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Effects of Entomopathogenic Nematodes on Suppressing Hairy Rose Beetle, *Tropinota squalida* Scop. (Coleoptera: Scarabaeidae) Population in Cauliflower Field in Egypt

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***Abstract*—**The potential of entomopathogenic nematodes in suppressing *T. squalida* population on cauliflower from transplanting to harvest was evaluated. Significant reductions in plant infestation percentage and population density (/m^2) were recorded throughout the plantation seasons, 2011 and 2012 before and after spraying the plants. The percent reduction in numbers/m^2 was the highest in March for the treatments with *Heterorhabditis indica* Behera and *Heterorhabditis bacteriophora* Giza during the plantation season 2011, while at the plantation season 2012, the reduction in population density was the highest in January for *Heterorhabditis Indica* Behera and in February for *H . bacteriophora* Giza treatments. In a comparison test with conventional insecticides Hostathion and Lannate, there were no significant differences in control measures resulting from treatments with *H. indica* Behera, *H. bacteriophora* Giza and Lannate. At the plantation season is 2012. Also, the treatments reduced the economic threshold of *T. squalida* on cauliflower in this experiment as compared with before and after spraying with both the two entomopathogenic nematodes at both seasons 2011 and 2012. This means an increase in the marketability of heads harvested as a consequence of monthly treatments.

***Keywords*—**Cruciferous plants, chemical insecticides, microbial control, Scarabiead beetles, seasonal monitoring.

I. Introduction

THE rose chafer or the hairy rose beetle, *Tropinota squalida* (Scop.), is known to heavily infest crucifers (canola, cabbage, cauliflower) especially at the flowering stage. It also infests many other plants and flowers. Ecological studies showed that adults of *T. squalida* preferred the top parts of plants and larvae were observed at 20 cm depth Rezk et al. [1]. The maximum infestation level in all seasons for all host plants studied occurred during March. [1]-[3] and Schmera et al. [4] reported that *T. hirta* was attracted to yellow; white or light blue colors with the light blue ones attracting more beetles and approved to be suitable for seasonal monitoring of the beetles.

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The control recommendation for populations of this insect pest over the years has been done using chemical insecticides such as Hostathion (40%) and Lannate (90%).

Due to all the various problems and side effects associated with the synthetic insecticides, bioinsecticides are being recommended as an alternative. The entomopathogenic nematodes of the family Heterorhaditidae are one of the potential alternatives. Entomopathogenic nematodes are obligate parasites kill insects with the aid of a mutualistic bacterium, which is carried in their intestine [5]. The nematodes complete 2-3 generations within the host, after which free living infective juveniles (IJs) emerge to seek new hosts Poinar [5], Villani and wright [6]. Entomopathogenic nematodes are effective at controlling a variety of economically important pests including the larvae of several weevil species (Coleoptera: Curculionidae) such as the Diaprepes root weevil, *Diaprepes abbreviate*s (L.) [7].

The potential for control of *T. squalida* larvae and adults on cauliflower plants with entomopathogenic nematodes appears to be poorly treated in the literature.

Our objective was to explore the potential of *H. indica* Behera and *H. bacteriophora* Giza as Egyptian isolates compared with two of synthetic insecticides in reducing *T. squalida* population on cauliflower plants at El-Nobaria District in Egypt, during the plantation seasons 2011 and 2012.

II. Materials and Methods

The experiments were conducted during 2011 and 2012 from January to May at the National Research Centre farm in El-Emam Malek village, 130 km northwest of Cairo. Seedlings of the locally popular cauliflower (*Brassica oleraceae* var. *botrytis*) were raised in the field and transplanted bare rooted at the four to five leaf stage into the different plots of the experiments. Cultural practices (Fertilization, irrigation, weeding, etc.) were carried out according to recommendations of the horticulture division of the Ministry of Agriculture.

A. Nematodes

Strains of H. *Indica* Behera and *H. bacteriophor*a Giza were selected and cultured in the last instars larvae of G.

mellonella according to procedures described in Kaya and Stock [8]. Nematodes were stored for up to 3 weeks prior to use.

1. Determination of Infestation Levels and Population Densities during 2011 and 2012 Plantation Seasons

The cauliflower plants in this test were cultivated in an area of 75m^2, divided into equal plots of 1.5×1.5m. Plots consisted of three rows with 50cm row spacing and 30cm plant spacing. Plots were arranged into three blocks (one for the untreated plants, one for the treated plants with *H. Indica* Behera and one for the treated plants with *H. bacteriophor*a Giza of three plots each. All the treatments were assigned to the plots in a randomized complete block design with three replications.

