EVALUATION THE ROLE OF DIFFERENT MORTALITY FACTORS ON *CEROPLASTES FLORIDENSIS* COMSTOCK POPULATION IN CITRUS ORCHARDS AT MANSOURA DISTRICT, DAKAHLIA GOVERNORATE, EGYPT.

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Abstract

Two hymenopterous parasitoids were found associated with the Florida wax scale (FWS), *Ceroplastes floridensis* Comstock on navel orange and mandarin trees namely *Microterys flavus* (How.) and *Tetrastichus ceroplastae* (Gir.) during the two years of study (2001/2002 and 2002/2003). Two peaks of parasitoids activity (parasitism %) were recorded yearly. These peaks were recorded on the 7th of April (34.9%) and 14th July (13.5%) during the first year, while in the second year were on the 20th of April (37.3%) and 27th July (8.1%). The parasitoids exhibited the highest activity during spring season especially on orange trees.

Six insect predators were found associated with FWS on the infested leaves. They were *Chilocorus bipustulatus* L., *Rodalia cardinalis* Muls., *Coccinella undecimpunctata* L., *Cydonia vicina isis* Gr., *Paederus alferii* Koch. and *Chrysoperla carnea* Steph. . The activity of predators on FWS population was obviously low as the percentage of predatism which was 1.5±1.7 and 1.2±1.3% during the first and second year.

Two species of entomopathogenic fungi, namely *Fusarium* sp. and *Cladosporium* sp. were isolated from FWS for the first time in Egypt. The percentage of diseased scales was 7.0±2.3 and 6.5±1.8% all over the first and second year of investigation.

Parasitism contributed with relatively high percentage to the total population mortality, in comparison with diseased or predaceous scales factors, during the two years of study. Diseased scales followed parasitism in contribution of the total mortality, while, predatism represented the lowest contribution mortality factor on *C. floridensis*.

INTRODUCTION

The Florida wax scale (FWS), *Ceroplastes floridensis* Comstock (Homoptera, Coccidae) is widely distributed in tropical and subtropical regions around the world (Ashmead, 1980). It attacks a great number of host plants especially *Citrus* spp.. Its main damage is due to the copious production of honey dew (especially in the large populations) which serves as a substrate for various sooty mould fungi (Podoler *et al.*, 1981).

According to Smith (1986), the application of insecticides is frequently followed by recurrent infestations. In spite of a wide spread application of insecticides which used to control this pest in Egypt, it still cause considerable damage to its plant hosts.

Therefore, during the past decade, intensive efforts have been done in Egypt to improve the biological control and to devise an integrated control program for citrus pests.

In order to achieve a successful biological control in any area, searching for the well established and most efficient biological agents should be involved (Hendawy, 1999). So, the present study aimed to evaluate the role of the natural enemies (parasitoids, predators and entomopathogens) which associated with FWS populations and compare their roles to determine the most effective factor on FWS population in citrus orchards at Mansoura district, Dakahlia governorate.

MATERIALS AND METHODS

Studying the role of insect natural enemies (parasitoids and predators) and pathogens of Florida wax scale (FWS), *Ceroplastes floridensis* Comstock population on navel orange orchards were carried out during two successive years (2001/2002 and 2002/2003) in the Experimental Farm of the Faculty of Agriculture, Mansoura University.

1-Sampling program :-

Five navel orange trees, *Citrus sinensis* (Osbeck) (infested with FWS) homogenous in size and age were selected and marked for the present study. Samples were collected biweekly during two successive years from the 7th of April, 2001 till 22nd of March, 2003. Each sample consisted of 50 infested leaves (10 leaves/tree) collected from the cardinal directions (north, south, east and west) and

center of the trees (two leaves/direction). Leaves were covered with polyethylene bag on the tree and then pulled up and transferred to the laboratory for examination.

The insect predators, which observed on the collected samples in spot close to the coccid pest were recorded as field observations.

To evaluate the role of host plant species on the efficiency of the natural enemies on FWS population, additional leaf samples were collected monthly (from February till November 2002) from navel orange (*C. sinensis*) and mandarin (*Citrus reteculata* (L.)) trees. Each sample consists of 50 infested leaves from each of host plant. Leaves were collected as previously mentioned and transferred to the laboratory for examination.

2- Evaluation the role of different mortality factors :-

2-1- Parasitoid and predators :-

To estimate the role of insect parasitoids and predators on FWS population, leaves of each tree were investigated on both surfaces by the aid of stereo microscope. Scales were recorded as living, dead, predator-damaged and parasitized with living parasitoids or emerged holes.

To determine the parasitoid species, each sample was maintained in Petri dishes (10 cm in diameter), containing a piece of moistened cotton wool. After two weeks, each sample was re-examined to collect and count the emerged parasitoids for identification.

The percentage of parasitism (Par. %) and predatism (Pre. %) were calculated as follows :-

$$\text{Par. or Pre. \%} = \frac{\text{par. or pre.}}{N + D} \times 100$$

Where, par. and pre. are the number of parasitized and predeceased scales, while, N and D are the number of living and all dead scales.

