Efficacy of Entomopathogenic Nematodes Against Three Species of Stored Product Insects

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Abstract

Two species of entomopathogenic nematodes (EPNs), Steinernema riobravi, symbolized by Sr, and Heterorhabditis bacteriophora, symbolized by Hb, were tested by using five concentrations: 125, 250, 500, 1000, and 2000 an infectious phase, ml of the type Sr and four concentrations of 500, 1000, 2000 and 3000 infectious phase/ ml of the type Hb against Cowpea weevil Callosobruchus maculatus, Khapra beetle, Trogoderma granarium, and Rust red flour beetle Tribolium castaneum, using three exposure times of 24, 48, and 72 h. The mortality rate increased within increasing in concentrations and the exposure period. The results showed that the highest mortality rate was 16.67 whole insects recorded for the 2000 infectious phase/ ml with Sr nematodes after 72 hours post-treatment of C. maculatus. The results showed that the highest rate mortality, which is 20 larvae, was recorded for the infectious phase dose of the genus nematode (Hb), all of which are 500, 1000, 2000 and 3000 gasric phase / ml, and for all exposure periods used between 24 hours and up to 72 hours against the larvae of the T. granarium. The results showed that the highest rate of mortality 10.00 whole insects was recorded for the dose of 3000 Infectious stage / ml and at the exposure time 72 hours, while the lowest rate for this mortality of 1.33 adult insects for rusty flour beetle was recorded for the dose of 500 infectious stage / ml and for the exposure time only 24 hours. The results indicated that mortality rate was increasing against any increase in both the exposure time and the dose of nematodes of the genus Hb.

Keywords: Nematodes, Insects, EPNs.

1. Introduction

Nematodes have been used with unrivaled success in inhibiting the population growth of insect pests that live in the soil as well as in greenhouses [1,2]. There is currently a growing interest in biological control that reduces the negative effects of the environment [3-5], as explained by Georgis 1990. The insect-parasitic nematode possesses a very high efficacy as a biological control agent against economic insects, especially the nematodes belonging to the genus Steinernema and Heterorhabditis [6-8]. Nematodes have been used against different species of storing insects such as the rusty flour beetle Tribolium castaneum (Herbst), the Mediterranean flour moth Ephesia kuehniella, the Indian flour moth Plodia interpunctella, and the Makhna beetle being Trogoderma variable [9-11]. The researchers [25] have shown that nematodes need moisture to reach the target and cause an infestation in warehouse insects. It is known that the grains and the storage environment are dry. Therefore, the provision of an aqueous environment in the insecticide manufactured from the nematode is required so that the nematode can reach the target effect killing.

The researchers Shahina Fayyz and Salma Javed 2009 revealed that they tested seven types of nematodes belonging to the two sexes Steinernema and Heterorhabditis against each of the last stage and stage of the complete insect of the Chinese legume beetle Callosobruchus chinensis of the nematodes, the researchers found that the last stage of larvae is more sensitive to infection with the nematode than the adult stage.

According to the above, our study of this came to test the possibility of two nematodes against three types of store insects: the Southern Cowpea weevil C. maculatus, the T. granarium, and the rusty flour beetle T. castaneum.

2. Materials and Methods

Samples were taken from a depth of 10-15 cm from the surface of the soil and away from the roots and trunk of the tree by about 100 cm (the end of the tree's shadow) in the farms and countryside of Giza Governorate (Arab Republic of Egypt) by
Prof. Dr. Atef Sayed Abdel Razzaq. The samples were chosen randomly at a rate of 5-10 samples around each tree and each sample consists of (500-1250 gm) of soil. Samples were placed in a separate nylon bag and these data were recorded in the bag. The aforementioned samples were transferred to the insect-pathogenic nematode laboratories in the Pest and Plant Protection Department of the National Research Center/ Egypt. Each sample was placed inside a glass bottle with a capacity of 2000 milliliters and the larvae of the great wax worm were placed at a rate of (20 to 50) larvae on the soil, and then the bottles were covered with a cloth and tied with a rubber band and kept in a refrigerated incubator at a temperature of (20-25°C).

The samples were examined twice a week with moistening of the soil in case it was dry, and in the event of an infection of the waxworm larvae, the insects were transferred to the water trap and the water was examined after (7-10) days and when the active nematodes were present at the third age.

It is withdrawn for storage in a special container and placed in the refrigerator at a temperature of 10 °C until the transactions are carried out [12]. An experiment was achieved to study the effect of nematodes on insect species. Five concentrations of the nematode strain Steinernema reibavi (125, 250, 500, 1000 and 2000 gastric stages / ml) were attended, while only four concentrations of the nematodes of the genus Heterohabditis bacterophora (500, 1000, 2000, and 3000 gastric stages / ml) were present. Concentration, for each type of nematode, and each of the three types of store insects Petri dishes of 8 cm capacity were used, and two layers of tissue paper were placed at the bottom to retain the moisture needed by the nematodes when treating. Only 1 ml of each concentration was added to each Petri dish. The three comparison replicates were treated with only 1 ml of water.