2. Comparable Effects of Entomopathogenic Nematodes with the Conventional Insecticides

The cauliflower plants in this test were cultivated in an area of 125m^{2,} divided into equal plots of 1.5×1.5m. Plots consisted of three rows with 50cm row spacing and 30cm plant spacing. Plots were arranged into five blocks (one for Hostathion treatment, one for Lannate treatment, one for *H. indica* Behera treatment, one *H. bacteriophor*a Giza and one for untreated plants) of three plots each. All the treatments were assigned to plots in a randomized complete block design with three replications.

3. Experimental Compounds Used

a) The entomopathogenic nematode, *H. Indica* Behera isolate, was used at a concentration of 1500 infective juvenile (Ij's)/ml.
b) The entomopathogenic nematode, *H. bacteriophor*a isolate, was used at a concentration of 1500 infective juvenile (Ij's)/ml.
c) The conventional insecticide, Hostathion, (E.C.40%) at the recommended concentration of 1.00 L. /400L.H_2O/F., and Lannate (90% Powder) at the concentration of 1.2Kg/400 H_2o/F.

A wetting agent and a sun screening substance were used at the rate of 3% for each spray. The control treatment received only water and the wetting agent and sun screening substance. All compounds were diluted in water and a twenty liter sprayer was used to apply the different compounds. Spraying buds and flowers of the cauliflower plants were started at the second week of January 2011 for the first experiment and for four times with one week interval for the second experiment and Percentage insect – infesting plants and insect population densities were recorded before and one week after each spraying time, adults were collected from flowers while larvae the soil under checked plants. Ten randomly selected plants were sampled in each sampling area for each plot.

B. Statistical Analysis

All data were subjected to Analysis of Variance (ANOVA) and means separated by Duncan's multiple range–test. Percentage data were arcsine-transformed before analysis.

III. Results and Discussions

A. Effects on Infestation Levels and Population Densities during 2011 and 2012 Plantation Seasons

In cauliflower plantation season 2011, the percentage of plants infested showed a maximum infestation level of 50.6% during March 2011 and a lower infestation level of 15.8% during January, before spraying with the entomopathogenic nematode, *H. indica* Behera. After spraying, the reduction in percent infestation compared with that before spraying were evident in all months of the plantation season 2011 (Table I). The population density of *T. squalida*/m^2 before spraying with entomopathogenic nematode, *H. indica* Behera showed insignificant differences between the higher records of March and April 2011, 2.47 and 2.06, respectively. After spraying with *H. indica* Behera, the highest population density was recorded during April.

The protection of plants by the application of *H. indica* Behera resulted in a highly significant reduction in population density after spraying during March with 64.4% reduction, followed by a 45.63% reduction in the population density during April, Table I.

Table II showed that cauliflower infestation levels were considerably high in March and April as compared with the other months and ranged between 10.9 and 55.10% before spraying with *H. bacteriophora* Giza throughout the 2011 plantation season. The population density was significantly low in January as compared with that at March and ranged between 0.34 and 2.01, respectively.

Plots treated with *H. bacteriophora* Giza during the growing period had significantly lower *T. squalida* infestation levels and population densities than that recorded before spraying (Table II).

In the next season of cauliflower plantation 2012, the rate of plant infestation was lower before spraying with *H. indica* Behera as compared with that before spraying in the 2011 plantation season. This reached in some months to more than half with a range of infestation between 9.32 – 41.22%. This range was significantly lowered after spraying with *H. indica* Behera recording a range of 6.30 – 15.57% (Table III). The population density showed a reduction after spraying compared with that before spraying with a maximum reduction in population of 94.7% during January. At the late season the reduction in population density was 77.55% Table III.

The same trend was also, recorded with *H. bacteriophora* Giza treatment (Table IV). The decrease in infestation level was most obvious during the late season 2012. The population density was low initially after spraying at the beginning of the season with at least a threefold decrease as compared with that before spraying, Table IV. The percentage reduction in population after spraying was significantly higher 83.33% during February 2012 as compared with that recorded for January, March and April. A lower degree of reduction (64.91%) was recorded during the second season at May as

compared with that for the other months and for the other treatment.

