

CONTENTS

Subject	Page
INTRODUCTION.	1
REVIEW OF LITTERATURE.	4
1- Parasitoids associated with the citrus leafminer population.	4
1-1- <i>Cirrospilus</i> species.	4
1-2- <i>Pnigalio</i> species.	18
1-3- <i>Citrostichus phyllocnistoides</i> Narayanan.	25
1-4- <i>Sympiesis</i> sp. .	29
1-5- <i>Baryscapus</i> sp..	31
2- Predators associated with the citrus leafminer population.	33
2-1- <i>Chrysoperla</i> species.	33
2-2- <i>Orius</i> species.	36
2-3- Other predators.	37
3- Entomopathogens associated with the citrus leafminer population.	39
3-1- Bacterial species.	39
3-2- Fungal species.	41
MAREIALS AND METHODS.	44
1- Ecological studies.	44
1-1- Sampling program.	44
1-2- The role of natural enemies on CLM population.	45
1-3- Effect of temperature and relative humidity on CLM natural enemies.	46
1-4- Searching rate and mutual interference of <i>Chrysoperla carnea</i> Steph. and <i>Orius</i> sp.	46
2- Evaluation of CLM entomopathogens.	47
2-1- Isolation and identification of microorganisms.	47
2-2- Pathogenicity and toxic effect of microbial isolates to CLM.	48
3- Biological studies on CLM parasitoids (<i>Cirrospilus ingenuus</i> Gahan and <i>Pnigalio</i> sp.).	50
3-1- Rearing techniques.	50
3-2- Parasitoids life and fecundity table studies.	51
3-3- CLM life table.	52

RESULTS AND DISCUSSION.	54
I- Ecological studies on natural enemies associated with <i>P. citrella</i> .	54
1- CLM parasitoids.	54
1-1- <i>C. ingenuus</i> .	56
1-2- <i>C. pictus</i> .	66
1-3- <i>Pnigalio</i> sp..	76
1-4- <i>C. phyllocnistoides</i> .	86
1-5- <i>Sympiesis</i> sp..	96
2- Predators.	111
2-1- Seasonal activity of predators on CLM population.	111
2-2- Synchronization between CLM population and predators' activity.	113
2-3- Effect of mean temperature and relative humidity on predators' activity.	113
2-4- The searching rate and mutual interference values of <i>C. carnea</i> and <i>Orius</i> sp..	115
3- CLM pathogens.	120
3-1- Seasonal activity of entomopathogens on CLM population.	120
3-2- Synchronization between host population and entomopathogens' activity.	123
3-3- Effect of mean temperature and R.H. % entomopathogenic activity.	123
3-4- The common effect of host density and weather factors (T & R.H.%) on entomopathogens' activity.	125
4-Efficiency of the different mortality factors on CLM population.	126
II- Pathogenicity and toxic effect of microbial isolates to CLM.	132
1- Pathogenicity of bacterial and fungal isolates.	132
2- Toxic effect of the bacterial and fungal suspension on CLM populations under field conditions.	133
III- Biological studies on CLM parasitoids.	136
1- Life table of the parasitoids, <i>C. ingenuus</i> and <i>Pnigalio</i> sp..	136
2- Egg laying behavior of the parasitoids.	139
3- The age-specific fecundity tables of the parasitoids (<i>C.ingenuus</i> and <i>Pnigalio</i> sp.) and their CLM host.	140
SUMMARY.	146
CONCLUSION.	155
REFERENCES.	156
ARBIC SUMMARY.	

Summary

The present work was carried out in the Experimental orchard at Mansoura University, Faculty of Agriculture during two successive seasons (2000/01 and 2001/02).

The aims of this study were to survey and evaluate the role of parasitoids, predators and pathogens associated with the citrus leafminer, *Phyllocnistis citrella*, to add some knowledge concerning the population fluctuation and activity of these natural enemies; also to investigate the role of different mortality factors on the total pest population mortality. In addition to, evaluate some biological characteristics of the main parasitoids.

The obtained results could be summarized as follow :

I- Ecological studies :

1- CLM parasitoids :

Six aphelinid parasitoids namely, *Cirrospilus ingenuus*, *C. pictus*, *Pnigalio* sp., *Citrostichus phyllocnistoides*, *Sympiesis* sp. and *Baryscapus* sp. were recorded associated with the CLM immature stages (larvae and pupae), while, no parasitoids on CLM eggs were recorded during the both years of study (2000/01 and 2001/02). The ectoparasitoid, *C. ingenuus* was the most dominant parasitoid, while *Baryscapus* sp. was rarely.

