it is shown in Table (1), Ordram and Machete caused no mortality among both L_2 and L_4 of C. pipiens when the larvae were exposed to concentrations corresponding to the field application rate 0.003 ml/L, (R). Doubling the dosage to 2R resulted no mortality with the exception of 4 % death recorded among L_4 treated with

Table (1) : Mortality % among L_2, L_4 and pupae of Culex pipiens exposed to two herbicides, i.e. Ordram and Machete in different concentrations for three successive days. Concentrations equivalent to the recommended field rates are marked as (R).

Tested mosquito stage	Concentrations	Mortality %					
		Ordram			Machate		
		24 h	48 h	72 h	24 h	48 h	72 h
L_2	0.02 ml/L	0	12	46	28	42	68
	0.01 ml/L	0	2	4	20	30	44
	0.006 ml/L	0	0	0	0	0	0
	0.003 ml/L (R)	0	0	0	0	0	0
	0.002 ml/L	0	0	0	0	0	0
	Control	0	0	0	0	0	0
L_4	0.02 ml/L	14	26	60	32	48	64
	0.01 ml/L	12	14	18	20	36	40
	0.006 ml/L	0	0	0	0	0	4
	0.003 ml/L (R)	0	0	0	0	0	0
	0.002 ml/L	0	0	0	0	0	0
	Control	0	0	0	0	0	0
Pupa	0.02 ml/L	0	0	0	6	6	6
	0.01 ml/L	0	0	0	0	0	0
	0.006 ml/L	0	0	0	0	0	0
	Control	0	0	0	0	0	0

Table (2) : Mortality % of C. pipiens (L_2 & L_4) exposed to Ronstar in different concentrations for three successive days. Concentrations equivalent to the recommended field rates are marked (R).

Tested mosquito stages	Concentration	Mortality %		
		24 hours	48 hours	72 hours
L_2	0.008 ml/L	44	44	44
	0.004 ml/L	12	22	40
	0.002 ml/L	0	0	0
	0.0009 ml/L (R)	0	0	0
	0.0005 ml/L	0	0	0
	Control	0	0	0
L_4	0.008 ml/L	20	26	26
	0.004 ml/L	10	12	12
	0.002 ml/L	0	0	0
	0.0009 ml/L (R)	0	0	0
	0.0005 ml/L	0	0	0
	Control	0	0	0
Pupae	0.008 ml/L	0	0	0
	0.004 ml/L	0	0	0
	0.02 ml/L	0	0	0
	Control	0	0	0

Table (3) : Corrected mortality % among L_2, L_4, pupae and adults of Culex pipiens exposed to two herbicides, i.e. Stomb 330 E and Rifit 550 EC in different concentrations for three successive days. Concentrations equivalent to the recommended rate are marked as (R).

tested mosquito stage	Concentration	Mortality %							
		Stomb 330 E				Rifit 550 EC			
		1 h	24 h	48 h	72 h	1 h	24 h	48 h	72 h
L_2	0.08	100				100			
	0.04	78	84.1	88.1	90.1	0	100		
	0.02	0	69.6	80.2	86.7	0	42.0	76.0	86.0
	0.01 (R)	0	44.9	66.2	66.2	0	20.0	30.0	34.0
	0.005	0	28.6	45.6	57.8	0	10.0	14.0	18.0
L_4	0.08	100				100			
	0.04	0	26.7	60.7	76.7	0	100		
	0.02	0	13.3	27.3	35.3	0	55.1	77.1	89.1
	0.01 (R)	0	22.2	24.4	24.4	0	18.2	38.4	54.4
	0.005	0	2.2	8.2	8.2	0	10.2	16.2	20.2
Pupae	0.01 (R)	0	16.0	10.1	12.2	0	4.0	6.3	6.3
Adult	0.01 (R)	0	0	20.0	20.0	0	0	0	20.0

Machete after 72 hrs. of exposure. At higher concentrations, i.e. 0.01 and 0.02 ml/L which are equivalent to 4R and 8R, respectively, noticeable mortalities were observed. However, L_2 appeared less susceptible to the high concentrations of Ordram than L_4. The mortality reached 46 % after 3 days among L_2 treated with 0.02 ml/L while the same dosage caused 60 % mortality among L_4 after an equal period. As for Machete, there were no big differences between susceptibility of the both tested larval instars, with mortality reaching 68 % and 64 % on the third day among L_2 and L_4 when treated with a concentration of 0.02 ml/L which is equivalent to 8-fold the application rate.

Similarly, Ronstar did not cause any mortality among L_2 and L_4 of C. pipiens when used at a concentration of 0.0009 ml/L (= R concentration). Increasing the herbicidal material to a rate of 0.008 ml/L (= 8R) caused initial mortality of 44 % among L_2. However, this mortality did not change during the following two days. On contrary to what was reported for Ordram, fourth-instar larvae of C. pipiens showed less resistance than those of second instar when treated with high concentrations of Ordram (Table, 2).

On the other hand, Stomb 330 EC and Rifit 550 EC demonstrated considerable larvicidal activity against both 2nd and 4th instar larvae of C. pipiens 24 h after treatment

with concentrations equivalent to applied field rates (0.01 ml/L). Data presented in Table (3) obviously showed that the recorded mortalities were proportional to the concentrations used. The exposure of L_2 to 0.01 ml/L of Stomb 330 E resulted in 44.9 % mortality after 24 hr, that increased to 66.2 % after three days in the same treatment which, in turn, caused 22.2 and 24.4 % mortality among L_4 on the 1st and 3rd day, respectively. Stomb 330 E showed stronger insecticidal power than Rifit 550 EC when used in similar concentrations. A concentration of 0.01 ml/L of this compound killed 20 % of L_2 on the 1st day increased to 34 on the 3rd day. The same concentration killed 18.2 and 54.4 % of the treated L_4 on the 1st and 3rd day, respectively. Increasing the concentrations of the herbicides caused higher mortalities among treated larvae. Very high concentration (0.08 ml/L) of these two compounds caused 100 % death within only one hour in both L_2 and L_4 (Table,3). Moreover, the two tested compounds seemed to keep their larvicidal action even in lower concentrations (= ½R) with relatively less mortality in Rifit than in Stomb in the case of L_2, but vice versa in the case of L_4.

