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Influence of Different Species of *Steinernema* and *Heterorhabditis* on *Galleria mellonella* and *Spodoptera littoralis*

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ABSTRACT

Entomopathogenic nematodes (EPNs) can be used efficiently as biological control agents against specific insects. *Galleria mellonella* (Gm) and *Spodoptera littoralis* (Sl) are considered important agricultural pests. The aim of this study was to evaluate the susceptibility of their larvae to ascending levels of four species of EPNs, *Steinernema carpocapsae* (Sc), *S. arenarium* (Sa), *Heterorhabditis bacteriophora* (Hb) and *H. indica* (Hi) as well as the ability of nematodes to penetrate and reproduce within their hosts. Fifth larval instar of Gm and Sl were used to evaluate the effect of four inoculum doses [200,400,800, and 1600 infective juveniles (IJs)] of each nematode species. Percentage of insect mortality (% M) and the nematode-rate of reproduction (Rr) were calculated after 24 and 48 hrs. The data confirmed that *H. bacteriophora* was the highest effective species on both insects. The % M was 75.7, 79.7, 82.3, and 83% after 24 hrs and 98.0, 99.3,100 and 100% after 48 hrs in Gm for these doses, respectively. On the other hand, the corresponding mortalities were 75.0, 78.3, 81.7, and 83.8% after 24 hrs and 97.3, 98.3, 100 and 100% after 48 hrs in Sl. The highest initial EPN population that could penetrate the insect (pi) was achieved by Hb in Gm (295 IJs) at 1600 IJs, while it was 221.7 IJs in Sl larvae at the same inoculum. The highest final population and Rr were obtained at the inoculum level of 200 and 400 IJs. in Gm and Sl, respectively. The study documented that *Heterorhabditis* and *Steinernema* can be used effectively in Gm and Sl management. Moreover, Hb is the most suppressive species tested against the two lepidopteran hosts.

Keywords**:** *Heterorhabditis*, *Steinernema*, *Galleria mellonella*, *Spodoptera littoralis*, biological control.

INTRODUCTION

The Egyptian cotton leaf worm *Spodoptera littorales* (Lepidoptera: Noctuidae) is one of the most economically important insects (Zhou et al., 2010). It is a polyphagous insect pest, feeding on most vegetables in addition to other many horticultural and field crops. It causes considerable loss in their production all over the world (Abd El-Razik & Mostafa, 2013) especially in the Middle East and Africa (Pineda et al., 2007; Shairra and Nouh, 2014). The level of damage depends on the host plant type, plant stage and the initial population densities (Ahmad et al., 2005). Apiaries all over the world are suffering from parasites, predators, and diseases (Foley et al., 2014; Bray and Nieh, 2014). The greater wax worms usually infect the stored wax comb around the year causing great damage for the wax comb and economic losses for beekeepers. An important trend of recent research is focusing on finding an alternative method instead

of chemical insecticides which have hazard effects on the human health, non-target organisms and environment. Moreover, some insect races could develop resistance to the chemical insecticides.

Entomopathogenic nematodes (EPNs) are considered one of the safest and most effective bioagents especially for subterranean pests under natural field conditions (Akhurst and Smith, 2002; Grewal et al., 2005; Rojht et al., 2009). They may offer an alternative eco-friendly choice against some economic insect pests (Orozco. et al., 2014 and Gaugler, 2002) without any unfavorable effect on human or beneficial organisms (Van Zy and Malan, 2014), with relatively low costs (Mutegi et al., 2018). The genera *Steinernema* and *Heterorhabditis* are the most insecticidal nematodes used in insect pest control in the open field. These genera of nematodes carry the symbiotic bacteria *Xenorhabdus* and *Photorhabdus*, respectively, in their body (Ehlers, 2001). When the infective juveniles (IJs) enter the suitable insect host (Gozel and Gozel, 2016), the bacteria kill the insect host causing a septicemia usually within one to three days (Griffin et al., 2005).

Moreover, these EPNs can be applied using the pesticide machinery (Shapiro-Ilan et al., 2006), without inducing resistance in the populations of the insect pests (Shamseldean et al., 2009). For example, *H. indica* and *S. carpocapsae* are highly effective on *Spodoptera litura*, especially the young larval instars (Khan et al., 2018; Ibrahim and Shairra, 2011). Entsar and Sawsan (2016) controlled the greater wax larvae inside the beehives by using *H. bacteriophora.* Ibrahim et al. (2017) reported that *S. carpocapsae* is highly effective on *Agrotis ipsilon* and *S. littoralis* larvae in the open field, as well as *H. bacteriophora* and *S. monticolum* against the same insect pests (Sobhy et al., 2020).