2-2- Microbial agents :-

Living and dead FWS individuals from each sample that showed general infected symptoms were distinguished and put in sterilized tubes and transferred to Microbiological lab, (Microbiology Department, Faculty of Agriculture, Mansoura University) to isolate and identify the fungi and bacteria presented on the tested pest.

All infected individuals were counted and the percentage of diseased scales was calculated for each sample.

Isolation and identification of microorganisms :-

1- Isolation :-

The dilution plate method was used for the isolation of the insect microorganisms. The insect was crushed, then sterile water was added. The suspension was shacked well for 10 min, then diluted to the desired final dilution. One ml of the desired dilution was transferred aseptically into a series of Petri-dishes containing Czabek's agar (Rechigl, 1978). After incubation at 30±1°C for 3.5 days, plates were examined and the developed colonies were identified.

2- Identification :-

The developed microbial colonies were examined on Czapek's culture media. The fungi were identified with the help of Clemnents and Shear (1931) and Gilman (1957).

Daily records of mean temperatures and relative humidity obtained from the Agrometeorological station at El-Mansoura region to represent the climatic conditions effect, during 2001/2002 and 2002/2003 seasons.

Statistical analysis was done by using a one way ANOVA.

RESULTS

1- Parasitoids :-

The obtained data, in the two years of study, revealed that two parasitoids were recorded on FWS belonging to Order Hymenoptera. The first was *Microterys flavus* (How.) (Fam.: Encyrtidae) and the second was *Tetrastichus ceroplastae* (Gir.) (Fam.: Eulophidae). *M. flavus* was the main dominant parasitoid.

The seasonal activity of the parasitoids on FWS in response to host density was illustrated in Figure 1 (A and B) for the first and second year.

In the first year, the percentage of parasitism all over the year averaged 8.0±10.4%. On the 7th of April the percentage of parasitism was 34.9% (the first peak of activity), then it decreased gradually till the 16th of June (8.1 %). The second peak of activity occurred during the 14th of July (13.5 %) after gradual increase

during the second half of June, then, the parasitoids activity decreased gradually till zero % on 8th of September (Figure 1 A).

In the second year, the percentage of parasitism on FWS was relatively low (4.8±8.9%) in comparison with the first year. As shown in Figure (1 B), the parasitoids exhibited approximately the same trend of activity as in the first year. The parasitoids recorded two peaks of activity, the first occurred on the 20th of April (37.3%), while the second one represented by 8.1% on the 27th of July.

Synchronization between parasitoids activity and host population density :-

As shown in Figure (1 A and B) the synchronization between the host population and parasitoids activity is not good in both years. Statistical analysis indicated that there was a negative correlation in the first and second year as "r" values were -0.48** and -0.28.

Effect of mean temperature and R.H.% on the parasitoids activity:-

Statistical analysis showed that there is an insignificant correlation between the parasitoid activity and mean temperature in the first and second year as "r" values were 0.06 and –0.28, while, with mean relative humidity there is negatively high significant correlation in the first year (r = -0.56**) and negatively insignificant correlation in the second year (r = -0.22).

Effect of host plant on the parasitoids activity :-

The average percentages of parasitism on orange trees were 2.7±3.1, 24.7±15.9, 12.3±7.8 and 0.2±0.3% in winter, spring, summer and autumn seasons, respectively, while on mandarin trees, these rates were 1.0±1.7, 15.7±16.7, 11.4±8.6 and 0.1±0.2%, respectively, which indicate that the activity of parasitoids on orange trees was higher than mandarin trees, and that spring was the favourable season for the parasitoids activity followed by summer season for the two host plants (Figure 2).

2- Predators :-

During the two years of investigation, sex insect predaceous species were recorded on the infested orange trees with FWS. These were the coccinellids, *Chilocorus bipustulatus* L., *Rodalia cardinalis* Muls., *Coccinella undecimpunctata* L. and *Cydonia vicina isis* Gr.; the staphylinid, *Paedrus alfirii* Koch and the chrysopid,

Chrysoperla carnea Steph.. Also, data revealed that, *C. bipustulatus* and *C. carnea* were the most dominant numerous predators.

The seasonal activity of all insect predators (Predatism %) on FWS in the first and second year was illustrated in Figure (3).

The percentage of predatism, all over the year, averaged 1.5±1.7% and 1.2±1.3% at the first and second year. As shown in Figure (3), the activity of all predators was slight in the two years of study. The highest activity was recorded in May (4.3 %) and July (2.7 %) during the first and second year.

3- **Entomopathogens :-**

Two species of entomopathogenic fungi, namely *Fusarium* sp. and *Cladosporium* sp. were isolated from FWS for the first time in Egypt during the present study.

Seasonal activity of the entomopathogens on FWS population :-

The seasonal appearance of naturally infected scales with entomopathogenic fungi in response to FWS population was illustrated in Figure 4 (A and B) for the first and second year of study.