4.1.3. Effect on pupae :

No mortality was recorded among pupae of C. pipiens when exposed to concentrations reached 4-fold the application

rates of Ordram, Machete and Ronstar for 3 successive days. In one expetion,a concentration of 0.02 ml/L of Machete, which equal 8 times the applied rate, caused 6 % mortality among the treated pupae one day after exposure. No increase in mortality was observed during the next two days. However, R concentrations of both Stomb and Rifit caused mortality among treated pupae. Stomb killed 6, 10.1 and 12.2 % of pupae exposed to 0.01 ml/L on the 1st, 2nd and 3rd days, respectively. Rifit seemed to be less active in terms of pupicidal power; its R concentration killed 4 and 6.3 % of the treated pupae on the 1st and 2nd days, respectively. No increase in the mortality was observed on the 3rd day. Results of the effect of the tested herbicides on mosquito pupae came in the same trend of their effect on the larvae.

4.1.4. Effect on adults :

Newly-emerged adults of C. pipiens were tested against the herbicides which proved to have obvious insecticidal action against larvae and pupae of the same species when used at the regular dosages, i.e. Stomb 330 E and Rifit 550 EC. Mosquito adults were allowed to feed upon sugar solution mixed with 0.01 ml/L of the both two herbicides. No mortality was recorded in the case of Stomb one day after treatment. Two days later, there was 20 % mortality

among treated adults. In the case of Rifit, similar result was obtained but after three days of exposure.

In conclusion, the obtained results indicate that the tested herbicides could be divided into two groups in terms of their insecticidal activity against mosquito when used at the field recommended dosages :

1) Mosquito-non-killing chemicals, which include Ordram, Machete and Ronstar, and
2) Mosquito-killing chemicals, which include Stomb 330 E and Rifit 550 EC.

Accordingly, when applied for weed control in rice fields or any other aquatic habitat, the two latter compounds will act as double-purpose pesticides; they will kill the target weeds and, at the same time, help in suppressing mosquito populations in the place. For the first glance, it is economically good to hit two birds with one stone. But from the ecological point of view it is necessary to go beyond this point. The impact of such chemicals on the other associate fauna, especially mosquito aquatic predators should be examined.

4.2. Impact of the Tested Herbicides on Mosquito Predators and Other Aquatic Insects :

Six aquatic insects belonging to four insect orders were subjected to the present set of experiments; May fly nymphs of Polymetarsus sp. (Ephemeroptera : Ephemeridae); dragonfly nymphs of Crocthemis erythraea (Odonata : Libellulidae); damselfly nymphs of Ischnura senegalensis (Odonata : Agrionidae); nymphs and adults of the backswimmer Anisops sardea (Heteroptera : Notonectidae); nymphs and adults of the water boatman Sigara mayri (Heteroptera : Corixidae) and larvae of the non-biting midge Chironomus sp. (Diptera : Chironomidae). At least three of these insects, i.e. C. erythrae, I. senegalensis and A. sardea are known as effective predators feeding upon mosquito larvae and sometimes pupae (e.g. Bates, 1965; Bay, 1974; and Abou Bakr, 1984). Meanwhile, there is a strong evidence that the water boatman S. mayri may play a considerable role as a mosquito predator in the aquatic environments (Agami, personal communication). Meanwhile, nymphs of Polymetarsus sp. and larvae of Chironomus sp. are major co-inhabitants in many mosquito breeding sites in Egypt. However, our laboratory observations throughout the course of the present investigation did not prove that nymphs of Polymetarsus sp. could be considered as predators of mosquito larvae. But the significance of both the

chironomid midges and the Ephemerid nymphs lies in their status as bottom dwellers in the aquatic habitats which usually receive the final products of the majority of environmental pollutants.

All experiments were carried out under laboratory conditions where temperature ranged between 18 and 17°C with an average of 22.5 °C. Results are summarised in Tables 4, 5, 6, 7 and 8. Data presented in these tables indicated that all the tested five herbicides have no deleterious effect on nymphs of Polymetarsus sp., C. erythraea and I. senegalensis when tested at concentrations equivalent to those applied in weed control programs. Moreover, doubling these rates did not result any mortality after 3 days of exposure. There were only two exceptions; the first was when a concentration of 0.001 ml/L (= 2R) of Ronstar caused 10 % mortality among I. senegalensis nymphs, after three days of treatment (Table, 5). The second was in the case of using 0.006 ml/L (= 2R) of Machete against nymphs of both Polymetarsus sp. and C. erythraea; 10 % mortality was recorded for the two insects on the 3rd day of exposure. Nevertheless, the aforementioned three species showed obvious resistance against the five herbicides at 2-fold the field rates, even in the case of Stomb 330 E and Rifit 550 EC which previously

proved to have strong insecticidal actions against mosquitoes. On the other hand, the tested herbicides caused different rates of mortality when tested against nymphs and adults of the backswimmer A. sardae, the water boatman S. mayri and the larvae of Chironomus sp. However, the response of A. sardea considerably varied according to the tested stage. Nymphs of this backswimmer were obviously more sensitive than their adults in all cases. For instance, Ordram caused 60 % and 100 % mortality after 3 days of exposing A. sardea nymphs to ½R and R concentrations, respectively. A higher concentration (= 2R) of the same compound caused 80 % and 100 % mortality among these nymphs on the 1st and 2nd day of exposure, respectively. The mortality among the untreated control did not exceed 10 %. Adults of A. sardea were not affected with the treatment of Ordram at the rate of field application. However, rising the concentration to 2-fold resulted in 10, 20 and 40 % mortality on the 1st, 2nd and 3rd day, respectively. No mortality was recorded among the untreated adults (Table, 4). When treated with Ronstar, adults of A. sardea showed response similar to what was recorded for Ordram at the concentration of 1R. But when higher concentration was used (2R) comparatively less mortality was observed, reaching 10 % of the treated backswimmers on the 3rd day of exposure (Table, 5). Nevertheless, immature stages of

Table (4) : Mortality (%) among certain aquatic insects that may associate with mosquito larvae in their habitats when exposed to different concentration of Ordram for 24, 48, and 72 hours.

