J. Agric. Sci. Mansoura Univ., 30 (4): 2217 - 2227, 2005

BIOLOGICAL STUDIES ON *Eretmocerus mundus* (HYMENOPTERA: APHELINIDAE), A PARASITOID OF *Bemisia argentifolii* (HEMIPTERA: ALYRODIDAE), AND ITS ROLE IN PEST POPULATION REGULATION ON TOMATOES.

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ABSTRACT

The Aphelinid parasitoid *Eretmocerus mundus* Mercet is considered the most effective parasitoid against *Bemisia argentifolii* Bellows & Perring, a serious pest in greenhouses and outdoor crops, in Egypt. Studies were carried out to determine some biological characters of *Er. mundus* under two temperature regimes 25 and 30 °C and 75% R.H. The life cycle of the parasitoid shortened at 30 °C (22.3±3.89 days), become longer (27.8±5.2 days) at 25 °C. Also, the female longevity lasted 9.2±2.45 and 12.55±3.4 days at 30 °C and 25 °C, respectively. At 30 °C, female progeny was 58.5±1.88 progeny, while it was 44.89±1.32 at 25 °C. In addition, the statistical analyses revealed that the parasitoid life cycle, female longevity and progeny rats were varied significantly according to the temperature regime used.

Constant number of *Er. mundus* was released against various numbers of its host. Higher parasitism percentage (87.5%) was recorded with the ratio of 1 parasitoid: 5 *B. argentifolii* nymphs. The parasitism percentages decreased when host numbers increased. When parasitoids released at ratios of 1: 10, 1: 15 and 1: 20, the parasitism percentages decreased to 65.96%, 55.5 and 40.4%, respectively. This means that increasing host numbers from 5 to 20 *B. argentifolii* nymphs, nymphal mortality decreased 6.5 times in comparison with 1: 5 ratio.

Keywords: *Eretmocerus mundus*, parasitoid biological characters, parasitoid releasing, Biological Control, Silverleaf whitefly, *Bemisia argentifolii*, Biotype B, tomatoes.

INTRODUCTION

Bemisia argentifolii Bellows & Perring, emerged recently as a one of serious pest of vegetable, ornamental and agronomic crops world-wide since 1986. The pest caused vital problems that led to threatening the agricultural industries (Gerling and Mayer, 1995). Their problems caused by three means, a) reducing crop yield, b) acting as vectors of viral pathogens, and c) contaminated the crop by sooty mould resulted from the honeydew excreted by the insect pest. The aleyrodid *B. argentifolii* is frequently found in greenhouses for ornamental plants (Gerling and Mayer, 1995). This insect produce equivalent damage to vegetable crops, and even has comparable levels of resistance in fields which pesticides frequently applied (Dittrich *et al.*, 1990). Factors that responsible for acceleration of *B. argentifolii* status are; a) rapid multiplication in warm climates (being thermophilic insect), b) higher fecundity compared with other biotypes, c) small number of natural enemies association as a result of pesticide usage (Hafez *et al.*, 1983), d) widest host range (Abdel-Baky, 2001). Destruction of pest natural enemies after insecticides usage may be one of important factors contributing to insect outbreaks (Gerling and Mayer, 1995).

Natural biological control is the backbone of IPM programs. Thus, ecological data on *B. argentifolii* and their parasitoids are essential for development of existing control measures and development of new control strategies in IPM (Botto, 1999; Stansly *et al.*, 2004). In Spain, a study of the parasitoids of *Bemisia* spp. was carried out in protected tomato crops. In fields where IPM was practiced, *Er. mundus, Encarsia lutea* and *En. transvena* were found, with *Er. mundus* being the predominant species (Rodriguez-Rodrigez *et al.*, 1994). In fields where chemical control methods had been applied, no parasitoids were found.

Er. mundus Mercet is a solitary parasitoid and being the most abundant indigenous nymphal parasitoid of *B. argentifolii* (Tellez *et al.*, 2003; Urbaneja and Stansly, 2004). The parasitoid is the important natural enemies of *Bemisia* nymphs and recorded from many parts of the Mediterranean basin (Simmons *et. al.*, 2002; Ardeh *et al.*, 2005). Therefore, *E. mundus* proved to be the most efficient whitefly parasitoids, while the other species showed only medium performance (Onillon and Maignet, 2000). *Eretmocerus* species are arrhenotokous, but Australian strain of *Er. mundus*, is thelytokous (McAuslane and Nguyen (1996); De Barro *et al.*, 2000). De Barro *et al.*, (2000) considered thelytokous populations are the best candidate for biological control of *Bemisia* spp. world-wide.

