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EVALUATION OF SOME ENTOMOPATHOGENIC NEMATODES AS BIOLOGICAL CONTROL AGENT AGAINST THE APPLE CLEARWING MOTH BORER, *Synanthedon myopaeformis* BORKHAUSEN (LEPIDOPTERA: SESIIDAE) ON APPLE TREES

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ABSTRACT

The main object of this study was the control of the sesiid borer *Synanthedon myopaeformis* (Lepidoptera) with entomogenous nematodes of the genera *Steinernem*a and *Heterohabditis*.

Three species of entomopathogenic nematodes were evaluated for suppression the population of the apple clearwing borer (ACB; *Synanthedon myopaeformis)* attacking apple trees *(Pyrus malus)* in Fayoum and Giza Governorates throughout 1998 and 1999, respectively utilizing *Steinernema carpocapsae, S. glaseri,* and *Heterorhabditis bacteriophora.*

In field trials, which were located in Fayoum, utilizing entomopathogenic nematodes and the organophosphorus Basudin “Diazinon” 60% EC, nematodes were applied as bark spray in spring (early April) and autumn (early September) as two application methods (sprays wetting bark and spray dry bark) using a knapsack sprayer. Examination of pupal skins revealed that nematode reduced ACB population in all treatments, with control ranging from 28.8% to 85.9%. Spring treatments were preferable to application since they significantly gave high control (61.5 – 85.9%, 50.9 – 65.0% and 32.5 – 46.6% for *S. carpocapsae, S. glaseri,* and *H. bacteriophora,* respectively) as compared to autumn treatments(51.4 – 63.7%, 42.7 – 55.1% and 28.8 – 33.7%, respectively). Also, wetting the tree bark prior to nematode application significantly reduced the number of pupal skins as compared to nematode treatment applied to dry bark. Basudin treatment showed relatively low reduction in ACB pupal skins relative to the control

In Giza, trunks of the grafting knots of infested apple trees were treated as trunk sprayer and a nematode-water-suspension of a 'collar' consisting of sponge - rubber material. The ‘collar' application reduced the host larvae with 95.7, 86.2 and 54.7% for *S. carpocapsae, S. glaseri,* and *H. bacteriophora,* respectively whereas trunks treated with the knapsack-sprayer resulted in mortality rates of 85.6, 65.1 and 37.5%, respectively .

In all experiments the mortality rate due to treatment was strongly depended on the humidity conditions. This effect could be excluded at the time of application using wetting the bark and the collar techniques.

INTRODUCTION

Apple trees, *Pyrus malus*., are grown extensively in different Governorates of Egypt. However, a shortcoming of apples is the susceptibility to attack by the apple clearwing moth borers (ACB), *Synanthedon myopaeformis* Borkhausen (Lepidoptera: Sesiidae*)* and the leopard moth borer, *Zeuzera pyrina* L. (Lepidoptera: Cossidae).

The clearwing borer, is a key pest of apple trees in Fayoum and Giza Governorates. Although *Z. pyrina* also commonly attacks apple trees, morphologically, *S*. *myopaeformis* is now known to be a distinct species. Its distribution ranges from Alexandria to Assiout where It attacks apple, pear, peach, quince, plum, apricot and others (Wilson, 1977).

A 1997 survey showed that approximately 50 percent of the apple orchards in Fayoum and Giza Governorates were attacked by clearwing borer (ACB).

The 1997 and 1998 phenology of *S*. *myopaeformis* in apple orchards at Fayoum and Giza Governorates by counting the numbers of pupal skins on trees and recording the males captured in synthetic pheromone traps showed that the adults started to emerge from mid - February till early December. The majority of captured adults occurred in spring (from mid – March till early May) and autumn (from early October till mid – November).

Day flying females deposit eggs on tree branches and trunks, principally at crotches and wounds from previous pruning cuts (Bone and Kochler, 1991). Hatching larvae tunnel through the bark into the phloem and cambium tissues - where they feed - forming irregular galleries and could be found by removing the infested areas of the bark, which could be easily detached due to the work of the caterpillars. The externally visible symptom of injury is a slight sap flow mixed with brown frass at the penetration site. Galleries are extended almost to the bark surface prior to pupation. An empty pupal skin can be found protruding from a circular hole in the bark after emergence of the adult moth (Solomon 1975 and Wilson 1977). Mining causes branch dieback, disfiguration, structural weakening, and death of trees. There is one generation a year.