Insect	Stage	Mortality %											
		0.002 ml/L (½R)			0.003 ml/L (R)			0.006 ml/L (2R)			Control		
		24h	48h	72h	24h	48h	72h	24h	48h	72h	24h	48h	72h
Polymetarsus sp.	Nymph	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Crocthemis erythraea	Nymph	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Ischnura sengalensis	Nymph	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Anisops sardea	Nymph	0.0	60.0	30.0	0.0	30.0	100.	80.0	100.		10.0	10.0	10.0
	Adult	0.0	0.0	0.0	0.0	0.0	0.0	10.0	20.0	40.0	0.0	0.0	0.0
Sigara mayri	Nymph	60.0	80.0	80.0	40.0	80.0	80.0	10.0	90.0	90.0	10.0	10.0	10.0
	Adult	91.7	91.7	91.7	66.7	83.3	83.3	91.0	100.		0.0	25.0	25.0
Chironomus sp.	Larva	0.0	0.0	0.0	0.0	20.0	30.0	0.0	20.0	30.0	0.0	0.0	0.0

Table (5) : Mortality (%) among certain aquatic insects that may associate with mosquito larvae in their habitats when exposed to different concentration of Ronstar for 24, 48, and 72 hours.

Insect	Stage	Mortality %											
		0.005 ml/L (½R)			0.0009 ml/L (R)			0.01 ml/L (2R)			Control		
		24h	48h	72h	24h	48h	72h	24h	48h	72h	24h	48h	72h
Polymetarsus sp.	Nymph	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Crocthemis erythraea	Nymph	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Ischnura sengalensis	Nymph	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	10.0	0.0	0.0	0.0
Anisops sardea	Nymph	0.0	40.0	40.0	40.0	80.0	80.0	60.0	80.0	80.0	10.0	10.0	10.0
	Adult	0.0	0.0	0.0	0.0	0.0	0.0	0.0	10.0	10.0	0.0	0.0	0.0
Sigara mayri	Nymph	50.0	70.0	70.0	10.0	50.0	90.0	40.0	80.0	90.0	0.0	10.0	10.0
	Adult	8.3	50.0	66.7	58.3	83.3	83.3	50.0	58.3	75.0	0.0	16.7	25.0
Chironomus sp.	Larva	0.0	0.0	0.0	0.0	0.0	0.0	0.0	10.0	10.0	0.0	0.0	0.0

Table (6) : Mortality (%) among certain aquatic insects that may associate with mosquito larvae in their habitats when exposed to different concentration of Machete for 24, 48, and 72 hours.

Insect	Stage	Mortality %											
		0.002 ml/L (½R)			0.003 ml/L (R)			0.006 ml/L (2R)			Control		
		24h	48h	72h	24h	48h	72h	24h	48h	72h	24h	48h	72h
Polymetarsus sp.	Nymph	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	10.0	0.0	0.0	0.0
Crocthemis erythraea	Nymph	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	10.0	0.0	0.0	0.0
Ischnura sengalensis	Nymph	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Anisops sardea	Nymph	30.0	30.0	30.0	40.0	50.0	50.0	50.0	80.0	80.0	0.0	10.0	10.0
	Adult	0.0	0.0	0.0	10.0	20.0	20.0	-	60.0	90.0	0.0	0.0	0.0
Sigara mayri	Nymph	30.0	60.0	70.0	90.0	100.		60.0	100.		16.7	16.7	16.7
	Adult	100.			100.			100.			0.0	0.0	0.0
Chironomus sp.	Larva	20.0	20.0	20.0	50.0	90.0	90.0	100.			0.0	0.0	0.0

Table (7) : Mortality (%) among certain aquatic insects that may associate with mosquito larvae in their habitats when exposed to different concentration of Stomb 330 E after 1, 24, 48 and 72 hours.

Insect	Stage	Mortality %															
		0.0005 ml/L(½R)				0.01 ml/L (R)				0.02 ml/L (2R)				Control			
		1h	24h	48h	72h	1h	24h	48h	72h	1h	24h	48h	72h	1h	24h	48h	72h
Polymetarsus sp.	Nymph	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Crocthemis erythraea	Nymph	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ischnura sengalensis	Nymph	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Anisops sardea	Nymph	0	100			0	100			100				0	10	10	10
	Adult	20	30	30	30	60	90	90	90	90	100			0	0	0	0
Sigara mayri	Nymph	0	30	70	100	0	60	100		0	100			0	0	0	0
	Adult	0	100			100				100				0	25	25	25
Chironomus sp.	Larva	0	0	0	0	0	0	10	10	0	10	30	50	0	0	0	0

Table (8) : Mortality (%) among certain aquatic insects that may associate with mosquito larvae in their habitats when exposed to different concentration of Rifit 550 EC after 1, 24, 48, and 72 hrs.