In the first year, the percentage of diseased scales with entomopathogenic fungi averaged 7.0±2.3%. The highest percentages of diseased scales were recorded on 21st April (8.8 %), 14th July (14 %) and 23rd March (8.3 %). Mean temperature and R.H. % were (21.5°C & 64.0%), (28.5°C & 72.0%) and (18.0°C & 71.0%), respectively.

In the second year, the percentage of scales killed by the pathogenic fungi averaged 6.5±1.8%. Seasonal prevalence of entomopathogenic fungi attacking FWS had the same trend as in the first year. The highest percentage of diseased scales was recorded on 6th April (11.5%), 10th August (10.3%) and 22nd March (7.9%). Mean temperature and R.H. % were 17.0°C & 66.0%, 30.0°C & 67.0% and 16.3°C & 71.0%, respectively.

Synchronization between host population and fungal activity :-

As shown in Figure (4 A and B) the synchronization between the FWS population and percentage of diseased scales is not good in both of the two years. Statistical analysis indicated that there was negatively insignificant correlation in the first and second year as "r" values were –0.09 and –0.23, respectively.

Effect of mean temperature and R.H. on fungal pathogenic activity :-

Statistical analysis showed that the correlation coefficients between the percentage of diseased scales and mean temperature were insignificantly positive in the first year (r = 0.21) and negative in the second year (r = –0.15) while, there was positively insignificant correlation with mean humidity in the two years (r = 0.12 and 0.02).

4- Efficiency of different mortality factors on FWS population :-

Data illustrated in Figure 5 (A and B) indicate the contribution of different mortality factors (parasitism, predatism, pathogenic and other mortality factors) to the total mortality of FWS population in the two years of study.

In the first year, the average percentages of parasitism, predatism, pathogenic and mortality due to other mortality factors were 8.0±10.4, 1.5±1.7, 5.4±1.8 and 19.8±16.0%, respectively (Figure 5A), while, in the second year (Figure 5B) they were 4.8±8.9, 1.2±1.3, 5.2±1.8 and 22.0±14.2, respectively.

As shown in Figure (5), parasitism contributes with relatively high percentage to the total mortality followed by diseased scales in comparison with predatism. Mortality due to other mortality factors recorded the highest contribution of the total population mortality. Seasonal fluctuations of parasitism percentage were correlated with changes of total population mortality in the first and second year.

Statistical analysis (Table, 1) indicated that in the first year there is a positive highly significant correlation between both the percentage of parasitism and diseased scales with total population mortality ($r = 0.53^{**}$ and 0.79^{**}). Also, in the second year the r-values were 0.80^{**} and 0.49^{*} for the mortality caused by parasitism and diseased scales. Data in the same table showed that, there was no correlation between the changes of the total mortality and predatism (Table, 2) in both years of study.

Data represented in Table (2) indicate the average percentage of mortality caused by each factor in spring, summer, autumn and winter seasons.

As shown in Table (2), in the first year the average percentage of parasitism was highest in spring followed by summer, which occurred the two peaks of parasitism. While, diseased scale showed similar percentages in all seasons of the year and predatism was highest in spring followed by winter season. On the other hand, mortality percentage due to other mortality factors was highest during summer

followed by autumn season. This results may be due to the high temperature in summer and low humidity in autumn.

In the second year (Table, 2) the average percentages of parasitism and diseased scales had approximately the same trend as in the first year, while, predatism and other mortality were highest in spring.

DISCUSSION

1- The role of insect parasitoids as mortality factors on FWS population :-

During the course of this study the, encyrtid, *Microterys flavus* (How.) and the eulophid *Tetrastichus ceroplastae* (Gir.) were reared from *Ceroplastes floridensis* Comstock at Mansoura region. Other authors were recorded them to attack FWS in Egypt at Alexandria (Hafez *et al.*, 1987) and at Kafr El-Sheikh (El-Agamy *et al.*, 1994 and Hendawy, 1999); in Israel (Ben-Dov, 1972 and Argov *et al.*, 1992) and in China (Huang* and Huang**, 1988).

The activity of parasitoids had two peaks of activity (in April and July) as well as in Israel (Ben-Dov, 1972) who recorded two peaks in April-May and July-August, while, in Egypt, at Alexandria these peaks were recorded in June and January (Hafez *et al.*, 1987) and at Kafr El-Sheikh, (El-Agamy *et al.*, 1994) recorded three peaks of parasitoids activity (in October, March and June). These differences may be attributed to the variation of environmental factors. The parasitoids activity was decreased sharply after each peak, especially during autumn season and the first half of winter (from the first week of September to the third week of January) where the percentage of parasitism was approximately zero. This reduction of activity may be due to the absence of susceptible stages (3rd instar nymphs and adult females). This suggestion agrees with Ben-Dov (1972) in Israel.