The standard control strategy for clearwing moth borers involves using a pheromone trap to detect the first male appearance, and then spraying bark with a residual insecticide (like synthetic pyrethroids, organochlorines or organophosphates) 10-14 days later, just before egg hatch (Nielsen, 1978). Newly hatched larvae that contact the insecticidal barrier as they chew through bark to reach cambium will die. The use of these products is not in accordance with the principles of integrated fruit production. Furthermore, the treatment often results in an insufficient control of the pest insect. However, once larvae are under the bark, pesticide treatments are ineffective.

The use of entomopathogenic nematodes in the families *Steinernematidae* and *Heterorhabditidae* has been shown to be effective in controlling clearwing borers (Kaya 1985, Kaya and Brown 1986, Georgis and Gaughler 1991 and Gill *et al.* 1992). The advantages of active movement, broad host range and high pathogenicity in addition to the insensibility to many pesticides (Kovacs, 1982) substantiate the application of nematodes as an environmentally safe method of interest for both biological as well as integrated cultivation programs. The moist, humid larval galleries are ideal for nematode searching and survival (Kaya and Brown 1986). Nematodes are applied directly on the woody portions of trees with conventional spray equipment. The nematodes enter the borer feeding gallery, search, and attack borer larvae. Larvae are typically killed within 48 hours of attack by the *Xenorhabdus* bacteria symbiotically sustained by the nematode (Kaya and

Gaugler, 1993).

Nematodes have limitations to their use for insect control. Low humidity (below 60%), and high temperature (above 27 °C) are detrimental to entomopathogenic nematodes (Kaya 1977). Therefore, it is important to investigate the influence of temperature and humidity at the time of application on the efficiency of nematodes in searching and parasitizing *S. myopaeformis* larvae in their moist galleries.

This study was initiated to determine the effectiveness of three entomopathogenic nematodes of the genera *Steinernema* and *Heterorhabditis*, *i.e. Steinernema carpocapsae, S. glaseri* and *Heterorhabditis bacteriophora* as possible biological alternatives to chemical control of ACB larvae on apple trees. Field tests were carried out against larvae of *S. myopaeformis* in Fayoum and Giza Governorates. Data was evaluated, to determine the effectiveness of the 3 species of nematodes applied in different times with different techniques.

MATERIAL AND METHODS

The experiments of this study were conducted in Fayoum and Giza Governorates. The available nematodes used for this study were supplied by International Company for Bio-agriculture. The products contained 10.000.000 nematodes (J3) immobilized in an agar.

Nematodes were stored in the refrigerator at 14°C till application. Prior to application, they were mixed in the shade (according to label directions) with 2 liter of sterilized distilled water to make spray solution. Nematode activity in the spray solution was observed under magnification prior to application. It was determined that 1000 ml of spray solution was sufficient to treat each individual tree to a point of runoff using a Cooper Pegler CP-3 knapsack sprayer. All nematodes were applied at the rate of 5000 nematodes /1 ml of spray solution between 7.00 am and 10.00 am. The temperature and relative humidity were measured at the start and end of treatments.

Fayoum trials were carried out to evaluate the efficacy of two application methods conducted in two different times with the 3 species of nematodes.

Fayoum trials were located in an orchard where apples ("Anna" cultivar) were the predominant trees. Over 90% of the trees were infested with ACB. The damage was extensive enough that the trees were condemned and they be removed and destroyed. The trees exhibited upper canopy dieback and had numerous entry sites on the main trunk at crotches and on branches at branch junctures. An abundance of active brown frass and oozing of the sap was found extruding from the openings during the summer months. Also, large numbers of empty pupal skins could be seen from 0.6 cm circular exit holes.

The developmental cycle of clearwing moth favors nematode-treatments in spring or early autumn, *i.e.* after emergence of adults or prior to pupation. Thus, two application methods were applied throughout 1998 in two different times, *i.e.* spring and autumn trials. Twenty-four trees, which had at least two major scaffold branches with brown fresh frass/ooze, were selected for each trial (time). The trial design was a randomized complete block with 3 replications (trees) per treatment (Table 1).