Insect	Stage	Mortality %															
		0.005 ml/L(½R)				0.01 ml/L (R)				0.02 ml/L (2R)				Control			
		1h	24h	48h	72h	1h	24h	48h	72h	1h	24h	48h	72h	1h	24h	48h	72h
Polymetarsus sp.	Nymph	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Crocthemis erythraea	Nymph	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ischnura sengalensis	Nymph	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Anisops sardea	Nymph	0	95	95	95	0	100			100				0	10	10	10
	Adult	0	0	0	0	0	10	20	20	0	0	60	80				
Sigara mayri	Nymph	0	20	50	70	0	10	70	80	0	80	100		0	0	0	0
	Adult	0	100			0	91.7	91.7	91.7	100				0	25	25	25
Chironomus sp.	Larva	0	0	70	90	0	40	70	100	0	0	100		0	0	0	0

A. sardea could not withstand the exposure to Ronstar even at levels 50 % less than those applied in the field; a mortality of 40 % was recorded on the 2nd day of treatment. This mortality increased to the double with doubling the concentration of Ronstar (= R), as the mortality recorded after one day was 40 %, and raised to 80 % on the 2nd day. However, increasing the concentration to 0.001 ml/L (= 2R) resulted in a higher initial mortality (60 %) among treated nymphs on the 1st day after exposure. Mortality increased on the 2nd day to 80 % whereas remained at this level on the 3rd day (Table, 5).

As regarding Machete, Stomb and Rifit, they proved to be very toxic for both nymphs and adults of A. sardea when used at the application rates (Tables 6, 7 and 8). Although adults of the tested backswimmers were not affected when exposed to one-half the field rates of Machete and Rifit, 20 % of the backswimmers died only one hour after being placed in water containing similar rate of Stomb 330 E. This mortality increased to 30 % after 24 hours and remained at this level until the 3rd day of the experiment. Exposing the adult backswimmers to concentrations equivalent to field rates caused 10 % mortality after 24 hours in case of both Machete and Rifit. At the same rate of treatment the two compounds caused death of 20 % of the treated bugs on the

3rd day (Tables 6 and 8). Increasing the concentration of Machete and Rifit to 2R resulted in killing 90 % and 80 % of the respected treated backswimmers on the 3rd day. Such a drastic lethal effect of Machete and Rifit was manifested in even a more rapid and dramatic manner among adults of A. sardea released in water containing 0.01 ml/L (= R) of Stomb 330 E. Sixty per cent of the treated insects died only one hour after exposure. One day later, the mortality jumped to 90 % (Table, 7). When higher concentration (= 2R) was tested, mortality after one hour reached 90 %, then increased to 100 % after 24 hours. Furthermore, the suffering of A. sardea nymphs was strikingly outstanding, especially in the case of both Stomb 330 E and Rifit 550 EC.

The strongest lethal effect on the nymphs was recorded for Stomb which killed all the individuals 24 hours after being exposed to a concentration of 5×10^{-4} ml/L which is 50 % less than that recommended for weed control. Rifit was slightly less lethal at the corresponding rate, killing 95 % of the nymphs after 24 hours. But when used at the field recommended rate, Rifit killed all the treated A.sardea nymphs presenting insecticidal power as ten times as that was recorded against the adults (Table, 8). In comparison with Stomb and Rifit, Machete comes in the third place, causing 30, 50 and 80 % mortality among nymphs exposed

for three days to concentrations equivalent to ½R, R and 2R, respectively (Table, 6). In all cases, mortality of the untreated backswimmer nymphs was 0, 10 and 10 % throughout the three successive days of the experiment.

The fate of both nymphs and adults of the corixid water boatman, S. mayri exposed to the tested "selective" herbicides was not better than that of the notonectid backswimmer. Data shown in Tables (4, 5, 6, 7 and 8) flagrantly prove the high and rapid lethal action of all tested compounds on both nymphs and adults of S. mayri even when these compounds were tested in rates equivalent to one-half the recommended herbicidal rates. Figures presented in the afore-mentioned tables showed a general trend of high mortality with the adults being more sensitive than their nymphs. It is noteworthy that the relatively high mortalities among untreated S. mayri adults -which ranged between 16.7 and 25 %- should not by any means devaluate the significance of the results obtained, in view of the known difficulty of maintaining waterboatman in the laboratory (Abou Bakr, 1984 and Tawfik, personal communication).

The effect of the five herbicides on the Chironomus midges noticeably varied. While Ronstar did not cause any mortality among Chironomus larvae in water treated

with 9×10^{-4} ml/L (= R) and only resulted in 10 % mortality after 48 h of exposure to 2R (Table, 5), the field recommended rate of Stomb 330 E caused only 10 % death after 48 hours. But the latter compound, when used in a higher concentration (= 2R), succeeded to kill 10, 30 and 50 % of the treated larvae after 24, 48 and 72 hours, respectively (Table, 7). Going up with the rest of the tested herbicides in an ascending manner, Ordram was more toxic to Chironomus larvae than the two compounds previously discussed (Table, 4). But, on the other hand, the toxicity of Ordram was much lower than that of Machete (Table, 6) and Rifit (Table, 8). The latter two compounds killed 90 and 100 % of the tested midges, respectively, after three days of being exposed to concentrations equivalent to those recommended for the field use. However, Rifit showed much stronger insecticidal power than Machete even when tested at concentrations one-half those of field application. The former compound killed 90 % of the larvae after 72 hours compared to 20 % killed by the latter after the same period.

Data obtained from the present experiments concerning the effect of the tested five herbicides on certain aquatic insect species revealed very interesting results. In addition to Tables (1-8), Table (9) summaries the information accumulated in this part in a qualitative manner to simply describe the situation of each tested insect species, and sometimes their different stages, versus the herbicides under investigations. As it is shown in this table, the tested compounds could be classified according to their mosquitocidal activity into two categories : a) those which did not kill C. pipiens at their field recommended rates, i.e. Ordram, Ronstar and Machete , and b) those which killed mosquito at the recommended rates, i.e. Stomb and Rifit. However, this classification was no longer valid when discussing the impact of the same compounds on the other aquatic insects concerned. For instance, all the given herbicides had no insecticidal action against Mayfly and odonate nymphs subjected to experiments. It is noteworthy that these three species are bottom dwellers in the aquatic habitats. But such an observation is not enough to extrapolate reliable deductions because a fourth, bottom dweller, i.e. Chironomus larvae, seemed to be very sensitive to all tested compounds. On the other hand, two of the major inhabitants of the fresh water ecosystems, i.e. the hemipterous A. sardea and S. mayri were drastically damaged by the herbicides tested.