1-1- *C. ingenuus* :

In the first year, larval, pupal and the total number of immature stages (larvae and pupae) had 5-6 peaks of abundance, while in the second year they had 6-7 peaks.

The highest abundance of the parasitoid population was coincided with the highest occurrence of the host, especially during the second year of study. Statistical analysis also indicated that there was a positive highly significant correlation between the parasitoids and its host populations during the first and second years (r-values were 0.592** and 0.628**).

The parasitoid population was positively correlated with the increase of temperature (r-values were 0.179^{ns} and 0.512**), while it exhibited negative response to the increase of relative humidity (r-values were -0.016^{ns} and -0.176^{ns}) during the two years of study.

The parasitism percentage estimated as the percentage of parasitized hosts was always higher than the calculated percentage as percentage of emerged parasitoid adults.

The relationship between parasitoid activity (Par. %) and each of host density (Nh) and the two weather factors (T & R.H. %) can be represented by the following equations :

$$\text{Par. \%} = -0.08 + 0.15\text{Nh} - 1.41\text{T} + 0.66\text{RH\%} \quad (\text{in } 1^{\text{st}} \text{ year})$$

$$\text{Par. \%} = 66.20 - 0.05\text{Nh} + 0.56\text{T} - 0.73\text{RH\%} \quad (\text{in } 2^{\text{nd}} \text{ year})$$

1-2- *C. pictus* :

In the first year, each of larval, pupal and the total number of immature stages had 6-7 peaks of abundance, while in the second year they had 7-8 peaks.

The obtained results showed that changes of the parasitoid population coincided with the changes of host population in both years ($r = 0.749^{**}$ and 0.533^{**}).

In respect to weather factors, the parasitoid population exhibited positive ($r = 0.527^{**}$ and 0.161^{ns}) and negative ($r = -0.288^*$ and -0.006^{ns}) response to increase of temperature and relative humidity in the first and second years.

The parasitism percentage calculated as the percentage of emerged parasitoid adults to the total host gave inaccurate value of the parasitoid activity.

The relationship between parasitoid activity (Par. %) and each of host density (Nh) and the two weather factors (T & R.H. %) can be represented by the following equations :

$$\text{Par. \%} = 44.01 + 0.03\text{Nh} - 0.30\text{T} - 0.36\text{RH\%} \quad (\text{in } 1^{\text{st}} \text{ year})$$

$$\text{Par. \%} = 40.43 - 0.01\text{Nh} - 0.26\text{T} - 0.31\text{RH\%} \quad (\text{in } 2^{\text{nd}} \text{ year})$$

1-3- *Pnigalio* sp. :

Larval and pupal populations had 5-6 peaks of abundance in the two years of study. Also, the total number of immature stages (larvae and pupae) had six to five peaks.

There is a positive correlation between the parasitoid and its host populations ($r = 0.476^{**}$ and 0.383^*) during the first and second year.

Pnigalio sp. population showed highly significant positive correlation ($r = 0.467^{**}$ and 0.497^{**}) with the increase of temperature, while it exhibited negative response with the increase of relative humidity through both years of study ($r = -0.328^*$ and -0.123^{ns}).

Parasitism percentages calculated as the percentage of parasitized hosts gave accurate value of parasitoids activity, while the percentage of emerged adults gave inaccurate value.

Multi regression analysis indicated that the relationship between parasitoid activity (Par. %) and each of host density (Nh) and the two weather factors (T & R.H. %) can be represented by the following equations :

$$\text{Par. \%} = 44.98 - 0.14\text{Nh} + 0.43\text{T} - 0.36\text{RH\%} \quad (\text{in } 1^{\text{st}} \text{ year})$$

$$\text{Par. \%} = 35.71 - 0.07\text{Nh} + 0.47\text{T} - 0.31\text{RH\%} \quad (\text{in } 2^{\text{nd}} \text{ year})$$

1-4- *C. phyllocnistoides* :

The parasitoid was recorded for the first time in Egypt. Seasonal abundance of the larval stage of *C. phyllocnistoides* recorded 5-6 peaks of abundance in both years. While, pupal stage and the total number of the both larvae and pupae exhibited three and seven peaks in the first and second years. In respect to effect of host density on parasitoid population, *C. phyllocnistoides* as anew species; statistical analysis indicated that there was an insignificantly negative correlation in the first year (-0.067^{ns}), while in the second year was highly significant positive correlation (0.684^{**}).