MATERIALS AND METHODS

1-Source and maintenance of entomopathogenic nematodes

Nematodes in this study defined as *Heterorhabditis bacteriophora* (Hb), *Heterorhabditis indica* (Hi), *Steinernema carpocapsae* (Sc), and *Steinernema arinarium* (Sa) were obtained from Plant Protection Department, Faculty of Agriculture, Ain Shams University, Shoubra El-Kheima. The nematodes were reared on the fifth instar larvae of the lepidopteran host, greater wax moth (*Galleria mellonella* L.). Insect larvae were placed in a 12-cm-diam Petri dish lined with a moistened tissue paper and exposed to about 150 IJs/insect larva at 25 ºC. After 48 hr., dead insect larvae were transferred to spongy trap dishes consisted of 10 cm length x 5cm width x 3mm thickness that was placed in a petri dish and kept in a plastic bag. The amount of water in wetted sponge was calculated approximately as 10 ml of water /1gm of sponge. They were maintained at 25 ± 2 ˚C (Kassab and Taha, 2016). After 8 days, the IJs began to emerge from the insect cadavers for collection and were used within 2 weeks of collection.

2-Rearing the insect host (the Greater wax moth, *G. mellonella*)

The larvae of this host were obtained from beehives, Faculty of Agriculture, Ain Shams, Shoubra El-Kheima, and reared on artificial feeding media in plastic jars at 25 ± 2 ºC in the laboratory and then eggs were gently removed and incubated in other rearing jars provided with the hatching medium (Kassab and Taha, 2016).

3-Rearing *Spodoptera littoralis*

Spodoptera littoralis was obtained from the Department of Plant Protection at Faulty of Agriculture, Ain Shams University. It was reared at the room temperature in plastic jars containing saw dust and fed on castor bean leaves (*Ricinus communis* L.). The pupae were transferred into transparent jars until emerging of the adult. Adults were fed on sugar solution. Deposited eggs were removed gently and transferred to the plastic jars where the hatching larvae were fed on the castor leaves again.

4. Laboratory bioassay

Susceptibility of *Galleria mellonella* and *Spodoptera littoralis* larvae to the entomopathogenic nematode species.

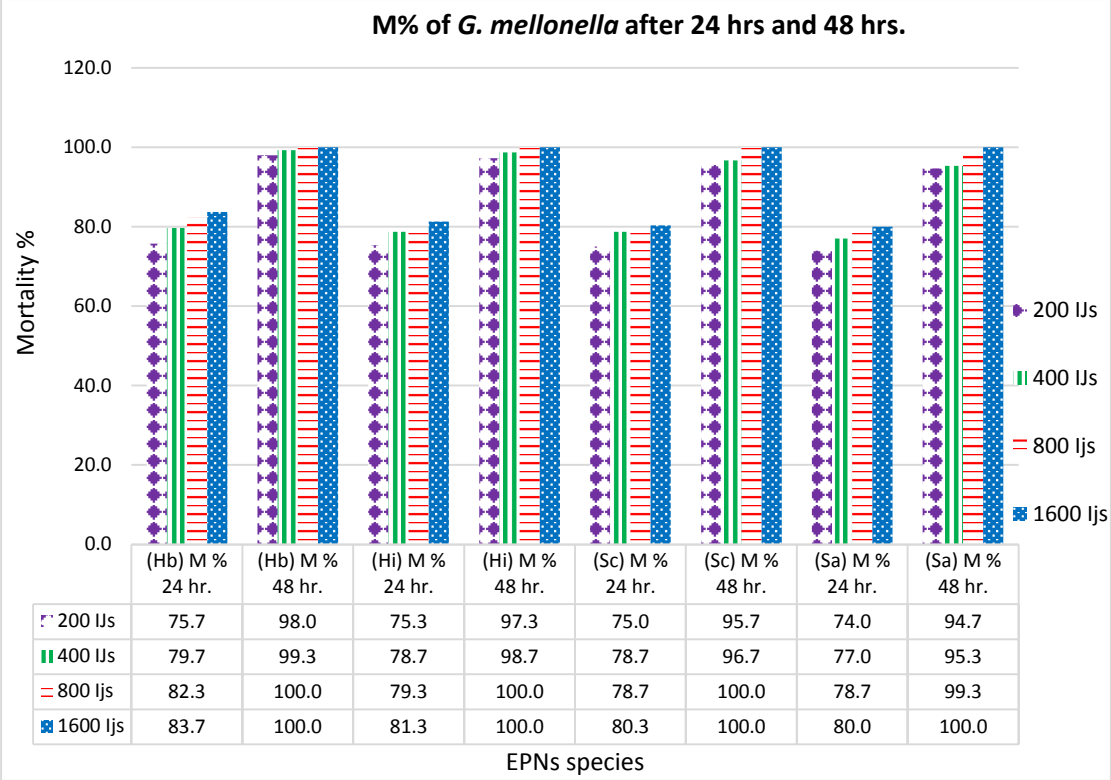
Five inocula for each of *H. bacteriophora*, *H. indica*, *S. carpocapsae*, or *Steinernema arinarium*, i.e., 0, 200, 400, 800, or 1200 IJs/ insect larva/petri dish were used to study the effect of the pathogenicity and productivity of these nematode species on the two different hosts, *G. mellonella* and *S. littoralis*, separately. One fifth instar larva was placed in a 10-cm-diam Petri dish lined with a moistened paper tissue and exposed to the previous nematode inocula while control replicates received only water. After 24 and 48 hr., the percentage of mortality (M%) was calculated for each treatment. After 48 hr. dead larvae of each insect were collected and divided into two groups. In the first group: the cadavers were dissected to estimate the percentage number of penetrating IJs (Pi%) per each larva according to the formula described by Caroli et al. (1996). In the second group, the cadavers were placed singly on the spongy trap for 10 days and the collected IJs were counted as the EPN-final population (Pf) and the rate of reproduction (Rr) was calculated in each treatment. All treatments were replicated 10 times and maintained at 25 ± 2 °C.