Emission of auditory stimuli from the host plant is the main factor in insect attractance (Labeyrie, 1976; Kydonieus and Beroza, 1982 and Abd El-Kareim, 1997). Differences in parasitoid female response to different host plant may explain variation of parasitized scales of FWS on orange and mandarin, which resulted in the reduction of egg laid (Abd El-Kareim, 2002) on mandarin.

2- The role of insect predators as mortality factors on FWS population :-

The insect predators, *C. bipustulatus*, *R. cardinalis*, *C. undecimpunctata*, *C. v. isis*, *P. alfirii* and *C. carnea* were recorded associating with FWS on navel orange trees. Similar finding was reported by Panis (1980), Abd-Allah (1988), Abd El-Kareim

(1992), Metwally *et al.* (1993) and Moustafa (1999). They reported that the above mentioned insect predators are among the main predators on citrus orchards.

The obtained results concluded that the activity of predators on the coccid scale, FWS population was obviously low. Also, Stansly (1984) mentioned that the coccinellid, *Pharascymnus numidicus* Pic. caused 2% mortality of the adult *Parlatoria blanchardi* Targ. in Niger and Stathas *et al.* (2003) who recorded approximately 6 % of mortality in *Ceroplastes sinensis* Del Guercio caused by *C. bipustulatus* and *Exochomus quadripustulatus* (L.) in Grecce.

3- The role of entomopathogens as mortality factors on FWS population :-

During the course of this study, two species of entomopathogenic fungi, namely *Fusarium* sp. and *Cladosporium* sp. were isolated from FWS for the first time in Egypt. *Fusarium sp.* and *Cladosporium sp.* are mycotoxin producing fungi (Opacka and Truszczynski, 1979 and Furlong *et al.*, 1995) and *Fusarium spp.* inhibits protein synthesis (Dowd, 1990). Also, in China Hui *et al.* (1999) isolated *Fusarium juruanum* from FWS, in addition to *Fusarium moniliforme* Sheld., *F. semitectum* Grenn. and *F. lateritium* Nees from *Ceroplastes destructor* (New.) and *C. sinensis* (Zheng *et al.*, 1990).

While, *Alternaria infectoria* Simmons and *Cladosporium cladosporioides* were isolated from *Ceroplastes rusci* L. in Egypt by Shabana and Ragab (1997).

The present results indicated that the diseased coccids had been recorded in low rates in both years. While, in India, Sharma and Dogra (1986) observed up to 13.6% mortality of *Eulecanium tiliae* (L.) was caused by the entomopathogenic fungus, *Rhinocladiella* sp. in the 2nd instar nymphs and in New Zealand, Lo and Chapman (1998) observed that the diseased (caused by *V. lecanii* and *Fusarium* sp.) was a greater mortality factor than parasitism in *C. destructor*. The differences between the present results and others may attributed to variations of climatic factors and / or the species of each of the pathogen agent and the coccid.

The obtained data showed that the synchronization between the FWS population and percentage of diseased scales is not good in both of the two years. These results may attribute to that the entomopathogen agents did not act in a density–dependent reaction. This suggestion agrees with Lo and Chapman (1998).

The weather factors (temperature and relative humidity) may have an effect on the activity of the entomopathogenic agents. Carruthers and Haynes (1986) and

Mcleod *et al.* (1998) reported that temperature is known to affect the rate of spore germination and the incubation period of many entomopathogenic fungi, thereby influencing the course of epizootics. Mortality and sporulation by fungi infection occurred mainly during night and early morning hours when humidity was much high (Steinkraus and Slaymaker, 1994).

In general, the present results indicated that parasitism and unknown mortality (other mortality factors) represented the highest contribution to the total mortality of *C. floridensis.* Similar results were obtained on the diaspidid species, *Aonidiella aurantii* (Mask) by Abou Hatab (1999) and *Chrysomphalus aonidum* (L.) (Moustafa, 1999).