Although the present results have considerable importance from the view point of environmental pollution and the possible interactions that could take place among the various factors envolved in both natural regulation and applied pest control, these results seem to pose more questions than the answers they may give. Needless to mention that these data need further interdisciplinary efforts of those interested in insect physiology, biochemistry, toxicology and behaviour as well as those concerned with biological control and environmental protection. Such efforts, as it is believed, are necessary for more thorough explanation of why some aquatic insects are harmfully affected by herbicides belonging to the same chemical group and why notonectid and corixid bugs are particularly more susceptible than the others.

The available published literature on the impact of herbicides on the environment does not give adequately helpful means for the understanding of the phenomena recorded during the present work. For instance, the herbicidal action of Ordram which belongs to thiolcarbamates is attributed to its interference with lipid biosynthesis in plants (Corbett, 1974 and Kearney and Kaufmann, 1975). Although Cremlyn (1978) reported insecticidal effects of some thiolcarbamate herbicides and referred

this property to the inhibition of acetylcholinesterase, the lack of insecticidal action of Ordram against mosquito, mayfly, dragonfly and damselfly nymphs and, in contrary, its obvious killing power against backswimmers, water boatmen and chironomid midges are still waiting for explanation.

As for Stomb 330 E, its relatively wider range of insecticidal activity shown herein may find some support in what was stated by Cremlyn (1978) when he discussed the toxicological properties of dinitrophenols. This author mentioned that these compounds and their derivatives may be used as insecticides. He added that they have high mammalian toxicities and they may cause environmental damage. The reports of Corbett (1974) about the contact poisonous effect of the same group and their interference with respiration through uncoupling oxidative phosphorylation with mitochondria may be of great value.

Oxadiazole compounds, to which Ronstar belongs, are still unclassified group (Anon., 1979), and there herbicidal activity may be similar to the carbamate compounds. However, classifying the tested herbicides according to their chemical groups does not seem to be alone a satisfactory basis for explaining the present results; there should be factors belonging to the insects themselves and

the resistance they have previously developed against the tremendous amounts of various pesticides released in their natural environments.

In general, the fact that some herbicides may have direct insecticidal activity finds support in some previous reports albeit on different insects species (Adams, 1960; Walker, 1965; McCraren et al., 1969 and Muller, 1971). However, there are much more reports on the indirect impact of such compounds on insects through the removal of vegetations or through the accumulation of dead plant materials in the aquatic habitats (Walker, 1963; Newman and Way, 1966; Muirhead-Thomson, 1971; Pimentel, 1971 and Frank, 1972).

Table (9) : Insecticidal effect of five herbicides on different stages of Culex pipiens and other associated aquatic insects including a group of mosquito predators. Data are presented in qualitative manner; R indicates to the concentration of herbicide that recommended for weed control; (+) indicates that the compound has insecticidal action while (-) means that it has not.

Tested herbicides	Mosquito					May-fly nymph	Dragon-fly nymph	Damsel-fly nymph	A.sardea		S.mayri		Chiro-nomus
	Eggs	L_2	L_4	P	A				N	A	N	A	L
Ordram	R^-	R^- $2R^-$	R^- $2R^-$	$2R^-$ $8R^-$	≠	R^- $2R^-$	R^- $2R^-$	R^- $2R^-$	$½R^{++}$ R^{++}	R^- $2R^+$	$½R^+$ R^+	$½R^+$ R^+	R^+
Ronstar	R^-	R^- $2R^-$	R^- $2R^-$	$2R^-$ $4R^-$	≠	R^- $2R^-$	R^- $2R^-$	R^- $2R^-$	$½R^+$ R^+	R^- $2R^+$	$½R^+$ R^+	$½R^+$ R^+	R^- $2R^+$
Machete	R^-	R^- $2R^-$	R^- $2R^-$	R^- $2R^-$ $4R^-$	≠	R^- $2R^-$	R^- $2R^-$	R^- $2R^-$	$½R^-$ R^+	R^+	$½R^+$ R^+	$½R^+$ R^+	R^+
Stomb	R^-	$½R^+$ R^+	R^+	R^+	R^+	R^- $2R^-$	R^- $2R^-$	R^- $2R^-$	$½R^+$ R^+	$½R^+$ R^+	$½R^+$ R^+	$½R^+$ R^+	R^+
Rifit	R^-	$½R^+$ R^+	$½R^+$ R^+	R^+	R^+	R^- $2R^-$	R^- $2R^-$	R^- $2R^-$	$½R^-$ R^+	R^+	$½R^+$ R^+	$½R^+$ R^+	R^+

≠ Adult not tested

4.3. Susceptibility of C. pipiens Larvae to the Infective Juveniles of the Entomogenous Nematode Neoaplectana carpocapsae :

The growing interest for using the entomogenous nematode Neoaplectana carpocapsae as a biological control tool for the suppression of several insect pests has attracted the attention of entomologists who are concerned with mosquito control. Some preliminary laboratory tests against various mosquito species have showed encouraging results (Poinar and Leutenegger, 1968; and Abou-Bakr and El-Kifl, unpublished data). Accordingly, second and fourth-instar larvae of laboratory-reared C. pipiens were subjected to bioassay experiments against different levels of N. carpocapsae infective stage suspended in distilled water. Data presented on Table (10) summarize the results obtained when L_2 were exposed to 10 concentrations of the dauer stage juveniles for successive 5 days. Mortality among treated larvae did not exceed 10 % even when very high rates were used. Although the parasitic nematodes could be detected inside the dead larvae, the nematode did not succeed to develop in the cadavers of L_2; subsequently, no progeny of N. carpocapsae could be produced. On the other hand, 4th-instar larvae of C. pipiens were apparently more susceptible to infection with N.carpocapsae

Table (10) : Mortality (%) among *C. pipiens* L_2 after exposure to different levels of infective stage of the nematode *N. carpocapsae*.