There were eight treatments, including three nematode species which applied for each trial as two application methods: 1) sprays wetting of the bark with water (wb) using a hand pump sprayer, and 2) sprays directly to dry bark, a traditional Basudin treatment (3 cm / 1 liter) - which was selected because of its widespread use - and a non-treated control (Table 1).

In spring trial, *S. carpocapsae, S. glaseri* and *H. bacteriophora* were applied on early April 1998 (following the beginning adult emergence) targeted newly hatched and early instar borer larvae within the tree cambium. The weather was cloudy, temperatures ranged from 20 °C to 23 °C, and relative humidity of approximately 80.0% at 7:00 am, falling to 69% by 10:00 am.

In autumn trial, nematodes were applied on early September 1998 (prior the adult emergence) targeting mid and / or late-instar borer larvae beneath the tree cambium before they pupated. The weather was sunny, clear, with temperatures of 26 °C to 30°C, and relative humidity falling from 70% to 56%.

All treatments were applied to runoff using a knapsack sprayer. Sprays were applied from ground level to about 1.5-meter height on the main trunk of each tree, below major scaffold branches.

To determine efficacy, baited pheromone traps with synthetic clearwing moth sex attractant - consisting of the (Z,Z) and (Z,E) isomers of 3,13-ODDA [3,13-octadecadienyl acetate] **-** were placed in the orchard 2 meters from the ground in early March 1998, and in early September 1998, and inspected them weekly until no moths were captured for at least 7 days. When the first male adult of ACB was captured (mid - March 1998 and early October 1998), selected trees were examined for exposed pupal skins. The pupal skins protruding from the bark were recorded weekly in spring (from mid – March till early May) and autumn (from early October till mid – November). All data were subjected to analysis of variance; significant means were separated by LSD, (P = 0.05).

Giza trials were made in spring of 1999 using “Collar technique” to overcome the unfavorable weather conditions (high temperature and low relative humidity) that prevailing at the time of nematode application on trunks. For this purpose, an attempt were carried out to evaluate the efficacy of “Collar-application trial” in comparison with **“**Trunk spray application trial” in reducing the chance of desiccation before the nematodes reached the caterpillars within the galleries, thus, reducing the alive larvae. (showing high parasitism)

Trunks of nine highly infested apple-trees (“Anna” cultivar, seven year old, grafted on MM 106) in an orchard located in Giza Governorate were selected at random for each trial (application method). The trial design was a randomized complete block with three replicates. There were three treatments including three nematode species for each trial.

The “trunk spray application” trial was carried out in early April 1999 with *S. carpocapsae, S. glaseri,* and *H. bacteriophora* (air temperature 20-25°C, RH 80-70%). Each trunk was treated with a knapsack sprayer using 1000 ml of spray solution containing 5000 nematodes/1ml. The amount of spray solution was sufficiently soaking the whole trunk. The field tests were assessed 2 weeks later by carefully dissecting the insect larvae out of their galleries with a knife and examining each larva for nematode infection.

The 7-year-old apple-trees (young trees) were characterized by their even trunk surface without any wounds, so most of the larvae of *S. myopaeformis* can be found in the non-lignified area of the grafting knot (Nachtigall and Dickler 1992). Therefore a 'collar' made of sponge rubber material (about 3 times of diameter of grafting knot x 1 cm thickness) was soaked in 15 ml per tree of nematode solution of *S. carpocapsae, S. glaseri,* and *H. bacteriophora*. The prepared 'collars' were fixed around the grafting knot immediately. A thin plastic foil was wrapped around each collar in order to prevent desiccation. All 'collars' were removed seven days post-treatment.

The 'collar-application' was conducted in early May 1999 at hot temperature and dry humidity (unfavorable weather conditions where air temperature ranged 30-34 °C and RH ranged 45-55%). Prior to nematode treatment and after the 'collars' had been removed, the frass of all galleries was brushed of carefully. Fresh frass appearing until late June 1990 indicated that these galleries were still active (*i.e*. larvae alive). Comparison of frass production was used as an index for control of *S. myopaeformis.*

The percentage of larval mortality was analyzed by the analysis of variance; significant means were separated by LSD, ($P = 0.05$).