Study of the effect of nematodes on the three insect species Twenty insects of the three modern species were used for each repeat, as well as the same number of comparison insects and were kept in a refrigerated incubator at a temperature between (20-25 °C) in the incubator with continued monitoring of the humidity level in the dishes, then the mortality rate was calculated after 24, 48 and 72 h.

2.1. Statistical analysis

The data were analyzed statistically using the Anova Table test of variance analysis and using Minitab statistical program. The mean of the coefficients was compared using the Duncan Multiple Range Test at a probability level of 0.05 [13].

3. Results and Discussion

The results recorded in Table (1) showed that the highest rate of killing was 16.67 whole insects, which were recorded after 72 h of treatment with nematodes, while the lowest rate of killing is 1.67 complete insects recorded after 24 h of treatment with the nematode of the genus Sr.

The results of the aforementioned table indicated that the rate of killing is increasing with the increase in the duration of exposure and the dose used from the infectious phase of the nematode.

| Exposure time Hour | Mortality ( %) | Treatments |
|--------------------|---------------|------------|
|                    | 125 | 250 | 500 | 1000 | 2000 | Control 0.00 |
| 24                 |     |     |     |      |      |             |
| a                  | 1.67| 3.45| 4.66| 8.67 | 12.67| 0.00        |
| b                  | 4.00| 5.33| 7.33| 11.33| 14.33| 0.00        |
| 48                 |     |     |     |      |      |             |
| c                  | 9.00| 10.33| 12.67| 14.23| 16.67| 0.00        |
| 72                 |     |     |     |      |      |             |
| Values followed by the same letter do not differ significantly from each other as a probability level of 5%.

The results recorded in Table (2) indicated that the highest rate of killing 14.00 whole insects was recorded for a dose of 3000 gastric phase/ml and after 72 hafter the date of treatment, while the lowest rate of killing of 3.33 whole insects was recorded at the 24h exposure period and for the dose 500 an infectious phase/ml used from the nematode genus (Hb). Thus, the results of the above table indicated that the killing rate was increased in exchange for increases in both the nematode dose and the exposure period used in an hour.
The effect of nematodes of the genus (Hb) against *Callosobruchus maculatus*.

| Exposure time (h) | Mortality (%) | Treatments | Dose (infectious phase/ml) |
|-------------------|---------------|------------|---------------------------|
|                   |               |            | 500 | 1000 | 2000 | 3000 | Control (0.00) |
| 24                | 3.33          | A           | 5.67 | 7.00 | 8.33 | 0.00 |
|                   | 6.67          | E           | 7.00 | 9.67 | 9.67 | 0.00 |
|                   | 9.00          | I           | 10.00| 10.00| 14.00| 0.00 |

Values followed by the same letter do not differ significantly from each other as a probability level of 5%.

Effect of nematodes of the genus (Sr) in killing larvae of the *T. granarium*. The results indicated in Table No. (3) that the highest rate of killing, which was 20 larvae, was recorded for the dose of 125 infectious stages/ml and the period of exposure only 24 hours. Thus, the results of the aforementioned table showed that the killing rate was increasing whenever there was an increase in both the duration of exposure and the dose used from the infectious phase of the nematode genus (Sr).

Effect of nematodes of the genus (Sr) in killing larvae of the *T. granarium*. The results indicated in Table No. (3) that the highest rate of killing, which was 20 larvae, was recorded for the dose of 125 infectious stages/ml and the period of exposure only 24 hours. Thus, the results of the aforementioned table showed that the killing rate was increasing whenever there was an increase in both the duration of exposure and the dose used from the infectious phase of the nematode genus (Sr).

Table 3. The effect of nematodes of genus (Sr) against *Trogoderma granarium*.

| Exposure time Hour | Mortality (%) | Treatments | Dose (infectious phase/ml) |
|--------------------|---------------|------------|---------------------------|
|                    |               |            | 125 | 250 | 500 | 1000 | 2000 | Control 0.00 |
| 24                 | 8.33          | a           | 7.33 | 5.33 | 8.67 | 12.67 | 0.00 |
| 48                 | 4.33          | D           | 6.67 | 9.00 | 10.00| 10.00| 0.00 |
| 72                 | 8.67          | e           | 9.33 | 10.00| 10.00| 10.00| 0.00 |