The response of the parasitoid populations to the increase of temperature varied from year to year. The correlation values were (-0.064^{ns} and 0.385^{*}) in the first and second years. While, *C. phyllocnistoides* populations exhibited negative response to the increase of relative humidity ($r = -0.013^{\text{ns}}$ and -0.197^{ns}) during both years of study.

In respect to the efficiency of the parasitoid on CLM population, there were highly significant correlations between the host and percentage of

parasitism. On the other hand, the percentage of parasitism as the percentage of parasitized host gave an accurate value for parasitoid activity.

The relationship between parasitoid activity (Par. %) and each of host density (Nh) and the two weather factors (T & R.H. %) can be represented by the following equations :

$$\text{Par. \%} = 27.42 - 0.10\text{Nh} - 0.17\text{T} + 0.06\text{RH\%} \quad (\text{in } 1^{\text{st}} \text{ year})$$

$$\text{Par. \%} = -2.01 + 0.01\text{Nh} - 0.20\text{T} + 0.30\text{RH\%} \quad (\text{in } 2^{\text{nd}} \text{ year})$$

1-5- *Sympiesis* sp. :

Seasonal abundance of *Sympiesis* sp. larvae, pupae and their total number showed 4-5, 3-4 and 3-5 peaks of abundance, respectively during the first and second year of study.

Changes of the parasitoid population did not coincide with the fluctuation of CLM population; correlation analysis indicated that there were insignificant correlations between the parasitoid and its host populations during the first and second year (r-values were -0.023^{ns} and 0.073^{ns}).

The parasitoid population was significant negatively (-0.285^* and -0.388^*) and positively (0.206^{ns} and 0.083^{ns}) correlated with the increase of temperature, while it exhibited positive response to the increase of relative humidity during both years.

Parasitoid activity (parasitism %) did not coincide with the host population.

In respect to the common effect of host density and weather factors (T & R.H.%) on parasitoid density, the relationship can be represented by the following equations :

Par. % = 51.38 + 0.03Nh - 1.70T - 0.13RH% (in 1st year)

Par. % = 15.65 + 0.04Nh - 1.69T + 0.37RH% (in 2nd year)

2- Predators :

During the course of this study, *C. carnea* and *Orius* sp. were as the important predators observed associated with CLM immature stages on the newly citrus infested leaves under field conditions.

The activity of predators on CLM population was low as the percentage of predatism was 3.41±2.60 and 3.69±4.26% in the first and second year. The highest activity had been recorded in April during the two years of study.

No synchronization between CLM population and percentage of predatism in both years as statistical analysis showed that correlation coefficients were $r = -0.049^{ns}$ and $r = -0.231^{ns}$ in the first and second years.

The percentage of predatism increased as the degree of temperature increased ($r = 0.153^{ns}$ and 0.032^{ns}) and the percent of relative humidity decreased ($r = -0.285^*$ and -0.522^{**}).

C. carnea larvae exhibited relatively higher searching rate than the adults of *Orius* sp. on the four host plants (navel orange, sour orange, lime and mandarin). In addition to, *C. carnea* showed relatively higher mutual interference values than *Orius* sp. on the different host plant species.

The highest searching rate had been achieved on navel orange, while the lowest rate on mandarin in the case of the two predators.

In respect to mutual interference values, the lowest interference value was obtained on lime and mandarin; while, the highest on navel orange.

3- CLM pathogens :

Two entomopathogenic bacteria (*Bacillus* sp. and *Pseudomonas* sp.) and two fungi (*Aspergillus flavus* Link and *Penicillium* sp.) were isolated from naturally infected *P. citrella* larvae and pupae.

The percentage of diseased insects were relatively low (5.75 ± 5.26 and 5.04 ± 4.32 %) and the highest activity occurred in January in the two years of study.

There are highly negative synchronizations between CLM population and percentage of diseased insects in both years of study.