5- Statistical analysis

The obtained data were statistically analyzed using analysis of variance procedure proposed by Snedecor and Cochran (1969). The differences between means were compared using Duncan's Multiple Range Test (Duncan, 1955).

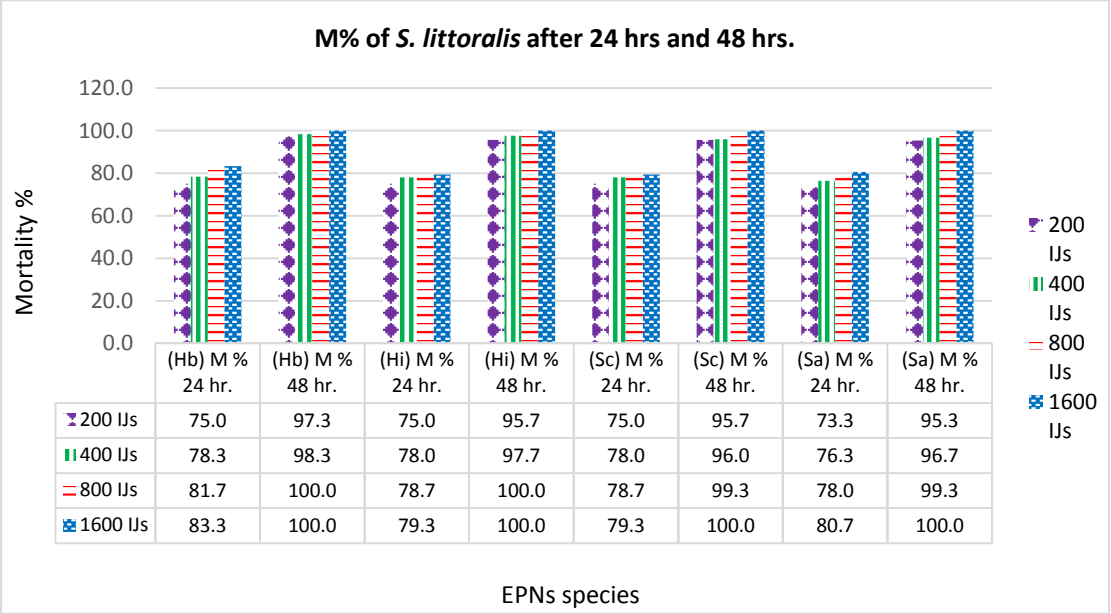
RESULTS

All applied species of EPNs were able to kill the two lepidopteran insects, *G. mellonella* and *S. littoralis*. Moreover, *G. mellonella* was more sensitive to the EPNs than *S. littoralis* larvae. Generally, the genus *Heterorhabditis* was more efficient than those of the genus *Steinernema*, However, *H. bacteriophora* was the most effective species than others. Data indicated that the highest percentage mortality was caused by *H. bacteriophora*, followed by *H. indica*, *S. carpocapsae* and *S. arinarium* in *G. mellonella* and *S. littoralis*. After 24 hrs, the highest M% was obtained by using the doses 800 and 1600 IJs with no significant differences between *G. mellonella* and *S. littoralis*. Moreover, the M% reached to 100% after 48 hrs in 800, and 1600 IJs per each insect (Figs 1& 2).