Values followed by the same letter do not differ significantly from each other as a probability level of 5%.
significant reduction in the size of the potato leaf total loss resulting from feeding the larvae when compared to their counterparts other than the comparative treatment. In Europe Our results are also in agreement with HEPES 2006, which showed that nematodes are better economically and environmentally than chemical compounds, as the effectiveness of nematodes has reached more than 60%. Effect of nematodes of the genus (Sr) in killing the rusty red flour beetle T. castaneum whole: The results recorded in Table (5) indicated that the highest rate of killing is 9 whole insects A dose of 2000 gastric phase / ml was recorded for the genus of nematodes (Sr) and the period of exposure 72h, while the lowest rate for this killing of 3 whole insects was recorded for the dose of 125 infectious phase/ml and the exposure period of 24 hours. Thus the results indicated that the killing rate was increasing in exchange for increases both in the period of exposure to the nematode and the dose of the gastric phase are used.

| Table 4. The effect of nematodes(Hb) against T. granarium. |
|---|---|---|---|---|---|
| Mortality( %) | Dose ( infectious phase/ml ) | Treatments |
| 24 | 13.67 | 13.67 | 19.33 | 19.33 | 0.00 |
| A | B | b | b | c |
| 48 | 20.00 | 20.00 | 20.00 | 20.00 | 0.00 |
| b | B | b | b | c |
| 72 | 20.00 | 20.00 | 20.00 | 20.00 | 0.00 |
| b | B | b | b | c |

Values followed by the same letter do not differ significantly from each other as a probability level of 5%.

| Table 5. Effect of Nematodes (sr) against Tribolium castaneum. |
|---|---|---|---|---|---|
| Mortality ( %) | Dose ( infectious phase/ml ) | Treatments |
| Exposure time Hour | 125 | 250 | 500 | 1000 | 2000 | Control 0.00 |
| 24 | 3.00 | 5.00 | 5.33 | 5.67 | 6.67 | 0.00 |
| A | B | B | b | C | g |
| 48 | 8.00 | 7.33 | 6.67 | 11.33 | 14.33 | 0.00 |
| D | D | E | f | H | g |
| 72 | 7.67 | 8.00 | 8.33 | 8.67 | 9.00 | 0.00 |
| D | D | D | d | d | g |

Values followed by the same letter do not differ significantly from each other as a probability level of 5% . Effect of nematodes (genus Hb) in killing red rust beetle T. castaneum: The results recorded in Table 6 showed that the highest rate of killing 10.00 whole insects was recorded for a dose of 3000 gastric stages/ml and at the exposure period 72 h., while the lowest rate for this killing of 1.33 adult insects for rusty flour beetle was recorded for the dose of 500 infectious stage / ml and the exposure period only 24 h. It was evident from these results that each of the killing rate was increasing in contrast to an increase in both the exposure period and the dose of nematodes of the genus (Hb).

| Table 6. Effect of Nematodes Sex (Hb) against T. castaneum. |
|---|---|---|---|---|---|
| Mortality( %) | Dose( infectious phase /ml ) | Treatments |
| Exposure time Hour | 500 | 1000 | 2000 | 3000 | Control 0.00 |
| 24 | 1.33 | 3.67 | 4.00 | 5.00 | 0.00 |
| A | b | b | c | f |
| 48 | 4.33 | 5.67 | 7.00 | 7.00 | 0.00 |
| D | d | e | e | f |
| 72 | 8.33 | 8.67 | 9.00 | 10.00 | 0.00 |
| F | f | h | h | f |

Values followed by the same letter do not differ significantly from each other as a probability level of 5% .

Our results agree with Atwa 2003 and Atwa and Shamsaldine 2008 that the only insect pathogenic nematodes can be relied upon in insects that inhabit hiding environments such as soil insects and tunnel insects as in the case of using nematodes in controlling the red palm weevil and the scarab insects that infect strawberries. The results showed a reduction in the red palm weevil population by between (75-92%) throughout the year by the method of injecting nematodes into the plant, as well as a reduction of 92% during the production season. Our results also agreed with [18], that S. riobravis nematode is the most effective and fastest killing nematode of late fifth and sixth larval ages. The cotton leafworm, Spodoptera littoralis, and so on, the nematodes of the genus Heterorhabditis have high efficiency and speed in killing the larvae of all kinds of insects under
study. Thus, the results of our study agreed with both [19,20], in the effectiveness of seven species of insect-pathogenic nematodes from two families, Heterorhabditidae and Steinernematidae, against the last age of larvae of the Spodoptera littoralis.

Conclusions: The results of the study showed that the nematodes showed a clear, definite, strong, and effective superiority in killing the entire three insect species that were studied, the southern cowpea weevil C. maculatus, Khapra beetle T. granarium, and Rust red flour beetle T. castaneum.

**Recommendations**

We recommended the possibility of using nematodes of both types in controlling the stored insects, Southern cowpea weevil C. maculatus, Khapra beetle T. granarium and red rusty flour beetle T. castaneum.

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