RESULTS AND DISCUSSION

Fayoum trials

Applications of entomopathogenic nematodes produced significant reductions in the number of emerging adults from trees treated at the 5000 nematodes/1 ml. Nematode treatments in spring and autumn caused approximately 28.8 % – 85.9 % reductions in the mean number of ACB pupal skins per tree as compared to the control (Table 1). The difference in the mean number of pupal skins per tree between spring and autumn trials was statistically significant ($F_{1,14}=11.39$, $P= 0.0452$).

Large numbers of pupal skins were recorded in autumn treatments as compared to spraying treatments. Autumn treatments of *Steinernema and Heterohabditis* nematode tree sprays targeting mid and / or larger clearwing borer larvae beneath the tree cambium showed moderate control levels, ranging from 51.4 – 63.7%, 42.7 – 55.1% and 28.8 – 33.7% for *S. carpocapsae, S. glaseri* and *H. bacteriophora*, respectively. Nematode treatments in the spring (targeting newly hatched and early instar borer larvae within the tree cambium) tended to give better control than autumn treatments. In spring trial, *S. carpocapsae, S. glaseri* and *H. bacteriophora* gave 61.5 – 85.9%, 50.9 – 65.0% and 32.5 – 46.6% control, respectively. These results support the results of previous investigations of entomopathogenic nematodes for control of clearwing moth borers (Davidson *et al*., 1992, Gill *et al*., 1992 and 1994, and Deborah *et al*., 1996).

Table (1): Control of the clearwing borer utilizing three nematodes species in spring (early April) and autumn (early September) of 1998.

Treatment	Spring		Autumn	
	Mean no. of pupal skins/ tree	% control	Mean no. of pupal skins/ tree	% control
S. carpocapsae (wb)	2.33 a	85.9	9.67 a	63.7
S. carpocapsae	6.33 b	61.5	13.00 b	51.4
S. glaseri (wb)	5.67 b	65.0	12.00 b	55.1
S. glaseri	8.00 c	50.9	15.33 c	42.7
H. bacteriophora (wb)	9.67 de	40.5	17.67 d	33.7
H. bacteriophora	11.00 e	32.5	19.00 d	28.8
Basudin	8.67 cd	46.6	19.00 d	28.8
Control	16.33 f	-	26.67 e	-
F	16.46**		11.24**	
$LSD_{0.05}$	1.36		2.11	

***wb: bark sprayed with water prior to nematode application.**

- Means followed by the same letter are not are not significantly different at 0.05 probability level.

**** Highly significant at 1% probability.**

Spring treatments may be preferable to autumn treatments since trees have sustained major damage as well as low spring temperature and high humidity are not as hostile to nematodes. In the spring, the treatments were applied at lower temperatures, higher humidity levels, and less intense sunlight. When treatments in autumn, the temperature was higher 6-7 °C, the relative humidity was lower by 10-13% and the sunlight was more intense. Perhaps these field conditions reduced nematode pathogenicity. In addition, it is suggest that nematode applications be applied in the spring when the frass is first detected. In the spring the larval galleries are short and closer to the bark surface making it easier for nematodes to contact the borer larvae which are smaller and more susceptible to nematode infection (Kaya, 1985).

These results are consistent with those from the California alder clearwing borer study where Kaya and Brown (1986) found that an October trunk spray with *Steinernema* nematodes targeting the sycamore clearwing borer did not give significant control. Also, Kaya (1988) achieved 77-84% and 60% control of *Synanthedon culiciformis* and *Synanthedon resplendens*, respectively using *S. carpocapsae* as spring bark sprays, while fall sprays failed to provide sufficient control.