Dauer stage/ 100 ml	Mortality %				
	Days after exposure				
	1	2	3	4	5
1,000	0	0	4	4	4
2,000	0	2	4	4	4
4,000	4	6	6	8	8
6,000	4	4	6	6	6
8,000	0	8	8	8	8
10,000	0	6	8	8	8
20,000	0	2	2	4	4
40,000	0	0	4	10	10
60,000	2	4	6	8	10
80,000	0	2	4	4	8
Control	0	0	0	0	0

Table (11) : Corrected mortality (%), among L_4 C. pipiens exposed to different levels of infective stage of the nematode N. carpocapsae for successive 6 days in the laboratory.

Dauer stage/ 100 ml	Mortality %					
	Days after exposure					
	1	2	3	4	5	6
1,000	0.0	0.0	0.0	0.0	0.0	0.0
2,000	0.0	0.0	4.1	10.1	10.1	10.1
4,000	0.0	2.1	10.3	16.3	18.3	34.3
6,000	0.0	2.1	18.4	32.4	46.7	46.7
8,000	6.0	10.2	32.6	32.6	42.8	48.8
10,000	4.0	12.3	42.9	44.9	46.9	50.9
20,000	0.0	20.8	42.3	50.3	52.3	54.3
40,000	6.0	31.0	57.5	61.5	69.7	77.7

dauer stage than L_2. Mortality ranged between 0 % at a level of 1,000 dauer stage (d.s.)/100 ml water and 69.7 % when 40,000 d.s./100 ml water were used for five days. This means that rising the concentration to 40 fold resulted in mortality among L_4 7 times greater than that recorded in L_2 using the same rate for similar period of time. Figures in the same table revealed that mortality increased from 34.3 to 77.7 % when numbers of d.s./100 ml water increased from 4,000 to 40,000 d.s., respectively. However, data shown in this table indicate that doubling the rate of d.s. results in slight increase in mortality among the exposed larvae. In spite of this relatively high percentage of parasitism among L_4 C. pipiens, no migrating progeny were obtained when the dead cadavers were placed in extraction chambers.

The failure of N. carpocapsae to parasitize 2nd-instar larvae of C. pipiens could be explained in view of the small size of the larval body at this developmental stage of the insect which is not big enough to harbour the parasite. The present observation contradicts the reports of Peterson and Willis (1970) concerning the infection og mosquito larvae by Romanomermis culicivorax. However, it confirms the reports of Dadd (1971) who stated that 1st and 2nd instar larvae of C. pipiens were unable to engulf

N. carpocapsae juveniles which were too large to negotiate the insect mouth parts. He added that of the many hundreds of nematodes that were ingested within 24 hours, only a few established themselves in haemocoel.

Meanwhile, the failure of N. carpocapsae to develop and propagate in the cadavers of the infected 4th-instar larvae indicates that although C. pipiens mature larvae are susceptible to the invasion by the infective juveniles of this entomogenous nematode, and although N. carpocapsae discharges the associated bacteria which is mainly responsible of the rapid kill of the larvae (Poinar and Thomas, 1966 and 1967), C. pipiens larvae seem to be unsuitable host for the development and propagation of N. carpocapsae. Such an observation, which was previously reported by Poinar and Leutenegger (1971), may have important implications if it is decided to use N. carpocapsae in the biological control of mosquito. However, further investigations are still needed to thoroughly clarify this point.

4.4. Effect of N. carpocapsae on certain mosquito predators and other associated aquatic insects :

One of the most important characteristics of a biological control agent is its specificity against the target

insect(s) and, subsequently, its safety to the non-target organisms and the other components of the given ecosystem. In view of this fact, it was planned to examine the infectivity of N. carpocapsae to a group of aquatic insects which oftenly coexist with C. pipiens in many mosquito breeding sites. As it was previously mentioned (c.f. p. 21) some of these aquatic insects are mosquito-predators. Water suspensions containing different rates of dauer stage juveniles of N. carpocapsae, ranged between 1,000 and 80,000 d.s./100 ml dist. water were bioassayed against nymphs of C. erythraea, I. senegalensis (Table, 12), nymphs of S. mayri and adults of A. sardea (Table, 13), nymphs of Polymetarsus sp. (Table, 14) and larvae of Chironomus sp. (Table, 15). Tested insects were exposed to the entomogenous nematode for five days in all cases except Mayfly nymphs (4 days) and Chironomus larvae (7 days).

No parasitism was observed among nymphs of either C. erythraea or I. senegalensis when they were placed in water containing 1,000 d.s./100 dist. water for 5 successive days. However, at a level of 4,000 d.s./100 ml 10 % and 20 % death were recorded among the nymphs of the respective two odonates. At higher densities of the entomo-parasitic nematode (e.g. 8,000 and 80,000 d.s./ 100 ml water) approximately similar mortalities were

Table (12) : Corrected mortality (%) among 3rd instar nymphs of C. erythraea and I. senegalensis after exposure to different levels of the infective juveniles of N. carpocapsae.

Dauer stage/ 100 ml	Days after exposure									
	C. erythraea					I. senegalensis				
	1	2	3	4	5	1	2	3	4	5
1,000	0	0	0	0	0	0	0	0	0	0
4,000	0	0	10.0	10.0	10.0	10.0	10.0	20.0	20.0	20.0
8,000	0	0	0	10.0	20.0	20.0	22.2	22.2	22.2	22.2
20,000	10	10	21.1	21.1	21.1	*	*	*	*	*
40,000	0	0	11.1	11.1	21.1	*	*	*	*	*
60,000	*	*	*	*	*	10.0	10.0	10.0	20.0	30.0
80,000	0	0	10.0	10.0	30.0	10.0	10.0	10.0	30.0	30.0

N.B. * = not tested

Table (13) : Corrected mortality (%) among 3rd instar nymphs of Sigara mayri and adults of Anisops sardea after exposure to different levels of infective stage of N. carpocapsae.

