

Effectiveness of Local and Commercial Isolates of *Heterohabditis bacteriophora* Nematodes in Controlling the Cucurbit Fly *Dacus ciliatus* (Loew)

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Abstract

The cucurbit fly is a pest with significant economic impact because it affects numerous agricultural crops and leads to fruit damage. This experiment was conducted to evaluate the efficacy of the local and commercial isolates of *Heterohabditis bacteriophora* nematodes on the larvae and pupae of the cucurbit fly insect *Dacus ciliatus*. Four concentrations were prepared for each isolate (10, 25, 50, and 100 infective juveniles/milliliter). The results showed clear variations in the isolates' ability to control the insects, and the effectiveness of the used concentrations varied in their impact on the mortality rate of the larvae and pupae. It was found that all concentrations could infect and control the cucurbit fly at both stages: larvae and pupae, but with varying percentages. Results showed that 100 IJs/mL scored the highest mortality rate compared to the other concentrations. The percentage of larvae and pupae mortality was higher when treated with the local isolate of nematodes compared to the commercial isolate at the same concentration.

Key words: *Heterohabditis bacteriophora*, *Dacus ciliatus*, Biocontrol.

1. Introduction

Cucurbits are prominent agricultural crops that are cultivated globally [1]. There are numerous pests that cause damage to these crops, but one of the prominent pests that attack cucurbits is the cucurbit

fly, *Dacus ciliatus*, which is considered one of the major species that infest these agricultural crops and belongs to the family Tephritidae [2]. This insect is considered a primary pest of cucurbits and a secondary pest of other vegetables [3]. The family

Tephritidae comprises 4500 known species, of which 250 species are considered major pests that cause significant damage to the cucurbits [1]. *Dacus ciliatus* (Loew) is a pest that affects the cucurbit plants in the Atlantic Islands, Africa, and East Asia [4]. The life cycle of this pest is like that of other species of *Dacus* insects, where females puncture the fruits and lay eggs inside them. This leads to their damage within ten days of fruit formation and sometimes even earlier [5].

Upon hatching, the larvae feed on the fruits for three instars before emerging from the fruit to transform into a pupa enclosed in an outer shell (pupal cocoon). The adults start emerging from the pupal cocoon to complete their life cycle. The life cycle of the cucurbit fly, *Dacus ciliatus*, is completed within 49 to 54 days at a temperature of 25 degrees Celsius [6]. Females start laying eggs from 10 to 13 days after emerging from the pupal cocoon. However, these timings can vary depending on the seasons, ranging from 5 to 6 days during the summer to over 30 days during the autumn. Based on data obtained from Egypt, females can deposit up to 210 eggs, which range in color from white to creamy yellow, over a period of three days [7].

2. Materials and method

2.1 Samples

Four types of samples were prepared for conducting the necessary tests and laboratory examinations, and appropriate measures were taken to ensure their proper preparation. The most important measures included providing a suitable environment and a suitable nutrient medium. The samples are.

- 1- Larvae of the cucurbit fly, *Dacus ciliatus*.
- 2- Pupae of the cucurbit fly, *Dacus ciliatus*.
- 3- Infective stages of the local nematode, *Heteroabditis bacteriophora*.
- 4- Infective stages of the commercial nematode, *Heteroabditis bacteriophora*.

The local isolation from nematodes *H. bacteriophora* is morphologically and molecularly characterized by the reported method of Al-Zaidawi and co-workers [8].

2.1.1 Preparation of the insect stages (larvae and pupae)

Infested fruits of cucurbits with eggs of the cucurbit fly *Dacus ciliatus*, were collected from local markets and placed in rearing cages with a 5 cm layer of soil to provide suitable conditions for larval development. The cages were placed in the laboratory under controlled conditions (25 degrees Celsius, 60 % relative humidity, and 14 hours of light). It is worth mentioning that test temperature and humidity level were specified to accommodate the exact actual environmental conditions under

which the insect is active throughout its ecological activity period. After emergence, the adults were transferred to cages equipped with a food source consisting of yeast and sugar, and cucurbit fruits were provided as a medium for egg laying.