Biological characteristics of *Er. mundus* have been studied under laboratory conditions (Gerling and Fried, 2000). Suitability of nymphal instars of *B. argentifolii* attacked by *Er. mundus* was assessed (Jones and Greenberg, 1998; Urbaneja and Stansly, 2004). Their studies comprised the host mortality; parasitoid survival, development time, and parasitoid fecundity. *Er. mundus* parasitize all instars. However, 2nd instar was preferred stage than 4th instar (Jones and Greenberg, 1998), since the 2nd instar exhibited the highest proportion of host mortality and parasitoid survival (Urbaneja and Stansly, 2004). Although 2nd and 3rd instars were clearly the most favorable host stage for *Er. mundus*, the parasitoid capacity to parasitize and develop on a wide range of host stages is a favorable characteristic for both rearing and field application (Tellez *et al.*, 2003). Jones and Greenberg, (1998) used two expression to distinguish between whitefly instars as a host suitability. A kionobiont expression used to refer to *Er. mundus* when parasitizes the 2nd instar only, because after parasitization, the host continues to feed, grow, and develop. However, when *Er. mundus* parasitizes 3rd and/or 4th instar nymphs, the parasitoid evidently stop development (idiobiont).

Gerling and Fried, (2000) reported that *Er. mundus* killed their hosts through feeding and mutilation. Older parasitoids (4-day-old) killed more hosts and than newly emerged or 2-day-old females. Also, the sex ratio of the progeny was about 60% females, whereas, the progeny of 10-day-old mothers was dominantly male (less than 20% females), possibly due to sperm depletion.

Relative humidity (RH) and host biotypes affect greatly the population and distribution of *Er. mundus* (Antony and Palaniswami, 2002). The population of *Er. mundus* was high in areas with low RH, ranged between 25 and 40%. These results showed that RH and host biotypes govern the general distribution of *Er. mundus*.

Because of the promising results obtained in the studies mentioned above, *Er. mundus* is considered one of the important whiteflies parasitoids to be used as part of IPM programs. Therefore, this study was conducted to shed lights on some biological characters of *Er. mundus* under two temperature regimes and its role in regulation of *B. argentifolii* population on tomatoes.

MATERIAL AND METHODS

Tomato plants were used as a host of *B. argentifolii* and accordingly rearing its ecto- endoparasitoid, *Er. mundus*, under laboratory conditions. Both of *B. argentifolii* and *Er. mundus* cultures were originally initiated from individuals collected from tomato fields, insecticides free, at Dakahelia governorate. Colonies of *B. argentifolii* and *Er. mundus* were kept under laboratory conditions at the room temperature and natural photoperiod. To obtain adult parasitoids, *B. argentifolii* nymphs with recognizable parasitoid pupae were stored out from the nymph samples. The parasitized *B. argentifolii* nymphs were kept for emergence in small tubes.

I. Biological characters of *Er. mundus*:

Two groups of pots (each, 10 pots) were cultivated by tomato seedlings, kept whiteflies free under screened cages until reaching a good vegetative development. Three full expanding leaves were selected from each pot. Couples of *B. argentifolii* adults were introduced to each leaf and left for 24 hours then *B. argentifolii* adults were removed. The number of eggs was counted on each leaf. The first group of pots was kept at 25 °C, while the others at 30 °C and RH 75%. Since *Er. mundus* prefers to parasitize the 1^{st} or 2^{nd} instars of *B. argentifolii* (Jones and Greenberg, 1998), once *B. argentifolii* reached to the 2^{nd}, the desire number of nymphs was left and then one couple of *Er. mundus* was introduced and kept only for 24 hours. The tomato leaves were left until emergence of adults of whitefly (WF) or parasitoid. The period from parasitoid eggs until adult emergence, adult fecundity and longevity and were recoded.

A: Parasitoid Longevity:

Longevity of *Er. mundus* fed on honey was determined in an environmental cabinet at the two previous temperature regimes and photoperiod of 12: 12 (L: D). Fifty newly emerged parasitoids were individually placed in Petri-dishes (9 cm diameter) with screened lids. The parasitoids adults were re-provisioned daily with honey smeared on the screened lids until they died.