TABLE I
MEAN INFESTATION LEVELS AND POPULATION DENSITIES OF *T. SQUALIDA* IN CAULIFLOWER CULTIVAR IN 2011 AND AS EFFECTED BY SPRAYING WITH *H. INDICA BEHERA*

Period	Before Spraying		After Spraying		% Reduction in Population Density
	% Plants Infested	Population Density/m^2 ± SE	% Plants Infested	Population Density/m^2 ± SE	
January	15.8	0.32 ± 0.12^{a}	12.39	0.26 ± 0.11^{a}	18.75
February	23.66	0.98 ± 0.14^{b}	15.48	0.70 ± 0.16^{b}	28.57
March	50.60	2.47 ± 0.70^{c}	38.66	0.88 ± 0.17^{c}	64.37
April	40.11	2.06 ± 0.12^{c}	19..22	1.12 ± 0.34^{d}	45.63
May	23.47	1.15 ± 0.65^{bc}	12.01	1.01 ± 0.22^{d}	12.17

Means within a column followed by a common letter are not significantly different at the 5% level

TABLE II
MEAN INFESTATION LEVELS AND POPULATION DENSITIES OF *T. SQUALIDA* IN CAULIFLOWER CULTIVAR IN 2011 AND AS EFFECTED BY SPRAYING WITH *H. BACTERIOPHORA GIZA*

Period	Before Spraying		After Spraying		% Reduction in Population Density
	% Plants Infested	Population Density/m^2 ± SE	% Plants Infested	Population Density/m^2 ± SE	
January	10.90	0.34 ± 0.12^{a}	11.65	0.30 ± 0.10^{a}	11.76
February	21.40	1.47 ± 0.19^{b}	17.32	1.00 ± 0.11^{b}	31.97
March	51.08	2.01 ± 0.02^{bc}	31.70	1.08 ± 0.32^{bc}	46.27
April	55.15	1.98 ± 0.15^{d}	33.40	1.70 ± 0.66^{cd}	14.10
May	30.20	1.71 ± 0.18^{bc}	14.15	1.18 ± 0.07^{bc}	31.00

Means within a column followed by a common letter are not significantly different at the 5% level

TABLE III
MEAN INFESTATION LEVELS AND POPULATION DENSITIES OF *T. SQUALIDA* IN CAULIFLOWER CULTIVAR IN 2012 AND AS EFFECTED BY SPRAYING WITH *H. INDICA BEHERA*

Period	Before Spraying		After Spraying		% Reduction in Population Density
	% Plants Infested	Population Density/m^2 ± SE	% Plants Infested	Population Density/m^2 ± SE	
January	9.44	0.19 ± 0.01^{a}	9.01	0.01 ± 0.01^{a}	94.7
February	17.70	0.27 ± 0.09^{ab}	14.01	0.02 ± 0.00^{a}	912.60
March	41.22	1.00 ± 0.21^{c}	15.57	0.14 ± 0.06^{b}	86.00
April	20.20	0.51 ± 1.01^{d}	14.21	0.19 ± 0.02^{b}	62.72
May	9.32	0.49 ± 0.18^{abd}	6.30	0.12 ± 0.01^{b}	77.55

Means within a column followed by a common letter are not significantly different at the 5% level

TABLE IV
MEAN INFESTATION LEVELS AND POPULATION DENSITIES OF *T. SQUALIDA* IN CAULIFLOWER CULTIVAR IN 2012 AND AS EFFECTED BY SPRAYING WITH *H. BACTERIOPHORA GIZA*

Period	Before Spraying		After Spraying		% Reduction in Population Density
	% Plants Infested	Population Density/m^2 ± SE	% Plants Infested	Population Density/m^2 ± SE	
January	11.04	0.32 ± 0.01^{a}	8.47	0.08 ± 0.11^{a}	75.00
February	16.75	1.02 ± 0.05^{b}	15.05	0.17 ± 0.30^{b}	83.33
March	32.30	0.75 ± 0.11^{bc}	20.45	0.23 ± 0.25^{c}	69.33
April	32.30	0.57 ± 0.02^{d}	15.30	$0.19 \pm 0..01^{b}$	66.67
May	21.80	0.57 ± 0.06^{d}	4.98	0.20 ± 0.11^{abc}	64.91

Means within a column followed by a common letter are not significantly different at the 5% level

B. Comparison of Entomopathogenic Nematodes with the Conventional Insecticides

The plots treated with *H. indica* Behera, resulted in significantly lower numbers of *T. squalida*/m^2 as compared to untreated plots and similar to that obtained by Lannate and with *H. bacteriophora* Giza treated plots. The percentage control of beetle on cauliflower plants with both entomopathogenic nematodes was lower as compared with that which resulted from treatment with Lannate and Hostathion in both seasons (Tables V and VI).

