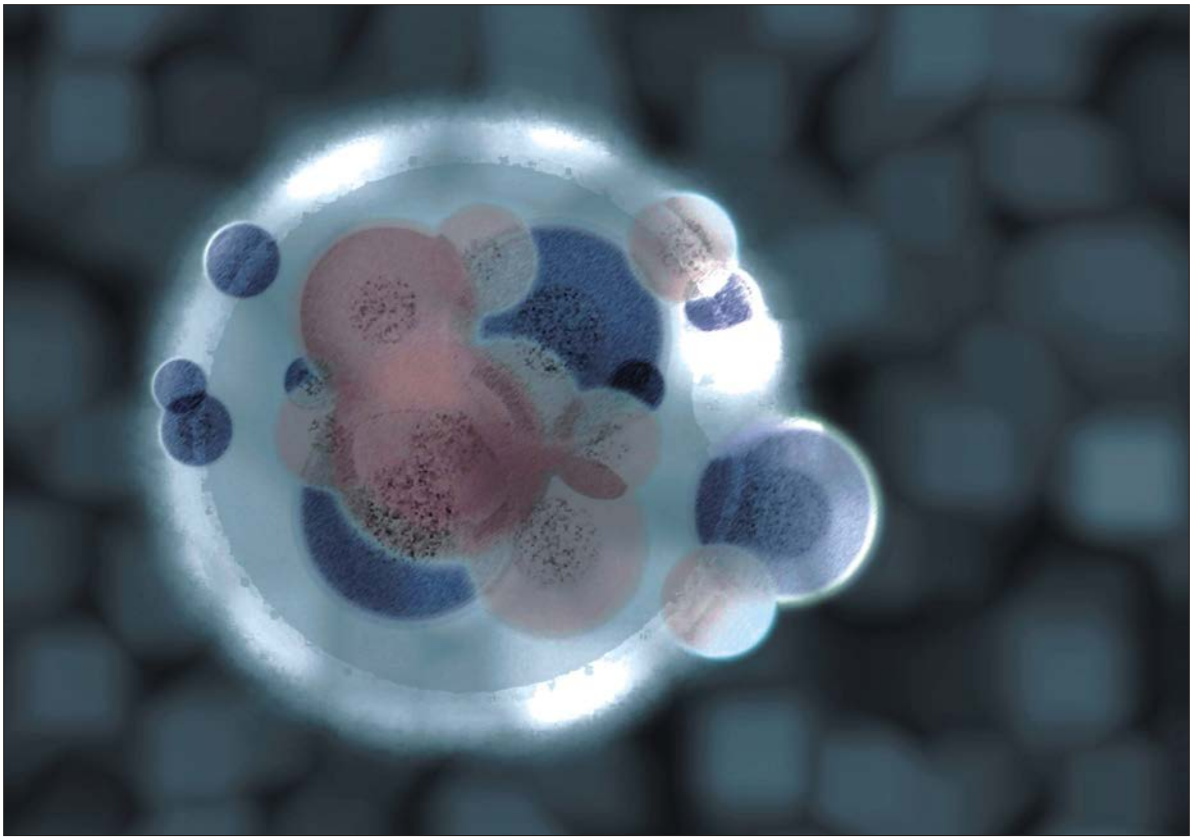

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Research Article

Effect of Entomopathogenic Nematodes, *Heterorhabditis bacteriophora* on *Galleria mellonella* in Bee Hives of *Apis mellifera*

Entsar H. Taha and Sawsan M. Abdelmegeed

Department of Plant Protection, Faculty of Agriculture, Ain Shams University, Egypt