B: Parasitoid Fecundity:

The experiment was performed at the previous conditions. Newly emerged females of *Er. mundus* were singly isolated in glass chimney sealed from the top with screened cover. Tomato pots with leaflets infested by the 2^{nd} (50-80 individuals) *B. argentifolii* nymphs were each inserted inside the glass chimney. The tomato plants were replaced daily with new similar pots until the death or escape of the parasitoid. Fecundity of *Er. mundus* adults were assessed by counting nymphs showing sign of parasitism and/or numbers of emerged of parasitoid adults.

II. Releasing efficiency of *Er. mundus* adults on *B. argentifolii* populations:

Tomato plants, that infested by desired numbers of *B. argentifolii* nymphs, were used to evaluate releasing efficiency of *Er. mundus* adults under semi-field conditions. Four releasing ratios were applied. The experimental unit of each ratio consisted of five pots screened with agryl tissue and replicated three times. The number of *Er. mundus* was kept constant against various numbers of *Bemisia* nymphs. When *B. argentifolii* 2^{nd} instar was reached, one mated parasitoid female (<two days old) was introduced per 5, 10, 15 or 20 *B. argentifolii* nymphs. After 24 h, the parasitoids were removed and plants were kept under semi-field conditions for insect and parasitoid development. A control treatment was left with no parasitoids release. Control treatment was also replicated three times.

Five days after releasing, numbers of the emerged whiteflies adults and its parasitoids were checked and counted. Number of *Er. mundus* adults was recorded at three days intervals until the end of the experiment or completing emergence of the parasitoid adults.

III. Statistical Analysis:

Statistical analyses were conducting using analysis of variance (ANOVA). Correlation Coefficient and regression analysis were also applied to determine the relationship among parasitized, non- parasitized nymphs and parasitism percentage (Costat Software, 1990).

RESULTS

I. Biological characters of *Er. mundus*:

Table (1) presents some biological characters of *Er. mundus* under two rearing temperatures. The life cycle of the parasitoid was shorter at 30 °C and lasted 22.3±3.89 days, while it become longer (27.8±5.2 days) at 25 °C. Female parasitoid lived shorter period (9.2±2.45 days) at 30 °C, in comparison with 12.55±3.4 days at 25 °C. In contrary, the higher progeny rate/female (fecundity) was 58.5±1.88 progeny (with an average 2.42 progeny/day) at 30 °C and receded to the lowest progeny/female at 25 °C female (44.89±1.32) with an average 2.22 progeny/day.

Also, the statistical analyses revealed that the parasitoid life cycle, female longevity and progeny rates were varied significantly according to the temperature regime used.

Table (1): Effect of rearing temperature on some biological characters of *Er. mundus*, a parasitoid of *B. argentifolii*.

Parameters	Temperature regimes	
	25 °C	30 °C
Life Cycle (in days)	27.8±5.2 a	22.3±3.89 b
Mean Female longevity (in days)	12.55±3.4 a	9.2±2.45 b
Female progeny (No.)	44.89±1.32 a	58.5±1.88 a
Average No. of progeny/day	2.22	2.42

[a] Means within a row followed by the same letter are not significantly different (P=0.05)

II. Releasing Efficiency of *Er. mundus* adults on *B. argentifolii* populations:

Parasitization activity by *Er. mundus* varied according to the releasing ratio (Table 2). Percentage of parasitism was higher, reached 87.5% with the ratio of 1 parasitoid: 5 *Bemisia* nymphs. When parasitoids released by ratio of 1: 10, the parasitism percentage recorded 65.96% under the laboratory conditions (Table 2). On the other hand, further decrease in percentage of parasitism was observed with increasing number *Bemisia* nymphs. The parasitism percentages reached to 55.5 and 40.4% when the parasitoid released ratios were 1: 15 and 1: 20, respectively.

Table (2): Effect of releasing ratios of *Er. mundus* on host population, number of emerged whiteflies, parasitoid adults, and parasitism percentages.

Parasitoid: Host Ratios	No. of *Bemisia* 2nd instar	*Bemisia* adults (No.)	*Er. mundus* adults (No.)	Parasitism (%)
1: 5	240	30 e	210 a	87.50
1: 10	235	60 d	175 b	65.96
1: 15	200	101 c	99 c	49.50
1: 20	250	168 b	82 c	32.80
Control	245	232 a	-	-

[a] Means within a column followed by the same letter are not significantly different (P=0.05).