T. squalida damages the heads and reproductive parts of flowers of several orchards trees and many ornamental bushes. It is active during daytime and hides in the soil during the night Schmera [4], Homonnay and Homonnayne [9]. Control of this pest is difficult, as most insecticides should not to be applied during flowering and because of their residual effects. So, the results in this study explore the importance of using the entomopathogenic nematodes to avoid the environmental hazards of chemical insecticides.

TABLE V
COMPARABLE EFFECTS OF H. INDICA BEHERA, H. BACTERIOPHORA GIZA, LANNATE AND HOSTATHION ON HAIRY ROSE BEETLE NUMBERS ON CAULIFLOWER PLANTS DURING THE PLANTATION SEASON 2011 IN NOBARIA DISTRICT

Treatment	No. of hairy rose beetle / m^2			% Control
	Larvae	Adults	Total	
Hostathion	8.35^{a}	9.38^{a}	17.73^{a}	75
Lannate	9.62^{ab}	8.55^{b}	20.01^{b}	70
H. indica Behera	9.83^{b}	12.30^{b}	21.32^{b}	54
*H. bacteriophor*a Giza	5.68^{c}	14.20^{c}	20.87^{c}	63
Control	17.33^{d}	15.65^{d}	33.55^{d}	

Means within a column followed by a common letter are not significantly different at the 5% level.

TABLE VI
COMPARABLE EFFECTS OF *H. INDICA BEHERA*, *H. BACTERIOPHORA GIZA*, LANNATE AND HOSTATHION ON HAIRY ROSE BEETLE NUMBERS ON CAULIFLOWER PLANTS DURING THE PLANTATION SEASON 2012 IN NOBARIA DISTRICT

Treatment	No. of hairy rose beetle / m^2			% Control
	Larvae	Adults	Total	
Hostathion	5.11^{ab}	10.32^{a}	13.40^{a}	78
Lannate	4.20^{b}	9.22^{b}	15.40^{b}	69
H. indica Behera	5.50^{b}	9.22^{b}	14.65^{b}	60
*H. bacteriophor*a Giza	4.65^{b}	8.65^{c}	15.72^{c}	68
Control	12.01^{c}	12.32^{d}	28.35^{d}	

Means within a column followed by a common letter are not significantly different at the 5% level

Data from treatments of cauliflower with *H. indica* Behera and *H. bacteriophora* Giza through the plantation seasons 2011 and 2012 explored a significant reduction in percentage of plants infested and population density/m^2 after spraying of plants each month as compared with that before spraying. Also, the percentage of reduction in population density was the highest in March during both the plantation seasons at both treatments as compared with that in the other months. Also, the results showed the importance of entomopathogenic nematodes in controlling larvae and adults of the hairy rose beetle where they gave control measure ranged between 54 – 68 % throughout both seasons. Previous experiments on how the entomopathogenic nematodes kill insects had been described by Gotz [10], Dunphy [11]. Our results showed that *H. indica* Behera was the most effective in reducing the infestation level, population density and control of adults and larvae of *T. squalida* on cauliflower plants as compared with the other entomopathogenic nematodes, *H. bacteriophor*a Giza. It is suggested that toxins in the bacteria and in their metabolites are responsible for the lethal effects observed. A patent has been applied in the USA for the use of toxins from *X. nematophilus* spp. for the insect control Ensign et al. [12].

The potential for use of nematode-bacterial complex in control of scarabaeid larvae has been demonstrated by Forschler [13]; Georges and Gaugler [14], Kline and Georges [15]. In the field [15] found that 310×10^{9} per ha of *H. heliothids* provided >60% control of *P. japonica* which was equivalent to the control achieved with labeled rates of chlopyrifos, trichlorfon and isofenfos.

It seems likely that the entomopathogenic nematodes could be used without normal toxicological data being required. The entomopathogenic nematodes used in this study can be diluted in water, so they can be washed away in the soil under heavy rains or irrigation and thus little environmental hazards besides it helps in the control of soil pests. From the present experiments we may conclude that mortality of *T. squalida* in the field could be increased by increasing entomopathogenic nematodes concentrations used. Entomopathogenic nematodes in this study reduced the damage caused by *T. squalid*a to cauliflower, so they can be greatly recommended for use in IPM strategies. These entomopathogenic nematodes as a biological control agent could alternate the use of hazardous chemical insecticides.

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