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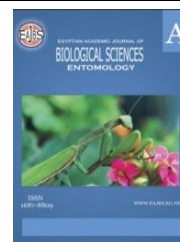
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## Mass Propagation of Two Entomopathogenic Nematode Species on Different Larval Species in Relation to The Resultant Yield of Nematode Juveniles

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### ABSTRACT

The 5<sup>th</sup> instar larvae of each of the four insect species were assayed about their capability to play as host for mass propagation of juveniles of two beneficial entomopathogenic nematodes (EPN) species; *Heterorabditis bacteriophora* and *Steinernema carpocapsae* in the laboratory at 25 °C and 65±5 % R.H. After exposure of larvae at the rate of 1000 IJs/ 5 larvae, daily microscope inspections were carried out until larval mortality and penetration of the new IJs out from the host larvae. Counts of the newly emerged juveniles were carried out periodically and successively (3, 6, 9, 12, 15, 20, 25 and 30 days after starting emergence). Larvae of *Galleria mellonella* manifested the shortest period from exposure to IJs until larval mortality (2 days), as opposed to 2.33 days in the case of *Spodoptera frugiperda*, 2.33 and 3 days after infection of *Spodoptera littoralis* larvae by *H. bacteriophora* and *S. carpocapsae* IJs, respectively, and 4.33 and 2.33 days, respectively for *T. molitor* larvae indicating the longest period after infection to mortality.

As for the period from the time of exposure until starting of IJs emergence, that was the shortest (7 days) in the case of *S. frugiperda* infected by either of the two EPN species and *G. mellonella* (by *S. carpocapsae*), while this period was the longest for *T. molitor* infested by *H. bacteriophora* and *S. carpocapsae* ( 12 and 14 days, respectively). After 30 days of starting emergence out of host larvae, the highest mean of the total number of *H. bacteriophora* juveniles (364767 IJs) resulted from *T. molitor* larvae, followed by 284680 IJs/ *S. frugiperda* larvae and 259817 IJs from *G. mellonella* larvae. While, in the case of *S. carpocapsae*; the highest number of harvested juveniles (341790 IJs) was produced from *G. mellonella* larvae, followed by 258363 IJs/ a *S. frugiperda* larvae and 246633 IJs/ a *S. littoralis* larvae.

As a general conclusion, for mass – propagation of *H. bacteriophora*, rearing on *T. molitor* larvae is recommended, followed by *G. mellonella*. On the same target for *S. carpocapsae*; to obtain the highest production of juveniles, rearing on *G. mellonella* larvae is the best followed by *S. littoralis*, then *S. frugiperda* larvae.

### INTRODUCTION

Entomopathogenic nematodes are effective bio-control organisms capable to infect and kill soil-dwelling and above-ground insect pests (Kaya and Gaugler, 1993 and Laznik *et al.*, 2010). These nematodes belong, mainly, to Steinernematidae and Heterorhabditidae

(Phylum: Nematoda). The two genera, *Heterorhabditis* and *Steinernema*, are the most important EPN Genera commonly used in pest management programs even though there are about 40 nematode families reported to control insect pests (Kaya and Koppenhöfer, 2004). Around 100 species of *Steinernema* and 26 species of *Heterorhabditis* have been recorded worldwide (Cimen *et al.*, 2016 and Shahina *et al.*, 2016). *Steinernema* and *Heterorhabditis* EPNs are known as parasites of lepidopteran, coleopteran larvae, and occasionally of orthopterans, dipterans, and hymenopteran (Klein, 1990; Poinar, 1990 and Grewal *et al.*, 2010).

The life stages of EPNs include eggs, juveniles and adults. The free-living, parasitic 3<sup>rd</sup> juvenile stage is known as the infective juvenile (IJ). The IJs of *Steinernema* and *Heterorhabditis* species transfer the symbiotic bacteria of the genus *Xenorhabdus* and *Photorhabdus*, respectively in the host's hemocoel (Campbell and Lewis, 2002). These two bacterial species multiply rapidly in nutrient-rich insect hemolymph and produce toxins, causing septicemia resulting in the death of the host within 24-48 hours (Bedding and Molyneux, 1982). The IJs further develop into adults and subsequently complete one or more generations depending on the conditions within the host (Poinar, 1990). These IJs penetrate the host's integument and seek out new hosts in the environment for their survival and development.

The two EPN genera, *Steinernema* and *Heterorhabditis*, are widely used for the biological control of many insect pest species. These beneficial EPNs are successfully mass-produced in vivo techniques using larvae of the greater wax moth. The produced IJs penetrate through the host integument to be harvested and stored to be later used for insects' biological control. The use of bio-agents for pest control is, generally, beneficial to reduce the intensive applications of chemical insecticides which have many disadvantages against the agricultural environment. (Manochaya, *et al.*, 2022).

The present study was carried out to investigate the possibility and volubility of mass-propagation of the two EPN species in larval bodies of 4 insect species be as *S. frugiperda*, *S. littoralis*, *T. molitor* and *G. mellonella*. The production rates of IJs in the larva of each of the four species were evaluated to be, qualitatively, compared to those produced from larvae of each of the other three host species hoping, to pinpoint the best host for economic mass propagation of either *H. bacteriophora* or *S. carpocapsae*.