2.1.2 Preparation of local and commercial isolates of *Heterohabditis bacteriophora* nematodes

Two isolates of *Heterohabditis bacteriophora*, the local and commercial isolates, were multiplied on last instar *Galleria mellonella* larvae under suitable conditions with a temperature ranging from 25 to 30 degrees Celsius and a relative humidity of up to 90 % according to methodology described by Hussein et. al [9]. After one week, the infective stages (IJs) were harvested by adding 5 mL of sterile distilled water to the Petri dish containing the nematode infective stages (IJs) and transferring it to a 20 mL sterile distilled water vial using a micropipette. The total volume of the nematode suspension was 25 mL.

Then, a 100 μ L was examined under a microscope to determine the number of nematode infective stages (IJs) per 100 μ L. The count of IJs for the local *Heterohabditis bacteriophora* isolate was 92, while for the commercial isolate, it was 85 IJs. The number of infective stages in the 25 mL suspension was determined using the following equation.

$$\frac{c1}{v1} = \frac{c2}{v2}$$

The helps calculate the number of nematode infective stages in a 25 mL nematode suspension based on the count of infective stages per 100 μ L and the volume of the examined suspension. This information is essential for determining the volume of the suspension containing the desired number of nematode infective stages. After accurately determining the volumes, the spray solution is prepared by adding sterile distilled water to the nematode suspension, resulting in a final volume of 1 mL for each concentration (10, 25, 50, and 100 IJs/mL). The specified concentrations for both local and commercial nematodes were determined because the study is conducted within the laboratory rather than the field and through which it is possible to control a limited number of insects, as the targeted number of pests is specified.

2.2 Laboratory experiments

A filter paper was placed in a Petri dish with a diameter of 9 cm, and the spray solution was distributed onto it. Ten insects (larvae or pupae) were collected from cucurbit fruits and placed in a Petri dish containing the nematode solution. Then Petri dish was covered with a ventilated cover, as shown in figure 1, and placed in an incubator at a temperature of 25 ± 2 degrees Celsius and a relative humidity of 90 %.

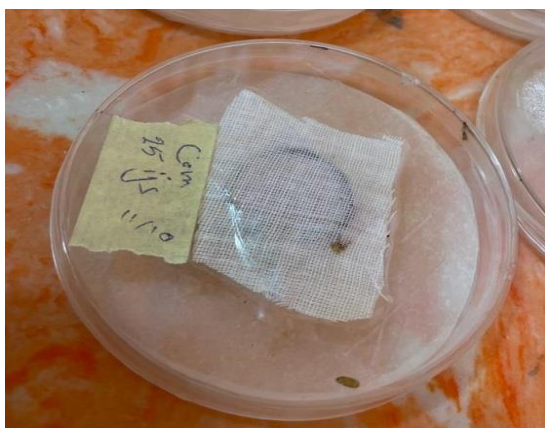


Figure 1: A ventilated petri dish.

The top surface of the incubator was designed to provide appropriate ventilation during the experimental period. Furthermore, there were three Petri dishes for each concentration, and each Petri dish contained ten larvae or pupae, depending on the targeted stage of the experiment. As for the control dishes, the filter paper was sprayed with 1 mL of sterilized distilled water, and the larvae or pupae (10 larvae or pupae) were placed on. The Petri dishes were then closed and placed in the incubator. After 48 hours, the larvae transformed into adults. After four days, the adults in each sample were examined under a microscope. This step allows for observing whether the pupae were infected with the nematode. After seven days of infection, the number of dead larvae and pupae were recorded, and the percentage of mortality was determined for each concentration and each isolate. The filter paper was used instead of soil in this study because it maintains the necessary moisture needed for the

growth, reproduction, and movement of nematodes.