Dauer stage/ 100 ml	% Mortality/ days after exposure									
	S. mayri nymphs					A. sardea adults				
	1	2	3	4	5	1	2	3	4	5
1,000	0	20.0	20.0	70.0	70.0	0	0	0	0	0
4,000	0	30.0	30.0	60.0	60.0	0	0	0	0	0
8,000	0	75.0	75.0	75.0	75.0	0	0	0	0	0
10,000	12.5	32.5	43.6	65.8	65.8	0	0	0	0	0
20,000	12.5	62.5	62.5	62.5	62.5	0	0	0	0	0
40,000	0	55.6	68.1	68.1	68.1	0	0	0	0	0
80,000	37.5	37.5	58.6	69.7	69.7	0	0	0	0	0

Table (14)): Mortality (%) among 3rd nymphs of Mayfly *Polymetarsus* sp. after exposure to different levels of infective stage of *N. carpocapsae*.

Dauer stage/ 100 ml	Mortality %			
	Days after exposure			
	1	2	3	4
1,000	0	40	70	70
4,000	0	40	60	80
8,000	0	20	60	90
10,000	0	30	70	100
40,000	10	70	90	100
Control	0	0	0	0

Table (15) : Mortality (%) among Chironomus sp. L_3 after exposure to different levels of infective stage of N. carpocapsae.

Dauer stage/ 100 ml	Mortality %						
	Days after exposure						
	1	2	3	4	5	6	7
1,000	0	0	10	10	20	35	35
2,000	0	0	30	40	60	80	80
4,000	0	20	35	45	65	70	90
6,000	0	10	50	80	90	90	100
8,000	15	25	50	80	80	85	90
10,000	5	15	45	65	85	95	100
20,000	0	15	55	80	80	90	90
60,000	0	60	85	90	95	100	
80,000	25	55	85	90	100		
Control	0	0	0	0	0	0	0

were recorded. However, mortality did not exceed 30 % at 80,000 d.s./100 ml water in the two concerned insects (Table, 12).

Nymphs of the corixid water boatman S. mayri seemed to be very susceptible to the invasion by the juveniles of N. carpocapsae even at the least rate used (Table, 13). Mortalities ranged between 60 and 75 % among the seven concentrations tested. However, increasing the nematode density above 8,000 d.s./100 ml water did not result in an increase of mortality on the 5th day of exposure. Death started to occur on the 1st and 2nd day of exposure. Dead boatman nymphs were dissected and the infective individuals of N. carpocapsae were detected in all cases indicating to 100 % parasitism among dead insects. On contrary, applying the same concentrations of the entomogenous nematode on the adults of the notonectid bug A. sardea resulted in no mortality even when the highest density (80,000 d.s./100 ml) was used (Table, 13); a result indicates that N. carpocapsae is safe to the mosquito-predator backswimmer A. sardea.

On the other hand, results shown in Tables (14) and (15) point to high susceptibility of both the nymphs of the Ephemerid Polymetarsus sp. and the larvae of Chironomus sp. to the invasion by N. carpocapsae. Mortality among

Mayfly nymphs ranged between 70 and 100 % when exposed to 1,000-10,000 d.s./100 ml water for 4 days (Table, 14) with death records starting on the 2nd day after treatment.

Larvae of Chironomus sp. showed similar susceptibility to the nematode infection, with progressive mortality records somewhat slower than that observed in the case of Mayfly. Nevertheless, a density of 4,000 d.s./100 ml caused 90 % mortality among Chironomus midges on the 7th day of treatment. Gradual higher densities caused mortalities ranged between 90 and 100 % (Table, 15).

Reviewing the data in Tables (12, 13, 14 and 15) indicates that the backswimmer A. sardea was the only tested insect which showed obvious resistance against N. carpocapsae infection. The other tested species showed different degrees of susceptibility. The most susceptible was Mayfly nymphs followed by Chironomus larvae, and nymphs of S. mayri, then come nymphs of dragonfly and damselfly. It is surprising to note that all the susceptible tested species have common ecological trait; all of them are bottom inhabitants occupying the bed of the water breeding sites. Such an observation, among other things, dictated the examination of the distribution of N. carpocapsae in the water after being sprayed on the water surface in a graduated cylinder with 30 cm deep.

Results of this experiment are presented in Table (16). According to this table, dauer stage juveniles of N. carpocapsae settle rapidly through the water column after being sprayed on the surface. During only 50 minutes samples taken from the water surface were free of nematodes while all individuals have already settled at the bottom.

Indeed, nothing is available in the published literature about the movement and distribution of N. carpocapsae when its infective juveniles are applied to water bodies. However, there are few articles dealing with the movements and migration of N. carpocapsae in the soil (Reed and Crane, 1967; Poinar, 1979; El-Kifl and El-Sherif, 1986; and Shroeder and Beavers, 1987).

Discussion of the results of this part reveals that although N. carpocapsae can be quite effective against the 4th-instar larvae of C. pipiens, its infectivity towards the associated aquatic insects may hamper its use in mosquito biological control. Although the fact that N. carpocapsae may attack insects from several orders has been mentioned by Poinar (1979) no previous reports on the effect of this entomogenous nematode on aquatic insects are available. The only related reports were exclusively limited to the mermithid nematode, Romanomermis culicivorax (Ignoffo et al., 1973 and Otieno,

1977). Hence, the present work may represent the first attempt mental impact of this biological control agent on the non-target insects. Thus, more caution must be excercised if such benificial insects are involved, especially, in the presence of predaceous aquatic insects which play an important role in the natural suppression of mosquito populations in their habitats.