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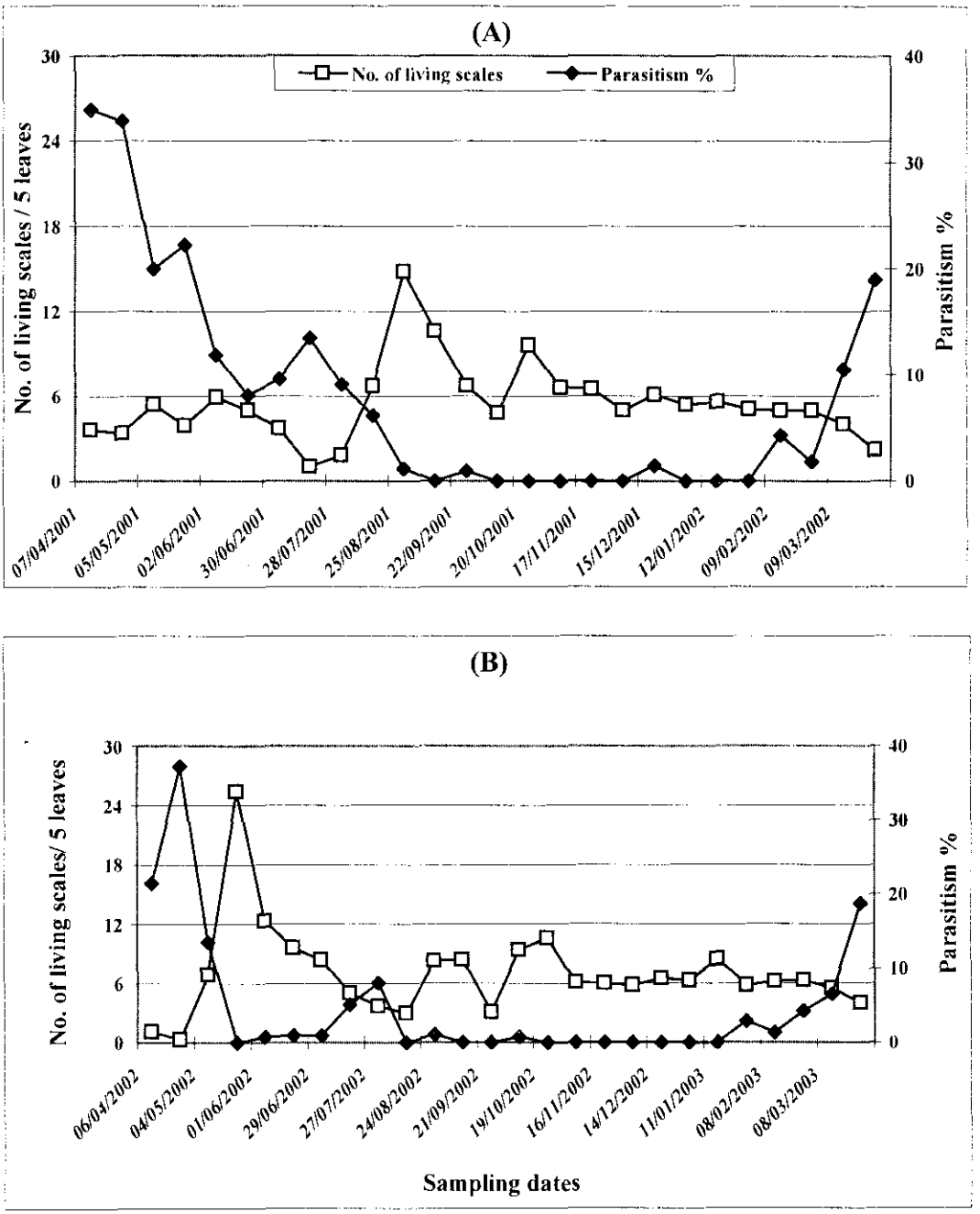

Figure (1): Seasonal activity of FWS, *C. floridensis* and its parasitoids in relation to host density on orange leaves during 2001/02 (A) and 2002/03 (B) at Mansoura district.

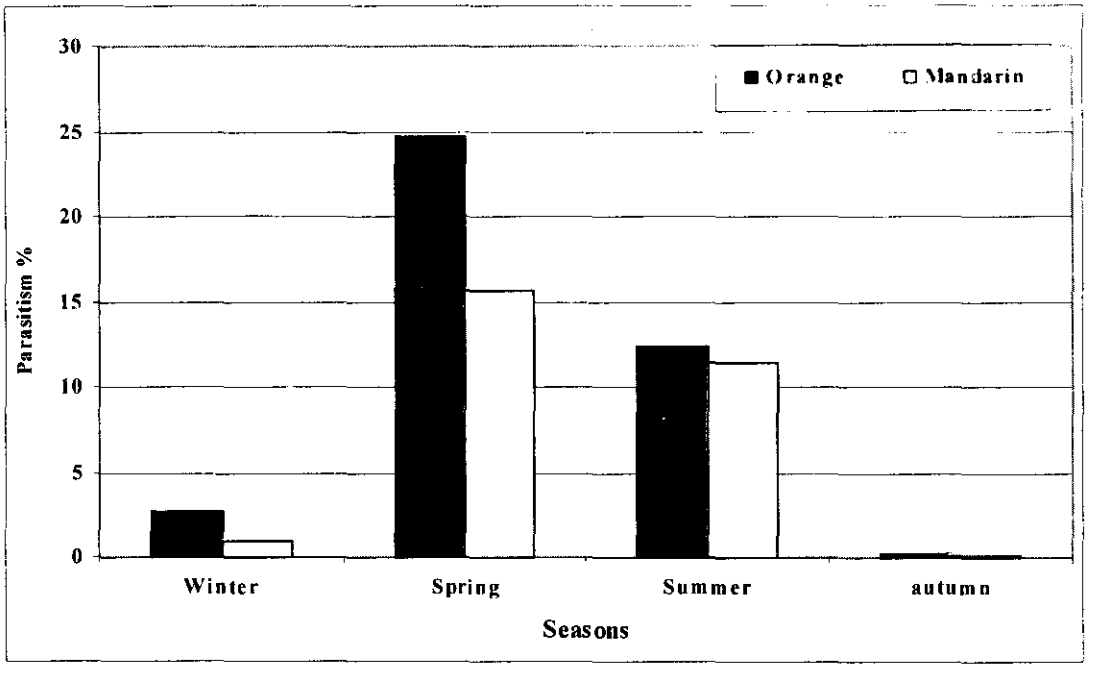

Figure (2): Effect of host plant on the activity of parasitoids associated with FWS, *C. floridensis* population at Mansoura district.