	(Hb) M % 24 hr.	(Hb) M % 48 hr.	(Hi) M % 24 hr.	(Hi) M % 48 hr.	(Sc) M % 24 hr.	(Sc) M % 48 hr.	(Sa) M % 24 hr.	(Sa) M % 48 hr.
200 IJs	75.7	98.0	75.3	97.3	75.0	95.7	74.0	94.7
400 IJs	79.7	99.3	78.7	98.7	78.7	96.7	77.0	95.3
800 Ijs	82.3	100.0	79.3	100.0	78.7	100.0	78.7	99.3
1600 Ijs	83.7	100.0	81.3	100.0	80.3	100.0	80.0	100.0

Figure 1: The effect of inoculum levels of *H. bacteriophora*, *H. indica*, *S. carpocapcae*, and *S. arinarium* on the mortality % of *Galleria mellonella* larvae after 24 and 48 hrs.



	(Hb) M % 24 hr.	(Hb) M % 48 hr.	(Hi) M % 24 hr.	(Hi) M % 48 hr.	(Sc) M % 24 hr.	(Sc) M % 48 hr.	(Sa) M % 24 hr.	(Sa) M % 48 hr.
200 IJs	75.0	97.3	75.0	95.7	75.0	95.7	73.3	95.3
400 IJs	78.3	98.3	78.0	97.7	78.0	96.0	76.3	96.7
800 IJs	81.7	100.0	78.7	100.0	78.7	99.3	78.0	99.3
1600 IJs	83.3	100.0	79.3	100.0	79.3	100.0	80.7	100.0

Figure 2: The effect of inoculum levels of *H. bacteriophora*, *H. indica*, *S. carpocapsae*, and *S. arinarium* on the mortality % of *Spodoptera littoralis* larvae after 24 and 48 hrs.

Table 1: Effect of inoculum levels of *H. bacteriophora*, *H. indica*, *S. carpocapcae*, and *S.arinarium* on the initial population (Pi), Pi %, final population (Pf), and rate of reproduction (Rr) in *Galleria mellonella* larvae.

Inoculum level	*H. bacteriophora*				*H. indica*				*S. carpocapsae*				*S. arinarium*			
	Pi	Pi %	Pf x10^3	Rr	Pi	Pi %	Pf x10^3	Rr	Pi	Pi %	Pf x10^3	Rr	Pi	Pi %	Pf x10^3	Rr
200 IJs	76.7 c	38.3 a	85.3 d	1145.1 b	78.3 c	39.2 a	81.3 c	1049.3 b	69.7 c	34.8 a	79.3 c	1150.3 b	64.3 d	32.2 a	79.3 c	1244.7 b
400 IJs	110.0 c	27.5 bc	173.7 a	1589.4 a	106.0 c	26.5 b	172.0 a	1651.3 a	99.0 c	24.8 b	167.3 a	1695.1 a	93.7 c	23.4 b	164.7 a	1767.5 a
800 IJs	230.3 b	28.8 b	142.7 b	626.5 c	227.0 b	28.4 b	126.0 b	561.1 c	224.3 b	28.0 b	106.0 b	476.5 c	220.0 b	27.5 b	91.3 b	418.2 c
1600 IJs	295.3 a	18.5 c	123.7 c	419.1 c	287.7 a	18.0 c	122.7 b	427.1 c	281.0 a	17.6 c	104.0 b	369.8 c	254.7 a	15.9 c	89.3 bc	350.8 c

Means followed by the same letter(s) within a column are not significantly different at 5% level of significance.

Table 2: Effect of inoculum levels of *H. bacteriophora*, *H. indica*, *S. carpocapcae*, and *S.arinarium* on the initial population (Pi), Pi %, final population (Pf), and rate of reproduction (Rr) in *Spodoptera littoralis* larvae.