Steinernema nematode treatments applied following the wetting of bark gave good control as compared to nematode treatment applied to dry bark. *S. carpocapsae* and *S. glaseri* with wetting of bark prior to application had significantly fewer pupal skins emerging per tree than application to dry bark (Table 1). In spring treatment, they provided 85.9% and 65.0% reduction in pupal skins per tree, relative to control as compared to 61.5% and 50.9% when treatments applied to dry bark. Results of autumn treatments showed similar tendency. However, application with *H. bacteriophora* did somewhat increase control (33.7% and 28.8% in spring and autumn, respectively), albeit not significantly. In this respect, Bedding and Miller (1981) found a reduced effectiveness of *H. heliothidis* compared to S. *carpocapsae* after treatment of blackcurrant cuttings infested with *Synanthedon tipuliformis.*

Wetting the tree bark prior to nematode application significantly enhanced nematode survival and control for *S. carpocapsae and S. glaseri*. The moist bark and amount of water applied with the nematodes may influence nematode survival, or perhaps facilitate entry into the galleries of borers. In comparison, nematode exposures to dry tree bark may have contributed to nematode mortality and reduced efficacy. Kaya and Brown (1986) determined that wetting the bark of sycamore trees prior to nematode application against the sycamore clearwing borer, may have enhanced spring nematode treatment results. In the same study, they reported that *Steinernema* nematode bark sprayer was not effective if the larval galleries were not sufficiently moist.

The results of wetting the bark obviously reflect sensibility of entomogenous nematodes to rapid desiccation often occurring on exposed surfaces like dry bark. J3, which did not reach their host until the spraying deposit evaporates, will dry up and die.

Basudin had significantly high pupal skins as compared to *Steinernema* nematodes. The Basudin treatment gave 46.6% and 28.8% reduction relative to the control in spring and autumn trials, respectively.

The ABC appears to be subject to attack by entomopathogenic nematodes because of its entry galleries, moist larval galleries, external bark openings, and frass-expelling behavior, which aid nematode survival and host searching efficiency. Since the concealed habitat of the ABC larva protects it from conventional insecticide applications, entomopathogenic nematodes offer a safe, potentially economical, and relatively effective means of reducing ABC larval populations after trees have been attacked, decreasing the need for protective chemical spays.

In conclusion, entomopathogenic nematodes, as a biological insecticide, have reached a level where they are both cost effective and practical to apply to control certain tree lepidopteran boring pests. This study demonstrates the validity of utilizing entomopathogenic nematodes for control of the ABC as trunk sprays under field conditions, with greater control achieved under condition of moist bark. It may be best to apply nematodes on pre-moistened bark in the morning or evening and on days, when humidity levels are higher to reduce nematode desiccation and mortality. These insect specific pathogens have many positive aspects including their ability to kill hosts within short time, ease of application and safety to humans and wildlife.

In addition, the chemicals must be applied as a preventative measure, thus timed to kill newly hatched borer larvae. Synthetic, long residual pesticides could possibly impact predator and parasite activity. Use of entomopathogenic nematodes for control of clearwing borers may be more compatible with insect predator and parasite activity.

Giza trials

Table (2) demonstrates the effectiveness of both described techniques (collar-application" and "trunk spray application") tested with *S. carpocapsae, S. glaseri,* and *H. bacteriophora* against *S. myopaeformis* hidden under the grafting knot. A direct comparison shows clearly the advantage of the 'collar' at hot temperature and low relative air humidities. At same concentration, a significant difference between the two methods could be obtained. The 'collar' reduced frass activity of the *S. myopaeformis* larvae with 95.7, 86.2 and 54.7%. On the contrary, trunks treated with the knapsack-sprayer resulted in larval mortality rates of 85.6, 65.1 and 37.5%, respectively.

Table (2): Percent reduction of *S. myopaeformis* larvae based on frass activity after application with three nematodes species at spring of 1999.

Nematodes	Application method	Mean no. of galleries / tree		% reduction in larvae
		Active	Inactive	
S. carpocapsae	collar	0.67	15.00 a	95.7
	sprayer	3.67	21.67 b	85.5
	F		12.69**	
	LSD 0.05		3.37	
S. glaseri	collar	2.67	16.67 a	86.2
	sprayer	7.37	13.67 b	65.1
	F		13.97**	
	LSD 0.05		1.44	
H. bacteriophora	collar	9.67	11.67 a	54.7
	sprayer	11.67	7.00 b	37.5
	F		19.67**	
	LSD 0.05		1.91	

- Means followed by the same letter are not are not significantly different at 0.05 probability level.
**** Highly significant at 1% probability.**

The spray treatment of the bark surface with *S. carpocapsae, S. glaseri,* and *H. bacteriophora* at low temperatures (20-25 °C) and relatively high humidities (70-80%) resulted in 37.5-85.5% reduction of S. *myopaeformis* larvae. Extremely hot temperature (30-34 °C) and dry weather conditions (45-55% RH) at the time of collar wrapped around the grafting knot in May 1999 not caused an immediate evaporation of the nematode. Therefore, high percentages of the host larvae (54.7-95.7%) were reduced.