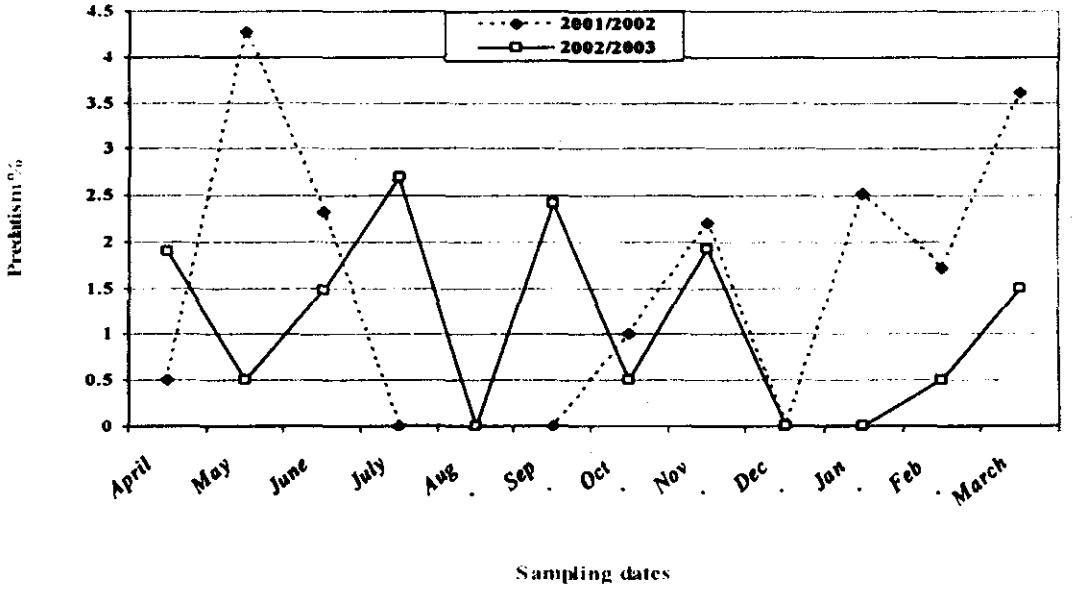

Figure (3): Seasonal activity of insect predators associated with FWS, *C. floridensis* on orange trees during two seasons 2001/2002 and 2002/2003.