Abstract

Background: Wax worms caused economically damage in storage wax, chemicals which uses in control caused bad effect on stored bee honey inside combs. The present study was carried out to investigate the effect of entomopathogenic nematodes (EPNs) on *Galleria mellonella* (Gm) in the natural bee hives. **Materials and Methods:** Seven different inoculum levels of *Heterorhabditis bacteriophora* (Hb) (100, 500, 1000, 2000, 3000, 4000 and 5000 Ijs mL^{-1}) were investigated on wax worms (Gm), larvae and adult of honey bees *Apis mellifera* inside the bee hives for 1 and 2 times. **Results:** The highest mortality percentage were 90 and 100% when 5000 Ijs mL^{-1}. were used for once and twice, respectively. On the other hand, the EPNs didn't effect on larvae or adult of honey bees. The initial population (Pi), final population (Pf) and rate of reproduction (Rr) were affected with the increase of EPNs inoculum levels and duplication of treatment. The Pi was 648.00 and 766.67 per insect cadaver at 5000 IJs for 1 and 2 times. The highest Pf was 61083.33 and 246166.7 when 2000 IJs were used for 1 and 2 times, respectively. **Conclusion:** The high concentration of EPNs cause 100% mortality for *G. mellonella* and there is no side effect on larvae or adult of honey bees. The EPNs can be used to control G. *mellonella* instead of chemicals without worried about honey bee.

Key words: Entomopathogenic nematodes, wax worms, honey bees, *Heterorhabditis bacteriophora*

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Corresponding Author: Entsar Helmy Taha, Department of Plant Protection, Faculty of Agriculture, Ain Shams University, Egypt Tel: 00201124373795

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Most apiaries in Egypt and all over the world are suffering from parasites[1,2], predators[3,4] and many diseases[5,6]. The wax worms infected the stored wax comb in the winter and caused great damage and economic losses for beekeepers. Many investigators were studied using of some plant volatile oils, plant extract and chemicals in order to control the wax worms[7-9]. Chemical pesticides are effect on bees and stored bee honey inside wax combs[10,12]. In recent years the biological control agent such as EPNs, *Steinernema* and *Heterorhabditis* are used to control many of insect pests above and below ground[13-16]. Shapiro-Ilan *et al.*[17] found that *H. indica* caused mortality in small hive beetles over than 78%.

The aim of this study is to control wax worms inside the bee hives using more safety agent: Entomopathogenic nematodes.

MATERIALS AND METHODS

Materials

Multiplication and maintenance of the entomopathogenic nematodes: Entomopathogenic nematodes (EPNs) of *Heterorhabditis bacteriophora* (Hb) was obtained from Plant Protection Department, Faculty of Agriculture, Ain Shams Univeristy, Shoubra El-Kheima. The nematodes were propagated on the greater wax moth (*Galleria mellonella* L.), a lepidopteran host that is highly susceptible to parasitic infection. Fifth instar of the insect larvae were placed in a 9 cm diameter petri dish lined with a moistened filter paper and exposed to 100 IJs at 25±2°C. After 48 h, dead larvae were transferred to the white trap dishes. After 6-10 days, IJ3s were collected. These IJs must be used for experiments during 2 week period.

Insect host (greater wax moth, *Galleria mellonella*): Larvae of *G. mellonella* were obtained from the same laboratory, Plant Protection Department, Faculty of Agriculture, Ain Shams University, Shoubra El-Kheima and reared on old bee wax in transparent plastic jars at 28±2°C, in the laboratory. Eggs that laid in masses were gently removed and incubated in other rearing jar provided with old bee wax.

Experimental methods

Effect of EPNs inoculum levels on the greater wax moth, *Galleria mellonella* within the hive: Seven increasing inoculum levels of *H. bacteriophora* (Hb), (100, 500, 1000, 2000, 3000, 4000 or 5000 Ijs mL^{-1}) were used to study the effect of the EPNs of Hb on *G. mellonella* Larvae withen hive. In order to do that, 24th larval instar of *G. mellonella* were added to a small combs (20×20 cm), after 2 days each combs was sprayed with 10 mL per side of nematode suspension or water as a control. Each treatment was repeated twice in a separate hive. After 3 days insect larvae were collected from the each comb and mortality percentages were calculated. Dead cadavers were divided into two groups, the larvae of the first one were dissected and invasive nematodes were counted (Pi), while the larvae of the second group were transferred individually into the white traps, After 6-10 days, the emerged IJs were collected (Pf) and the rate of reproduction (Rr) was calculated.