Data revealed that the non-parasitized nymphs were correlated by a strong relationship with the parasitized nymphs and parasitism percentage (Table 3).

Figure (1) shows the initial numbers of *B. argentifolii* nymphs that affected by releasing number of *Er. mundus*. When number of *B. argentifolii* nymphs was increased from 5 to 20 nymphs and parasitoid density was held constant (one female), the percentage of nymphal mortality decreased 6.5 times. The parasitism percentage was significantly highest (87.5%) at the lowest pest numbers. Meanwhile, the parasitism percentage was lowest (32.80%) at the highest population density of *B. argentifolii* nymphs (Table 2). The variations between the two values initial numbers of host nymphs and emerged host adults indicate the efficiency of releasing ratios (Fig. 1).

Also, population trend of *B. argentifolii* nymphs at various releasing ratios of *Er. mundus* was plotted in Figure (2). The host population trend was higher at zero time of treatment, then decreased sharply or gradually based on the parasitoid: pest ratio. Subsequently, the number of *Er. mundus* increased gradually based on the number of non-parasitized nymphs until the end of experiment.

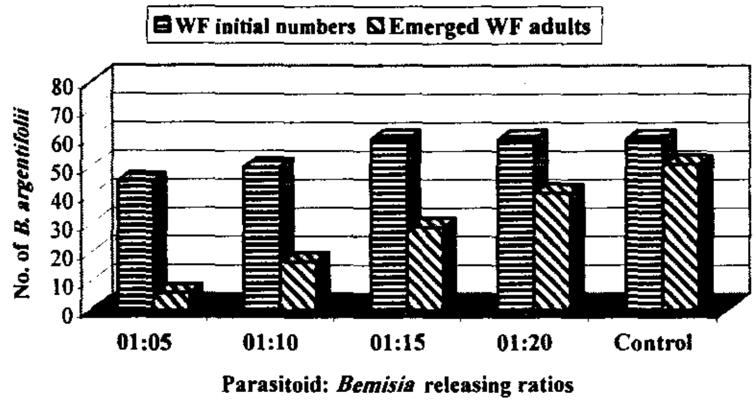

Fig (1). Initial numbers of *B. argentifolii* nymphs and number of emerged *Bemisia* adults as a result of releasing *Er. mundus* at various parasitoid-host ratios.

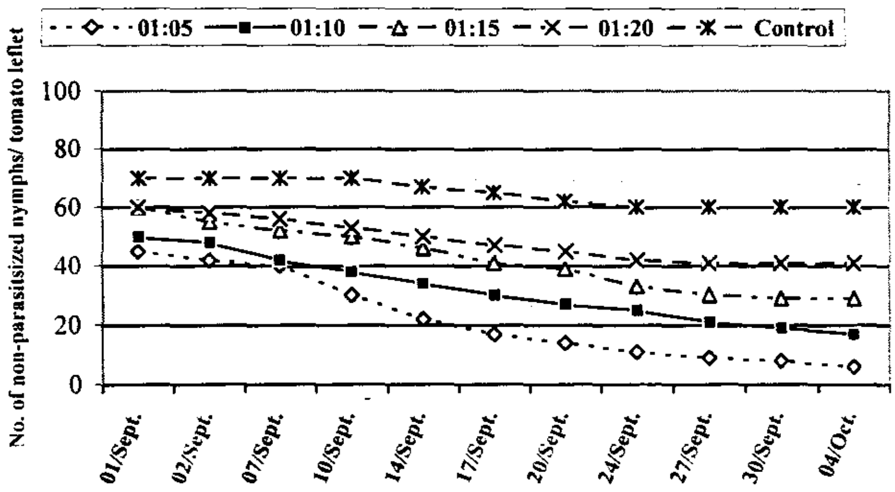

Fig (2). *B. argentifolii* population trend after releasing *Er. mundus* with varied numbers of the host in comparison with control treatment (Parasitoids free).

Table (3): Correlation coefficient and regression equations of the relation between parasitized, non-parasitized *B. argentifolii* nymphs and parasitism percentage by *Ertemocerus mundus*.