Inoculum level	*H. bacteriophora*				*H. indica*				*S. carpocapcae*				*S. arinarium*			
	Pi	Pi %	Pfx10^3	Rr	Pi	Pi %	Pf x10^3	Rr	Pi	Pi %	Pf x10^3	Rr	Pi	Pi %	Pf x10^3	Rr
200 IJs	48.7 d	24.3 a	73.0 ab	1501.2 a	46.7 d	23.3 a	47.7 ab	1027.6 a	45.7 d	22.8 a	45.0 a	1001.9 a	42.0 d	21.0 b	39.7 bc	941.0 a
400 IJs	94.0 c	23.5 a	81.3 a	870.8 b	94.3 c	23.6 a	56.3 a	600.1 b	93.0 c	23.3 a	53.3 a	573.5 b	90.0 c	22.5 a	54.0 a	601.1 b
800 IJs	119.3 b	14.9 b	68.3 bc	585.9 c	117.7 b	14.7 b	52.3 a	450.2 b	115.3 b	14.4 b	48.7 a	431.5 b	110.0 b	13.8 c	47.0 ab	428.0 c
1600 IJs	221.7 a	13.9 b	60.0 c	271.4 d	220.0 a	13.8 b	40.3 ab	183.7 c	216.3 a	13.5 b	28.0 b	129.7 c	218.3 a	13.6 c	34.0 c	155.7 d

Means followed by the same letter(s) within a column are not significantly different at 5% level of significance.

The results in Table (1) demonstrated that the penetration value was directly proportional to the nematode inoculum level in all nematode species in the tested insects. In this line, the highest value was obtained by the inoculum 1600 IJs especially with *H. bacteriophora*. Apparently, the penetration % was inversely proportional with the lowest inoculum levels. On the other hand, the Pf and Rr at the inoculum 400 IJs were significantly higher than the other inoculum levels and there were no significant differences between *H. bacteriophora* and *H. indica*. No significant difference was recorded between *S. carpocapsae* and *S. arinarium.*

Regarding *S. littoralis*, Table (2) demonstrated that Pi, Pi%, Pf, and Rr had the same trend as in the case of *G. mellonella*. However, all these values in *S. littoralis* were less than *G. mellonella*. Overall, *H. bacteriophora* was the highest efficient nematode than the rest.

DISCUSSION

Entomopathogenic nematodes proved high efficiency in killing several species of insect pests; some are specific to a particular insect host or even a specific developmental stage while others have a wide range of hosts (Odendaal et al., 2016; Williams et al., 2015). Hence, a few EPNs can be used as commercial products against some insects in the field application (Georgis et al., 2006; Koppenhöfer et al., 2020; Askary and Abd-Elgawad, 2021), and these is depending on the suitability of this host to the nematode species (Dillman et al., 2012). In this study, the four selected EPN species were highly effective in killing the two insect hosts, *Galleria mellonella* and *Spodoptera littoralis*. Moreover, *G. mellonella* was more sensitive to the EPNs than *S. littoralis* larvae. Differences in the susceptibility may reveal the mechanism of parasitism and this may be due to the relative susceptibility of the insect to the nematode species (Langford et al., 2014; and Hübner et al., 2017), and the tough cuticle of the insect (Garriga et al., 2018). Moreover, it may be attributed to the symbiotic bacteria within the EPN body (*Photorhabdus* and *Xenorhabdus* in *Heterorhabditis* and *Steinernema*, respectively) and the host immune system response (Eleftherianos et al., 2017) after being released in their hemolymph. This latter may have a toxic immunosuppressive effect on the insect host (Liao et al., 2017; Peña 2015). In addition, the cellular response of the insect hosts is highly different from one species to another (Alvandi et al., 2014; Rahatkhah et al., 2015; Lalitha et al., 2018). Also, the enzyme activity such as phenoloxidase and proteases may demonstrate such differences (Ibrahim et al., 2015; Ebrahimi et al., 2018).