Susceptibility of honey bee larvae: The same previous inocula of *H. bacteriophora* (Hb), were used to study the effect of Hb IJs on the larvae of honey bee. Data were collected in the same way until the end of larval stage.

Susceptibility of adult honey bee workers: Ten adult worker bees were collected and putted in a small glass jar with a piece of stalk, each jar received 5 mL of one of the previous inoculum levels. Each treatment replicated twice and the data were obtained in the same way.

Statistical analysis: The data of all experiments were statistically analyzed using analysis of variance to check the significance of the differences between treatments using SAS program (2005) and separation between means was applied by Tukey test.

RESULTS AND DISCUSSION

Effect of *Heterorhabditis bacteriophora* on larvae and adult workers of honey bees: The 3rd instar larvae and adult workers of honey bees did not affect by the deferent inoculum levels or the duplication of treatments with EPNs, mortality percentages were zero, while Baur *et al.*[18] found that, the *Heterorhabditis bacteriophora* and *Steinernema* spp. affected on the adult of bees and caused less than 10% mortality.

Effect of *Heterorhabditis bacteriophora* (Hb) on mortality of wax worms: Figure 1 shown that, the mortality in larvae of Gm in bee combs affected by different inoculums levels of Hb. The percentage of mortality increased with the increasing of EPNs inoculum levels and the duplication of

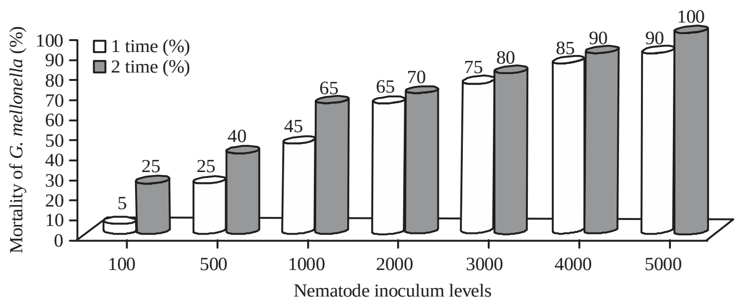

Fig. 1: Effect of different inoculum levels of EPNs *Heterorhabditis bacteriophora* (Hb) on *Galleria mellonella* (Gm) in bee hives. The same letters above bars have no significant differences, while different letters above bars indicate statistically signi cant differences in their effects

Table 1: Effect of different inoculum level of *H. bacteriophora* (Hb) on initial invasive populations (Pi) and final populations (Pf) withen *Galleria mellionella* in bee hives for 1 time

Nematode inoculum levels	Pi	Pi (%)	Pf	Rr
100	44.000±2.94	44.00±2.94	14833.33±1433.70	34017.93±5260.90
500	113.67±11.32^{ef}	22.73±2.26^{b}	26666.67±962.96^{cd}	23616.37±1647.16^{bc}
1000	187.00±12.83^{de}	18.70±1.28^{b}	53416.67±10985.47^{ab}	28325.63±4034.61^{ab}
2000	224.00±10.23^{d}	11.20±0.51^{c}	61083.33±5001.39^{a}	27283.39±2014.54^{abc}
3000	352.67±5.31^{c}	11.75±0.18^{c}	60366.67±8936.94^{a}	17128.65±2581.62^{cd}
4000	451.67±48.99^{b}	11.29±1.22^{c}	44500.00±4636.81^{abc}	10100.31±2286.43^{de}
5000	648.00±50.92^{a}	12.96±1.02^{c}	32833.33±3064.13^{bcd}	5094.660±611.50^{e}
F. value	113.84**	108.32**	17.05**	24.30**
LSD	95.079	5.5168	20788	10243

Numbers followed by the same letters did not differ significantly in their effects, while different letters had a statistically significant differences

treatments. The lowest percentage of mortality occurred when treated with 100 IJs (5 and 25% for 1 and 2 times, respectively). About 90% mortality occurred when treated with 4000 IJs for 2 times, while the same ratio was obtained with 5000 IJs for 1 time and 100% mortality occurred when treated with 5000 nematodes for 2 times.