Correlation Coefficient Parameters				
X variable	Y variable	Corr. (r)	Slope (b)	Y Int. (a)
No. non-parasitized nymphs	No. of parasitized nymphs	0.29562	0.7220	3.3051
No. of parasitized nymphs	Parasitism %	0.64160	0.9875	38.1315

Regression Analysis			
Variables	Regression Equation	R^2	Significant
Non- parasitized and parasitized nymphs	Y= 0.3687x + 1.554	0.5216	**
Parasitized nymphs and parasitism %	Y= 3.606x + 0.0297	0.9206	***

DISCUSSION

Evaluation and eventual use of *Eretmocerus mundus* as a biological control agent requires considerations of many factors, including the parasitoid biological aspects and its efficiency in reducing the whiteflies population (Ardeh *et al.*, 2005). The life history of *Er. mundus* is affected by temperature, R. H., host density, host size (Jones *et. al.*, 1999). Females of *Er. mundus* lay 81.1-247.5 eggs/wasp each, during the life time of 10-16 days under laboratory conditions (Gerling and Fried, 2000). The lower fecundity recorded in the present study may be due to the egg–laying behavior of *Er. mundus* females which oviposits its eggs externally beneath nymphs and not within them (Buncker and Jones, 2005). Therefore, Foltyn and Gerling (1985) considered *Er. mundus* as an eco-endoparasitoid, oviposition under 2nd or 3rd nymphal body, once hatched, the parasitoid larva burrowing through the ventral host surface. Consequently, there many scenarios for decreasing the parasitoid fecundity (expressed as total parasitoid adult emerged); i) emerging parasitoid larvae may be unable to penetrate *Bemisia* cuticle nymphs, so the parasitoid larva should be able to defeat the host defense (Tuda and Bonsall, 1999); ii) *Er. mundus* do not discriminate the parasitized hosts, so the same female of the parasitoid may lay more than one egg under the same nymph that leads to intra-specific competitions. Unlike, Sarhan (1976) reported that *Er. mundus* female deposites one egg/host nymph and in few cases two eggs were deposited under the same host but only one parasitoid adult is emerged, or/and iii) *Er. mundus* and *Er. eremicus* females share the same niche; this may be leads to super-parasitism or multi-parasitism (Ardeh *et al.*, 2005). Their finding may influence the sequence of importation and release of *Eretmocerus* species in biological control (Borgan and Heinz, 2002).

Regarding the effect of temperature, YuTong *et al.*, (2003) found that the developmental time of juvenile *Er. mundus* required 64 days at 15 °C, whereas the developmental time decreased to around 14 days at 32 °C. Similar effect was observed with our results, which *Er. mundus* life cycle decreased by 5.5 days when the temperature increased from 25 to 30 °C. Sarhan (1976) reported total duration of that Er. *mundus* under three temperature regimes of 29.8 °C, 18.6 °C and 11.6 °C were 17.9, 25.5 and

35.5 days, respectively. Urbaneja *et al.*, (2003) evaluated the longevity and fecundity of *Er. mundus* on tomato and pepper at 25 °C. Adult longevity was 10.1±1.0 days in pepper and 7.3±0.81 days in tomato. Fecundity (number of parasitized hosts) recorded 171.1±22.8 and 147.8±13.5 /female in pepper and in tomato, respectively. They also concluded that these values on any crop indicating the potential of *Er. mundus* to control *B. argentifolii*.

Releasing constant number of *Er. mundus*, against various host densities, resulted on different parasitism ratios. Increasing host densities from 1: 5 to 1: 20, decreased parasitism percentage from 87.50 to 32.80%. Thus, the emerged *B. argentifolii* adults was higher at ratio of 1: 20 (Fig. 2), but decreased at lower population density of the host. In addition, increasing parasitism level depends on the number of exposed WF nymphs, number of releasing parasitoid adults and, the behavior of parasitoid (Figure 2). This figure shows that the parasitoid number when reached to steady level under constant pest density, there's no change of the parasitism percentages. This point can be attributed to the parasitoid egg-laying behavior. In cages which parasitoid releases were made, whitefly nymphal densities were suppressed by 99.8 and 96.8 % with fixed and variable-rates of *Er. eremicus*, respectively (Driesche *et al.*, 2001). Their results indicated that whitefly suppression was not increased by concentrating the release of this parasitoid early in the crop.

Because of its high searching activity, its high host encounters rate and its favorable developmental and reproductive capabilities, *Er. mundus* is expected to be the most efficient *Bemisia* parasitoid of the five parasitoids species tested (Hoddle and Driesche, 1999). *Er. mundus* was very effective in controlling the first generation of *B. tabaci* during the spring season. Inclusion of *Er. mundus* into IPM programs in greenhouse should improve control of *B. tabaci* and consequently reduce the need for pesticides in this crop (Tellez *et al.,* 2003). Finally, the potential value of *Er.mundus* for the biological control of whiteflies in greenhouse crops having a high reproductive rate over a short period.