The percentage of diseased insects was increased as the degree of temperature decreased, while, the fluctuation of diseased insects was coincided with the changes of relative humidity in the both years.

4- Efficiency of different mortality factors on CLM population :

The combined parasitoids were the main mortality factor and contributed with the highest fate of the total mortality of CLM population. The average percentage of parasitization through the whole year was 33.29 ± 7.84 and 40.46 ± 7.47 % during the first and second year.

Entomopathogens occupied the second order of activity on CLM population. The average percentage of pathogens through the whole year was 5.69 ± 5.13 and 5.04 ± 4.32 % in the first and second year.

Mortality caused by predators represented by the lowest rate of contribution to the total mortality. The average percentage of predatism through the whole year was 3.41 ± 2.60 and 3.69 ± 4.26 % in the first and second year.

The relation between the different mortality factors {parasitism (Par.), predatism (Pre.), pathogenic (Path.) and unknown mortality (UK.)} and total population mortality (TM) could be represented by the following equations :

$$\text{TM\%} = 1.77 + 0.99\text{Par.} + 1.00\text{Pre.} + 1.00\text{Path.} + 1.00\text{UK. (in 1}^{\text{st}} \text{ year)}$$

$$\text{TM\%} = 6.45 + 0.86\text{Par.} + 0.75\text{Pre.} + 0.86\text{Path.} + 1.09\text{UK. (in 2}^{\text{nd}} \text{ year)}$$

II- pathogenicity and toxic effect of microbial isolates to CLM :

The pathogenicity of microbial pathogens was evaluated under laboratory conditions. *Bacillus* sp. was significantly more effective than other isolated pathogens (*Pseudomonas* sp., *A. flavus* and *Penicillium* sp., respectively).

The bacterial pathogens (especially *Bacillus* sp.) exhibited higher toxic activity against *P. citrella* population in comparison with pathogenic fungi under field conditions. The percentage of mortality cause by each pathogen increased as its concentration increase.

III- Biological studies :

1- Life table of the parasitoids, *C. ingenuus* and *Pnigalio* sp. :

The total developmental time was 9.48 days and the mortality (Qx) was highest among all stages, it was 0.299, 0.230 and 0.216 for larval, egg and pupal stages, respectively (for *C. ingenuus*). While, the total developmental time was 8.35 days and the mortality (Qx) was highest among egg stage (0.293) followed

by the pupal stage (0.288) while the larval mortality was the lowest (0.278) (for *Pnigalio* sp.).

Pre-oviposition, oviposition and post-oviposition periods of *C. ingenuus* and *Pnigalio* sp. lasted 1.80 ± 0.92 and 1.20 ± 0.79 , 8.90 ± 0.88 and 5.60 ± 0.84 and 1.90 ± 0.74 and 1.50 ± 0.53 days, respectively. Life span of parasitoid female and male was 22.08 and 16.38 (for *C. ingenuus*) and 16.65 and 13.75 days (for *Pnigalio* sp.).

2- Egg laying behavior of the parasitoids :

The average number of deposited eggs was 30.90 ± 3.81 per *C. ingenuus* female, while it was 52.30 ± 13.13 per *Pnigalio* sp. female.

3- The age-specific fecundity of the parasitoids, *C. ingenuus* and *Pnigalio* sp.:

C. ingenuus have one and quarter generation to one of the host, while, *Pnigalio* sp. have one and half generation to one of the host. The parasitoid, *C. ingenuus* can potentially produce 29 female progeny during the period of one generation of the host, while *Pnigalio* sp. produce 149 female progeny during the period of one generation of the host.

CONCLUSION

- 1) The ectoparasitoids, *C. ingenuus* and *Pnigalio* sp. appeared to be the main mortality factor on *P. citrella* populations. So, it could be used in IPM program for this pest.
- 2) The entomopathogenic bacterium *Bacillus* sp. promise to be a good biological control agent against the tested pest (CLM).
- 3) The host plant had an effective role on the predators (*C. carnea* and *Orius* sp.) searching characteristics.
- 4) Estimation of parasitoid percentage as parasitized hosts % gave an accurate value to CLM parasitoid activity.
- 5) For the success of IPM program it is essential to know several information concerning the biology of the pest and their natural enemies such as net reproductive rate (R_0), mean generation time (T) and the intrinsic rate of natural increase (r_m).