Effect of *Heterorhabditis bacteriophora* (Hb) on initial populations (Pi), final populations (Pf) within *Galleria mellionella* in bee hives for 1 time: Table 1 clearly show that, Pi, Pf and Rr were affected significantly by the different inoculum levels of Hb in Gm larvae for 1 time. The lowest mortality percentage was obtained at 100 IJs 44.00%, while the highest was obtained with 5000 IJs, 648.00%. There was no significance in Pi% with 2000, 3000, 4000 and 5000 IJs and the same data were obtained in final reproduction. In the case of the rate of reproduction the highest Rr were recorded in 100, 500, 1000 and 2000 IJs without significance (34017.93, 23616.37, 28325.63 and 27283.39, respectively).

Effect of *Heterorhabditis bacteriophora* (Hb) on initial populations (Pi), final populations (Pf) within *Galleria mellionella* in bee hives for 2 times: In the case of duplicated the treatments, initial population, final population and rate of reproductive of Hb were affected by different inoculum levels of Hb for 2 times. The initial population of Hb increased significantly when increased the inoculum levels of Hb. The lowest Pi was 52.32, while the highest was 766.67 with 100 and 5000 of Hb, respectively. The percentage of initial population was significant with 100, 500, 1000 and 2000 IJs but no significance with 2000, 3000, 4000 and 5000 IJs. The same data was obtained with the final population, the higher final population (246166.7) was occurred with 2000 IJs. The highest rate of reproduction was recorded with 100 and 500 IJs (125413.3 and 111383.1, respectively) without significance while the lowest ratio was found with 4000 and 5000 IJs (42205.4 and 31283.9, respectively) Table 2.

Even honey bee are not highly affected by entomopathogenic nematodes, other hymenopteran are more susceptible. For example, yellow jacket (*Vespula vulgaris* L.) and *V. pennsylvanica* (Saussure)[19] and imported red fire ant (*Solenopsis invicta* Burren)[20] can be controlled by using EPNs.

Bee hive conditions (temperature, moisture), behavioral differences between species[21] or physiological differences between species may be cause the differences in susceptibility to EPNs infection.

Table 2: Effect of different inoculum level of *H. bacteriophora* (Hb) on initial invasive populations (Pi) and final populations (Pf) withen *Galleria mellionella* in bee hives for 2 time

Nematode inoculums level	Pi	Pi (%)	Pf	Rr
100	52.330±3.68[e]	52.33±3.68[a]	65000.00±5715.5[c]	125413.3±19050.8[a]
500	134.67±6.18[e]	26.93±1.24[b]	150000.0±12267.8[b]	111383.1±7340.1[ab]
1000	271.67±37.28[d]	27.17±3.73[b]	235333.3±8993.8[a]	88268.80±12265.6[bc]
2000	346.67±8.58[d]	17.33±0.43[c]	246166.7±6637.4[a]	71097.70±3607.5 [cd]
3000	453.67±17.25[c]	15.12±0.57[c]	242333.3±13193.0[a]	53516.70±3953.3[de]
4000	570.67±15.75[b]	14.27±0.39[c]	241000.0±38893.0[a]	42205.40±6497.6[de]
5000	766.67±55.53[a]	15.33±1.11[c]	239000.0±41206.8[a]	31283.90±5592.1[e]
F. value	168.31**	84.20**	18.35**	26.16**
LSD	92.571	7.1722	78428	33391

Numbers followed by the same letters did not differ significantly in their effects while, different letters had a statistically signi cant differences

CONCLUSION

Entomopathogenic nematodes can use as an alternative control for *G. mellonella* in the natural environment of bee hives which are more safety than chemical to avoid the chemical side effect on honey bees. The highest mortality percentage can be obtained with a high inoculum level of EPNs even for 1 or 2 times.

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