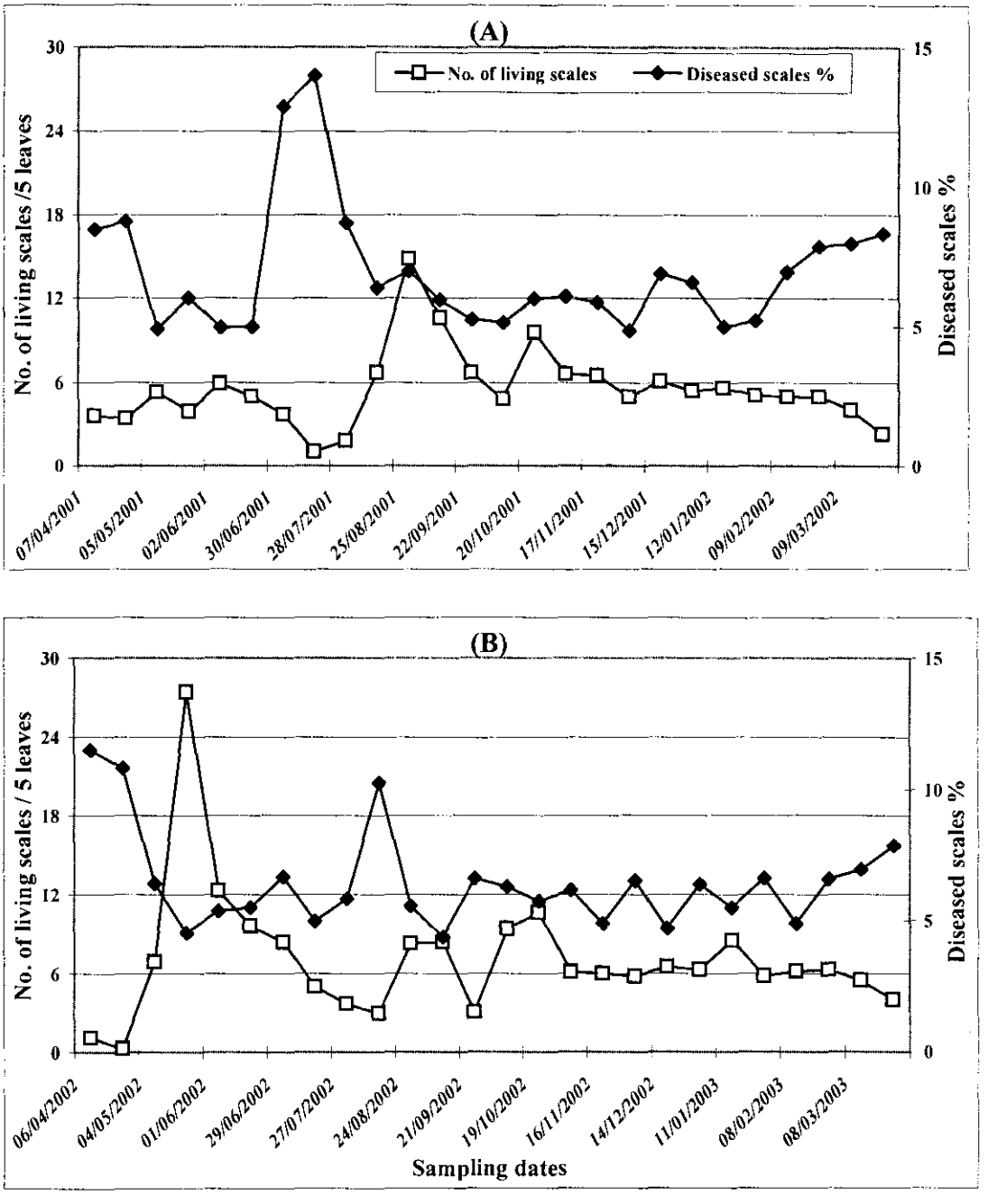

Figure (4): Seasonal activity of fungal pathogens in relation to host *C. floridensis* density on orange leaves during 2001/02 (A) and 2002/03 (B).