Moreover, differences in the susceptibility of the various tested aquatic insects to the infection of N. carpocapsae need some explanations. The fact that infective juveniles of N. carpocapsae settled rapidly in the water and accumulate at the bottom may explain why bottom-inhabiting insects were more susceptible to the nematode infection than those exist mainly near the surface of the water body including mosquito larvae. Taking the concentration of 40,000 d.s./100 ml water as one example; it caused 10 % and 18.3 % mortality among 2nd and 4th instar larvae of C. pipiens, respectively on the 5th day. The same concentration killed 21.1 %, 68.1 % of C. erythraea and S. mayri nymphs, respectively. Meanwhile, the same concentration resulted in 100 % mortality among Mayfly nymphs on the 4th day. Much less concentration, e.g. 6,000 d.s./100 ml water caused total kill among Chironomus larvae after one week. On the other hand, very high

concentration, i.e. 80,000 d.s./100 ml water did not result any mortality among adults of the backswimmer A. sardea. The comparison between these figures becomes much clearer in view of the position which is occupied in the water column by each of the tested insects. While A. sardea and C. pipiens tend to remain beneath the water surface the rest of the insects water under investigation are mainly bottom dwellers. This becomes more obvious when comparing between the susceptibility of two related hemipterous water bugs, i.e. A. sardea and S. mayri.

Although such an explanation may find support in the statements of Woodard and Fukuda (1977) that most insects avoid or escape nematode parasitism by their normal habits or behaviour, it is addmitted that this is not the only possible explanation. For instance, host escape, host age, cellular responses (Poinar, 1979), humoral responses (Cotz, 1969), normal habits or behaviour, the structure of the cuticle and conditions inside the digestive tracts (Woodard and Fukuda, 1977) could be of considerable value.

Table (16) : Settling rates of the dauer stage juvenile of *N. carpocapsae* during 60 minutes after being sprayed on the water surface (depth : 30 cm).

Sample/time	% of d.s. found	
	Surface	Bottom
Zero	99.90	0.10
5 min.	46.00	54.00
10 min.	26.53	73.47
20 min.	12.00	88.00
30 min.	11.00	89.00
40 min.	5.00	95.00
50 min.	0.00	100.00
60 min.	0.00	100.00

4.5. Effect of the tested 5 herbicides on the infectivity of *N. carpocapsae* :

Having examined the effect of the herbicides Ordram, Ronstar, Machete, Stomb and Rifit on both mosquito and some mosquito predators as well as certain mosquito-associated insects, and having in turn tested the infectivity of the entomogenous nematode *N. carpocapsae* on both mosquito and their associates, now it is the time for entering the final round. In the following few pages the effect of the five herbicides under investigation on the infectivity of *N. carpocapsae* is examined. Infective juveniles of *N. carpocapsae* were mixed with each herbicide in water dilutions equivalent to the field application rates. Juvenile nematodes were kept in the herbicidal solutions for 48 hours, then washed several times with distilled water, sieved and adjusted in water suspensions to get a nematode suspension of ca. 40,000 d.s./100 ml dist. water. The infectivity of *N. carpocapsae* was measured by bioassay against L_4 *Culex pipiens* for successive six days at 25°C. Similar untreated nematode water suspension served as control while equal numbers of L_4 *C. pipiens* in dist. water served as blank control. Data obtained from this experiment are summarized on Table (18). Mortality % recorded on the 6th day among mosquito 4th

Table (18) : Mortality % among L_4 C. pipiens exposed for 6 successive days to infective juveniles of N. carpocapsae (40,000 d.s./100 ml) previously kept for 48 hours in 5 herbicidal solutions at R concentrations.

Herbicides	Mortality %					
	Days after treatment					
	1	2	3	4	5	6
Ordram treated nematode	14	60	74	82	86	86
Ronstar treated nematode	20	64	80	84	86	86
Machete treated nematode	16	74	88	88	90	90
Stomb 330E treated nematode	20	54	72	76	78	78
Rifit 550 EC treated nematode	4	30	38	46	48	52
Untreated nematode+	32	54	66	76	82	84
Control++	0	0	0	0	0	0

+ Nematode + mosquito larvae without herbicide

++ Mosquito larvae + dist. water

instar larvae exposed to Ordram-, Ronstar-, Stomb-, and Rifit-treated nematodes were 86, 86, 90, 78 and 52 %; respectively. Untreated infective juveniles caused 84 % mortality among L_4, while there was no mortality among untreated control larvae. Statistical analysis proved that there were no significant differences between treatments in case of Ordram, Ronstar, Machete and nematode in dist. water. However, the differences between Stomb, Rifit and the other treatments were significant. Results of this experiments indicate that Ordram, Ronstar, Machete did not interfere with the viability of N. carpocapsae when the juveniles were placed in their herbicidal solutions for 48 hours, which means these three herbicides could be compatible with this entomogenous nematode. On the other hand, the presence of Stomb and Rifit negatively affected of N. carpocapsae in noticeable manner.

Since our previous experiments showed the unsuitability of L_4 of C. pipiens for the development and reproduction of N. carpocapsae, another experiment was carried out to examine the effect of the concerned 5 herbicides on the reproductivity of the nematode as well as the infectivity of the produced progeny. Infective juveniles were exposed to the herbicidal dilutions for 8 days. Then juveniles were washed and mixed in aqueous suspension

with soil to which full grown larvae of Spodoptera littoralis were added. Two days later complete infection could be detected among all the treated caterpillars. Moreover, successful extraction of new progeny could be achieved. When the new generation infective individuals were tested again against S. littoralis they could infect the larvae and reproduced inside their cadavers .

Although nothing is available in the published literature concerning the effect of herbicides on the entomogenous nematodes, the detrimental action of some herbicides on certain plant-parasitic nematodes is fairly known. However, very few reports on the interaction between insecticides and the entomogenous nematode N. carpocapsae point to the lethality of organophosphates and carbamates to the infective juveniles (Prakasa et al., 1975; Fedorko et al., 1977 a,b; Kamionek, 1979 and Hara and Kaya, 1982). Other reports concluded that N. carpocapsae could be compatible with ten insecticides commonly used in rice culture (Rao et al., 1975).