H. bacteriophora, do not show sufficient parasitation properties in the two application methods although the weather conditions were ideal for a both other nematodes treatments . A mean mortality of 54.7 and 37.5% was recorded for collar and trunk applications, respectively. Deseo *et al.* (1984) obtained a similar effect in Northern Italy with the strain IH of *Heterorhabditis.* Also, examining galleries of *Synanthedon tipuliformis* short time after nematode treatment, Bidding and Miller (1981) reported that a lower proportion of *H. heliothidis* entered the galleries compared to *S. bibionis.*

Sufficiently low temperature and high air humidity during and shortly after application are the minimum requirement for a successful treatment.

The disadvantages unfavorable weather conditions such as high temperature and drying effect of wind can be avoided by employing the suitable techniques of “wetting the bark prior to application” and “collar-application”. Both techniques require low labor input - where they are quick faster than gallery or soil injection - and proved to be successful for controlling ACB larvae. In this respect, Kaya and Brown (1986) reported that reduction of damage in commercial orchards by means of injecting the galleries of wood-boring insect larvae - as often described for ornamental plants is far more time-consuming. In addition, the frass galleries are often concealed under the grafting knot, thus being difficult to be recognized.

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تقييم فعالية بعض أنواع النيماتودا الممرضة كوسيلة للمكافحة البيولوجية لحفار ساق الحلويات رائق الأجنحة على أشجار التفاح

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كان الهدف الرئيسي من هذه الدراسة هو تقييم فعالية ثلاثة أنواع من النيماتودا الممرضة التابعة لجنسي *Steinernema*، و *Heterorhabditis* (*Steinernema carpocapsae*، *S glaseri*، و *Heterorhabditis bacteriophora*) في مكافحة حفار ساق الحلويات رائق الأجنحة على أشجار التفاح في محافظتي الفيوم خلال موسم 1998 والجيزة خلال موسم 1999 0

ولقد أظهرت التجارب الحقلية التي أجريت في محافظة الفيوم رشاً على الأشجار المصابة بالحفار - باستخدام الثلاثة أنواع من النيماتودا بالإضافة إلى البازودين - أن النيماتودا سببت إنخفاضاً في تعداد جلود العذارى تراوح من 28.8 % إلى 85.9 %0 وكان التطبيق الحقلي في الربيع أكثر معنوية في المكافحة (85.9-61.5%، 65.0-50.9%، و 46.6-32.5%) لكل من *S. carpocapsae*، *S. glaseri*، و *H. bacteriophora* على التوالي) بالمقارنة بتطبيق الخريف (63.0-51.4%، 55.1-42.7%، و 33.7-28.8% على الترتيب) 0 كذلك، فإن إبتلال القلف بالماء قبل المعاملة بالنيماتودا سبب إنخفاضاً معنوياً في تعداد جلود العذارى بالمقارنة بمعاملة القلف الجاف مباشرة0 وأظهرت المعاملة بالبازودين إنخفاضاً بسيطاً في تعداد جلود العذارى0

كما أظهرت التجارب الحقلية التي أجريت في محافظة الجيزة تفوق طريقة " الطوق Collar" المصنوع من مطاط إسفنجي في مكافحة اليرقات بنسب 95.7, 86.2 و54.7% لأنواع النيماتودا الثلاثة على الترتيب بالمقارنة بـ 85.6 ، 65.1 ، و37.5% على التوالي عند إستخدام طريقة رش القلف0

وقد أمكن توفير الرطوبة المناسبة في وقت المعاملة والضرورية لفعالية النيماتودا باستخدام كل من طريقة "إبتلال القلف بالماء قبل المعاملة بالنيماتودا" وطريقة " الطوق"0