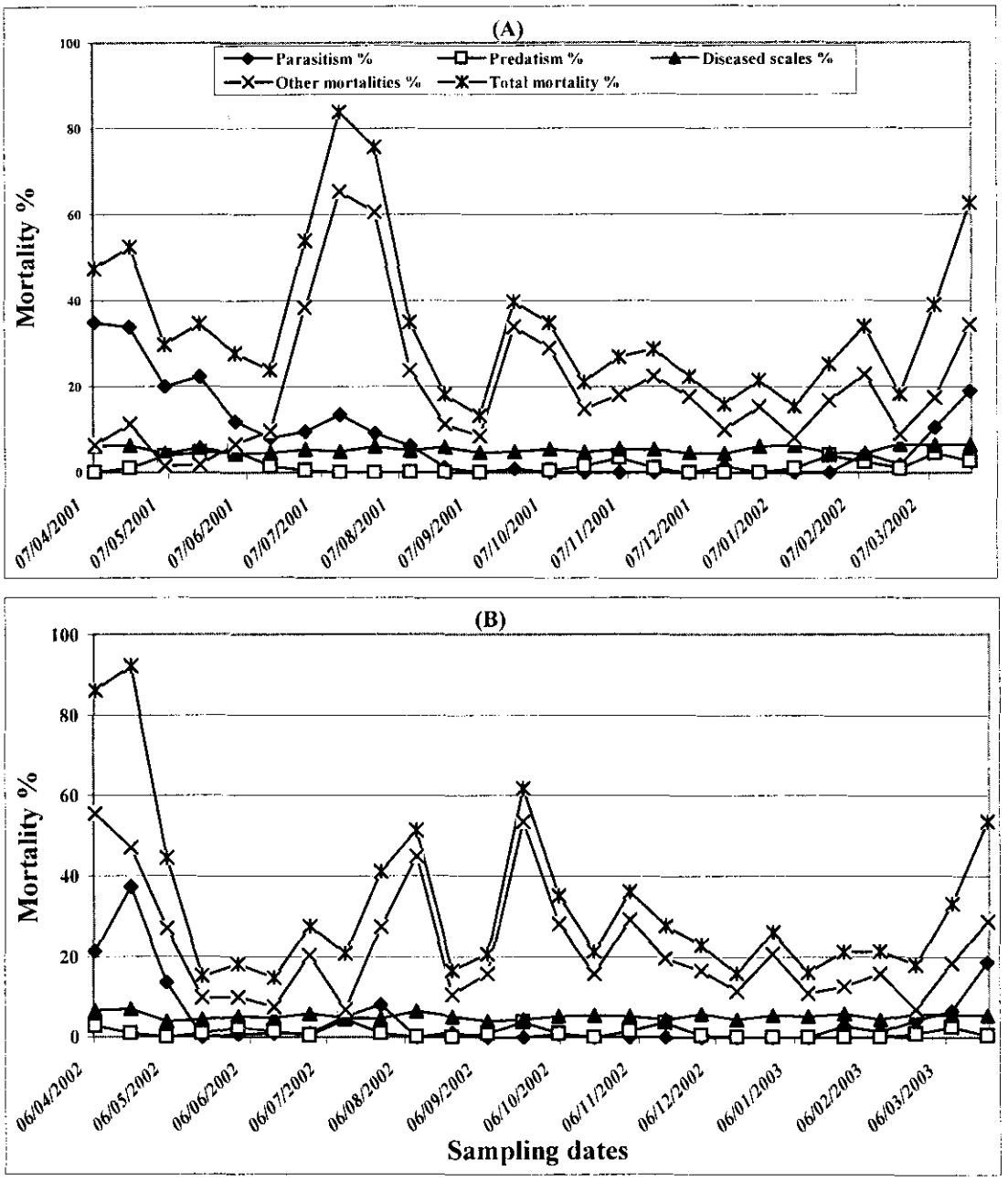

Figure (5): Mortality percentages caused by the natural enemies (parasitoids, predators and pathogens) and other mortality factors on FWS, *C. floridensis* population during 2001/02 (A) and 2002/03 (B).

Table (1): Correlation coefficient values (r) between changes of each mortality factor and total population mortality.

Mortality factor	2001- 2002	2002- 2003
Parasitism	+ 0.53**	+ 0.80**
Predatism	- 0.09	+ 0.19
Pathogenic	+ 0.79**	+ 0.49*
Other mortality factors	+ 0.94**	+ 0.93**

Table (2): Average percentages of mortality on *C. floridensis* population during all seasons of the first (2001-2002) and second year (2002-2003) of study.

Mortality factor		Season			
		Spring	***Summer***	***Autumn***	***Winter***
Parasitism	1st	27.8±7.7	8.5±4.0	0.2±0.4	1.1±1.6
	2nd	18.1±15.6	2.4±3.0	0.1±0.3	1.4±1.8
Predatism	1st	2.4±2.2	1.0±1.8	1.1±1.3	1.2±1.5
	2nd	2.0±1.2	1.4±1.6	1.7±1.5	0.2±0.4
Pathogenic	1st	5.7±1.9	5.2±1.6	5.1±1.3	5.3±2.0
	2nd	5.8±2.5	5.2±1.7	4.9±1.7	5.2±1.7
Other m. f.	1st	5.2±4.6	30.8±24.5	21.1±9.3	14.2±5.4
	2nd	34.9±20.4	18.2±14.0	25.6±13.6	13.1±4.7
Total	1st	41.1	45.4	27.4	21.8
	2nd	60.7	27.2	32.3	19.9

1st = the first year & 2nd = the second year & Other m. f. = Other mortality factors

تقييم دور عوامل الموت المختلفة على تعداد حشرة الموالح الشمعية في بساتين الموالح بمنطقة المنصورة – محافظة الدقهلية – مصر

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خلال عامين من الدراسة (٢٠٠١/٢٠٠٢ و٢٠٠٢/٢٠٠٣) تم تسجيل نوعين من الطفيليات على حشرة الموالح الشمعية بحقول البرتقال أبو سرة واليوسفي هما *Microterys flavus* (How.) و *Tetrastichus ceroplastae* (Gir.) . حيث تم تسجيل ذروتين لنشاط هذين الطفيليين في ٧ من ابريل (بنسبة تطفل ٤٣,٩%) وفى ١٤ من يوليو (١٣,٥%) وذلك خلال العام الأول بينما في العام الثاني تم تسجيل ذروتي النشاط في ٢٠ من ابريل (٣٧,٣%) و٢٧ من يوليو (٨,١%) . ووجد أن أعلى نشاط لهذين الطفيليين خلال فصل الربيع خاصة على أشجار البرتقال أبو سرة .

كما تم تسجيل ستة أنواع من المفترسات الحشرية مرتبطة بحشرة الموالح الشمعية هم *Coccinella undecimpunctata* L., *Rodalia cardinalis* Muls.، *Chilocorus bipustulatus* L.، *Cydonia vicina isis* Gr.، *Paederus alferii* Koch.، و *Chrysoperla carnea* Steph. . ولوحظ انخفاض نشاط هذه المفترسات على تعداد الآفة حيث بلغ متوسط نسبة الافتراس ١,٥±١,٧ و١,٢±١,٣% خلال العام الأول والثاني من الدراسة .

تم عزل نوعين من الفطريات الممرضة لهذه الآفة هما *Fusarium* sp. و *Cladosporium* sp. وذلك لأول مرة في مصر . وبلغ متوسط نسبة الحشرات المصابة بهما ٧,٠±٢,٣ و٦,٥±١,٨% خلال العام الأول والثاني من الدراسة .

كما أوضحت النتائج أن التطفل هو العامل الأكثر إسهاما في الموت الكلى لتعداد الآفة مقارنة بالمسببات المرضية أو الافتراس وذلك خلال عامي الدراسة يليه المسببات المرضية ، وكان الافتراس هو العامل الأقل إسهاما في الموت الكلى بتعداد الآفة .