3. Results

The results were recorded after seven days of the experiment, indicating the number of larvae and pupae that were killed due to nematode infection, as illustrated in tables 1, and 2, and figures 2, and 3. Based on the results shown in tables 1 and 2, it can be observed that as the concentration of nematodes increases, the incidence and mortality of larvae and pupae also increase. This leads to an increase in the efficiency of biological control against the pest. In general, the local isolate of *Heterorhabditis bacteriophora* nematodes is found to be more effective in the biological control of larvae and pupae compared to the commercial isolate. Microscopic examination also revealed that the infection by the local nematode isolate was denser and clearer compared to the infection by the commercial nematode isolate, as in figures 4 and 5.

Table 1: Mortality rate of larvae and pupae effected by Iraqi local IJs.

IJs Concentration, IJs/mL	Mortality Rate, %	
	Pupae	Larvae
10	6.66	13.33
25	16.66	30
50	36.66	46.66
100	56.67	63.33
Control	0	0

Table 2: Mortality rate of larvae and pupae effected by commercial IJs.

IJs Concentration, IJs/mL	Mortality Rate, %	
	Pupae	Larvae
10	3.33	6.66
25	10	13.33
50	20	26.66
100	40	43.33
Control	0	0

The mortality rate was highest at a concentration of 100 IJs/mL of Iraqi local *H. bacteriophora* for both larvae and pupae, it was 63.33 %, and 56.67 % respectively. This is since the number of nematodes in this

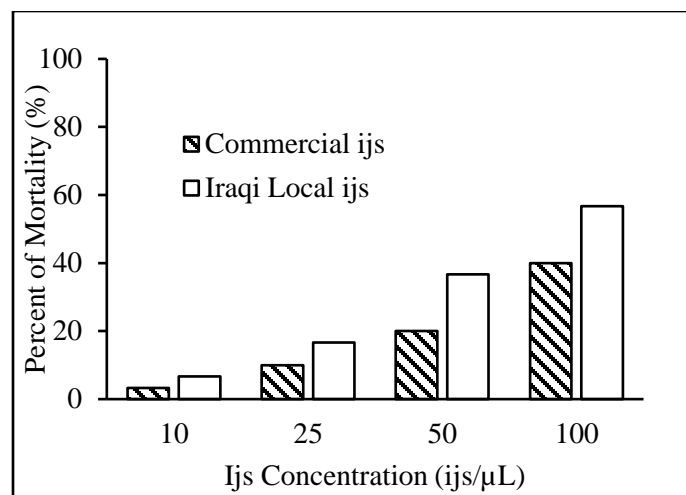


Figure 1: Mortality rate of pupae effected by commercial and Iraqi local *Heterohabditis bacteriophora*.

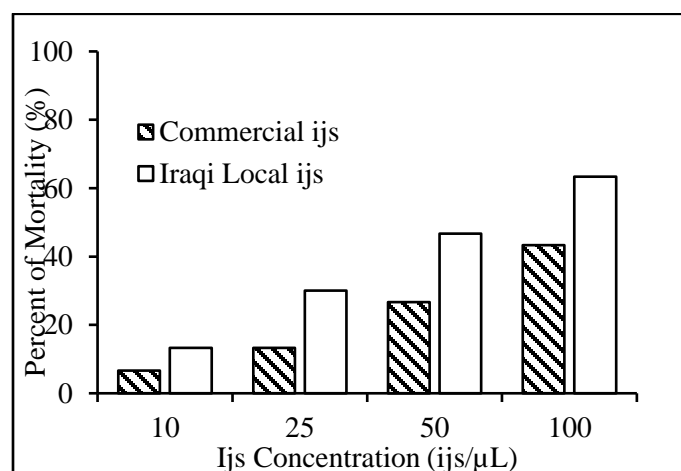


Figure 3: Mortality rate of larvae effected by commercial and Iraqi local *Heterohabditis bacteriophora*.

concentration is higher than in other concentrations. Conversely, the lowest mortality rate was observed at a concentration of 10 IJs/mL for larvae and pupae, The results indicate that a 100 % mortality rate is not achieved at the

current concentration of nematodes, and it is recommended to use higher concentrations to achieve optimal mortality rates. The mortality rate was highest at a concentration of 100 IJs/mL for both larvae and pupae because the number of nematodes in this concentration is higher than in other concentrations. The results clearly indicate that *Heterohabditis bacteriophora* nematodes are effective biological control agents against the larvae and pupae of the cucurbit fly *Dacus ciliatus*, given the favorable conditions for their growth. This is consistent with findings reported by Hussein et. al [10]. Moreover, Minas and co-workers [11] reported that the increased susceptibility of the larvae compared to the pupae is attributed to the larger natural openings in the body of cucurbit fly larvae and the weakness of their body wall, which facilitates infection by the nematodes.