ACKNOWLEDGMENTS

This work was funded by the Regional Council for Agricultural Research and Extension, Agricultural Research Center, Ministry of Agriculture and Land Recl., Egypt. We are grateful to Dr. Gregory Evans, Florida Department of Agriculture, Division of Plant Industry, USA, for identification of *Eretomcerus* species collected from Dakahelia governorate. Also, thanks are going to Mr. Mohamed M. El-Dessouky, Post-graduate student for his help during the course of study.

Dr. Essmat M. Hegazy, Professor of Economic Entomology Department, Fac. Agric., Alexandria Univ.; Dr Awad A. Sarhan, Professor of Economic Entomology Department, Fac. Agric., Suez Canal Univ. and Dr. Ahmed M. Abou El-Naga Professor of Economic Entomology Department, Fac. Agric., Mansoura Univ., are also acknowledged for critically reviewing the manuscript.

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دراسات بيولوجية على طفيل *Eretmocerus mundus*، (غشائية الأجنحة- أفيلنيدى) كأحد طفيليات الذبابة البيضاء المسببة لمرض الورقة الفضية (نصفية الأجنحة - أليروديدى)، ودوره في تنظيم تعداد تلك الآفة على الطماطم.

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يعتبر طفيل *Eretmocerus mundus* أحد أهم الطفيليات المؤثرة في مكافحة الذبابة البيضاء المسببة لمرض الورقة الفضية تحت الصوب وخارجها ولهذا ، فقد أجريت تلك الدراسة لتحديد بعض الصفات البيولوجية لهذا الطفيل تحت تأثير درجتى حرارة ٣٠، ٢٥ م٥ .

أوضحت الدراسة أن دورة حياة طفيل كانت أقصر (٢٢,٣± ٣,٨٩ يوم)عند التربية على درجة حرارة ٣٠م٥.بينما استغرقت فترة أطول (٢٧,٨±٥,٢ يوم) عند التربية على درجة حرارة ٢٥م٥. سجلت فترة حياة الأنثى ٩,٢±٢,٤٢ يوم على درجة ٣٠م٥، فى حين بلغت ١٢,٥٥±٣,٤ يوم على درجة ٢٥م٥. أما خصوبة الأنثى فاختلفت هى الأخرى حسب تأثير درجة الحرارة، حيث بلغت ٥٨,٥±١,٨٨ فرد على درجة ٣٠م٥ ، أما على درجة ٢٥م٥ كانت الخصوبة ٤٤,٨٩±١,٣٢ فرد.

تشير الدراسات الإحصائية إلى أن صفات الطفيل البيولوجية موضع الدراسة اختلفت معنويا تبعا لدرجة الحرارة المستخدمة فى التربية.

أما الجزء الأخر من الدراسة عنى بتقييم كفاءة هذا الطفيل فى مكافحة حوريات الذباب الأبيض تحت ظروف الأقفاص. تم إطلاق الطفيل بنسب ثابتة بينما اختلفت الكثافة العددية للعائل الحشرى (حوريات الذباب الأبيض). وقد تم الإطلاق بأربع معدلات هى: ١ طفيل: ٥ حوريات، ١ طفيل: ١٠ حوريات، ١ طفيل: ١٥ حوريه، و ١ طفيل: ٢٠ حوريه مقارنة بالكنترول (بدون طفيليات).

بلغت نسبة التطفل ٨٧,٥، ٦٥,٩، ٥٥,٥، و ٤٠,٢% عند الإطلاق بنسب ١: ٥، ١: ١٠، ١: ١٥، و ١: ٢٠، على التوالى، وهذا يعنى أن زيادة عدد حوريات الذباب الأبيض من ٥ إلى ٢٠ مع ثبات معدل إطلاق الطفيل (طفيل واحد فقط) قد أدى إلى انخفاض معدل التطفل بـ ٦,٥ مرة.

تشير أيضا الدراسة إلى أن عدد حوريات الذباب الأبيض الغير متطفل عليها ارتبطت بعلاقة معنوية جدا مع عدد الحوريات المتطفل عليها وكذلك النسبة المؤوية للتطفل.