As stated in the results, *Heterorhabditis* especially *H. bacteriophora* recorded the highest insect mortality percentage and the highest penetration value followed by *H. indica, S. carpocapsae* and *S. arinarium* in *G. mellonella and S. littoralis*. This superiority of *H. bacteriophora* may be attributed to the fact that *Steinernema* contain a venom with high amount of proteases viz. zinc carboxypeptidases, serine carboxy-peptidases, eukaryotic aspartyl proteases, trypsins (Peña 2015; Lu et al., 2017). For that, the insect may have the chance to challenge the nematode infection in the case of *Steinernema* by encapsulating or melanizing the nematode and/or their bacteria in the hemolymph and this may explain the superiority of *Heterorhabditis* in their lethal effect than *Steinernema* or their symbionts (Li et al., 2007). Moreover, it may be correlated to *Heterorhabditis* bacterial in producing several types of toxins as well as a large amount of urease in the insect haemocoel (Ferreira- DaSilva et al., 2000; Blackburn et al., 2016) and this will lead to the blockage of insect enzyme's activity. One or more of the previous factors could lead to a cardinal importance and

consequently to choose the suitable species of the EPNs to a specific target pest (Lewis et al., 1992; Lewis, 2002).

On the other hand, in most cases, when the EPNs find their host, the infection will occur, but the rate of reproduction and the final population of EPN is highly correlated to the relative host susceptibility as well as the EPNs species, the number and type of bacteria in within the IJs (Rahoo et al., 2016a, b, 2017b; Nabeel et al., 2018) and its initial population (Koppenhöfer and Kaya, 1995). In contrast, when IJs are applied in high numbers, it is likely that at least some hosts get infected by numerous nematodes. Yet, such multiple infections may lead to competition between nematodes.

CONCLUSION

Heterorhabditis spp. and *Steinernema* spp. confirmed their high suppression of *G. mellonella* and *S. littoralis* populations. Moreover, *H. bacteriophora* is the most suppressive species against the two lepidopteran hosts. So, it is documented herein to be used effectively in the management of these pests as an alternative method instead of chemical insecticides to avoid the chemical harmful effects on human health, nontarget organisms, plants, and environment.

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الملخص العربى

تأثير أنواع مختلفة من جنسى النيماتودا الممرضة للحشرات *Steinernema* and *Heterorhabditis* ضد كلا من دودة الشمع الكبيرة *Galleria mellonella* ودودة ورق القطن *Spodoptera littoralis*

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تعتبر النيماتودا الممرضة للحشرات أحد دعائم المكافحة الحيوية الفعالة ضد بعض الحشراتالهامة اقتصاديا وعلى رأسها دودة ورق القطن *Spodoptera littoralis* و دودة الشمع الكبيرة *Galleria mellonella* لما تسببه كلا منهما من أضرار اقتصادية فادحة.

لذلك تناول البحث اختبار كفاءة بعض الانواع من جنس *Heterorhabditis* وجنس *Steinernema* لتحديد ايهما اكثر كفاءة للتوصية باستخدامها فى التطبيق الحقلى وكانت الأنواع المستخدمة كالتالى: *Steinernema carpocapsae*(Sc), *S. arenarium*(Sa), *Heterorhabditis bacteriophora*(Hb) and *H. indica*(Hi) وباستخاد تركيزات متصاعدة من الأنواع الأربعة وهى (0,200,400,800, and 1600 IJs) ضد العمر اليرقى الخامس لكلا الحشرتين وتم تسجيل نسب الموت بعد ٢٤ ساعة و٤٨ ساعة كما تم تشريح اليرقات لمعرفة التعداد الاولى للنيماتودا التى دخلت كل حشرة وكذلك تم تسجيل أعداد النيماتودا التى خرجت من كل حشرة بعد فترة التكاثر (حوالى ١٠ أيام).

ومن النتائج يتضح أن النيماتودا *Heterorhabditis bacteriophora* كانت أكثر كفاءة من الانواع الاخرى حيث كانت النتائج كالتالى: 83% , 75.7,79.7,82.3 بعد ٢٤ ساعة و وصلت الى , 98.0,99.3,100.0 100.0% بعد ٤٨ ساعة فى دودة الشمع. بينما كانت 83.8 , 81.7 ,78.3, 75.0بعد ٢٤ ساعة ووصلت الى 100.0 , 97.3,98.3,100.0 بعد ٤٨ ساعة فى دودة ورق القطن. سجلت أعلى معدلات الدخول (Pi) فى دودة ورق القطن ودودة الشمع عند تركيز ١٦٠٠ يرقة نيماتودا وكان ٢٩٥ IJs و ٢٢١ IJs بالترتيب.وعلى العكس كانت نسبة ال (Pf) أعلى ما يمكن فى التركيز ٢٠٠ و٤٠٠ IJs .

واعتمادا على النتائج المتحصل عليه فانه من الافضل ان تستخدم النيماتودا من النوع *Heterorhabditis bacteriophora* ضد دودة ورق القطن والدودة القارضة بكفاءة أكثر من غيرها.