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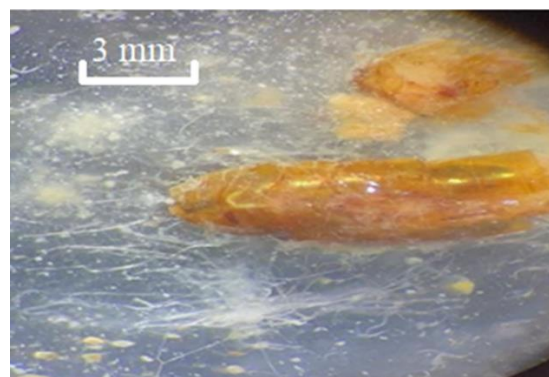


Figure 4: Infection density of commercial *Heterohabditis bacteriophora*.

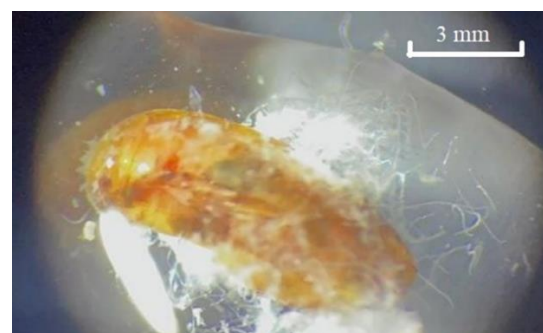


Figure 5: Infection density of commercial *Heterohabditis bacteriophora*.

larvae and the weakness of their body wall, which facilitates infection by the nematodes. Furthermore, Toledo et. al [12] linked the low susceptibility of cucurbit fly pupae to the small size of their respiratory openings and the hardness of their cuticle, which hinders the penetration of nematodes. Other studies have indicated an increased susceptibility of cucurbit fly larvae to infection by entomopathogenic nematodes compared to the pupae. This can be

attributed to several factors, including the developmental stage and activity level of each, their behavior in the soil, and the method they use to search for hosts, in addition to the size of their natural openings [13]. The results of this study indicate that the entomopathogenic nematode *H. bacteriophora* is effective against fruit flies and can be utilized in pest control programs. Numerous studies have also highlighted the efficacy of these nematodes in impacting all stages of this insect and other pests [10, 14]. A similar study conducted by El-Sabah [2] examined the impact of different concentrations (50, 100, 150, 200, 250 infective juveniles per square centimeter) of *Heterohabditis* nematodes against cucurbit flies (*Dacus ciliatus*). The study indicated that the *H. bacteriophora* effect increases with higher concentrations, and larvae were found to be more susceptible. The increased production of carbon dioxide was identified as a factor attracting the nematodes towards the larvae. This study was in line with a similar study conducted by El-Sabah [2] to examine the impact of various concentrations (10 IJs/mL) of *Heterohabditis bacteriophora* nematodes on fruit flies. The study indicated that the pupae exhibited lower sensitivity to the nematodes compared to the larvae. It also highlighted the high susceptibility of the larvae, attributed to increased release of carbon dioxide, which acts as an attractant for the nematodes, in addition to the larger size

of natural openings and the flexibility of the cuticle.

4. Conclusion

From the study results, it can be concluded that local nematodes are more efficient and effective in controlling cucurbit fly *D. ciliatus*, whereas commercial nematodes showed lower efficiency compared to the local ones. This can be attributed to the fact that local isolates are better adapted to environmental conditions. Additionally, it was found that larvae are more sensitive than pupae to biological control agents, which can be attributed to their direct exposure to the control factors, unlike pupae that are surrounded by a cocoon which protects them from external influences.

5. References

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