## MATERIALS AND METHODS

### EPNs and Insect Hosts' Culture:

Frist stock culture of *H. bacteriophora* 88 and *S. carpocapsae* (All) juveniles were brought from the Plant Protection Research Institute (ARC), Dokki, Egypt. Juveniles of each of the two EPN species were, separately, propagated in the biology laboratory of the plant protection Department, Faculty of Agriculture, Benha University on full- grown larvae of *G. mellonella* as described by Shairra, (2000).

5<sup>th</sup> instar larvae of four host species were assayed for the production of juveniles of each of the two entomopathogenic nematodes. These host species were the fall armyworm, *Spodoptera frugiperda*; mealworm, *Tenebrio molitor*; wax moth, *Galleria mellonella* and cotton leaf worm, *Spodoptera littoralis*. Larvae of each of the four host species were reared in the mentioned laboratory as followed:

### *S. frugiperda* and *S. littoralis*:

Larvae of *S. frugiperda* and *S. littoralis* were reared, separately under laboratory conditions in plastic containers (25×20×10 cm.) and fed on castor bean leaves until pupation. Pupae were collected and placed in plastic jars until adults' emergence. In the plastic jars moths fed on (10%) sugar solution on cotton pieces. Clusters of eggs were collected daily

and transferred to clean Petri dishes until hatching. Neonate larvae were fed on castor bean leaves until the 5<sup>th</sup> instar (the used instar for the experiments).

***G. mellonella*:**

*G. mellonella* larvae were reared in glass jars (8 cm diameter and 20 cm highest) containing an artificial diet consisting of (wheat flour, corn flour, milk powder, baking yeast powder, honey and glycerin) as described by Metwally *et al.*, (2012), and provided with perforated plastic covers. A piece of suitable size black muslin cloth was placed to cover the bottom of each jar for egg laying under laboratory conditions. Every 24 hours, the deposited eggs were collected and transferred into another clean jar and left until the eggs hatched under the same conditions. Freshly hatched larvae were placed in clean jars (5x15 cm) and provided with an artificial diet.

***T. molitor*:**

*T. molitor* larvae were reared in a plastic cage (25×20×10 cm.) with light pored covers. Adults were reared every 5 pairs in a plastic cage (25×20×10 cm.) for egg production. The eggs were incubated, and after hatching, the larvae were reared at 25 °C in a substrate with wheat bran.

**Exposure of Tested Larvae for Infection by EPNs Species:**

Five of the 5<sup>th</sup> instar larvae of each host insect species were placed on white paper tissue in a plastic cup (8×8×4 cm). A volume of 1 ml distilled water containing 1000 IJs of either of the EPNs was distributed to the 5 larvae. The same technique was repeated five times for each EPN species on the 4 insect species. All the treated larvae were daily observed and the time from treatment until larval mortality was recorded.

**Isolation and Counting of EPNs IJs Emerged from Every Insect Host Larva:**

At the time of larval mortality, larvae were transferred to other containers of the same size containing water and a plastic cover covered with gauze (white trap modified by Kaya and Stock, 1997), on which the dead larvae (cadavers) were placed to attract infecting nematodes to descend into the water. The numbers of IJs individuals of EPNs fallen in the distilled water were counted under an inspection microscope with the aid of an automatic counter after 3, 6, 9, 12, 15, 20, 25 and 30 days. Counting of IJs trapped in 20 ml distilled water for each count continued until no more IJs emerged from the host insect (cadaver) in the water. The total number of IJs/ every larva was calculated and averaged to facilitate a comparison between the four hosts' production of EPNs. Also, the total number of IJs emerged /larvae could be determined. Finally, the relation between the mean of weight/ larvae and the total count of emerged IJs was calculated for every host species.

**Statistical Analysis:**

Obtained data were analyzed using ANOVA with three factors at a 0.05 significance level. Pairwise comparisons were performed using LSD value for multiple comparisons. All statistical analyses were carried out using SPSS soft program.

## RESULTS

After releasing IJs of *H. bacteriophora* and *S. carpocapsae*, separately, on the 5<sup>th</sup> instar larvae of the fall armyworm, greater wax moth, mealworm and cotton leaf worm) for quantitative propagation of IJs, continuous daily microscopic inspections proved the susceptibility of all the tested larvae for infection by either of the two EPN species (Tables, 1 &2) and Figure (1).

**Weight of Used Host Larvae in Relation to Host Mortality and The Emergence of IJs of EPNs:**

The mean weights of larvae used for the experiment were recorded before infection to make a relation between host weight and the number of emerged nematodes/hosts. Mean

weight/5 larvae of four insect hosts larvae were 1.70 and 1.68 g/5 larvae of fall armyworm, 0.80 and 0.94 g/ for *G. mellonella*, 0.75 and 0.77 g/ for *T. molitor* and finally, 1.04 and 1.07 g for 5 of *S. littoralis* larvae used for infection with *H. bacteriophora* and *S. carpocapsae*, respectively.

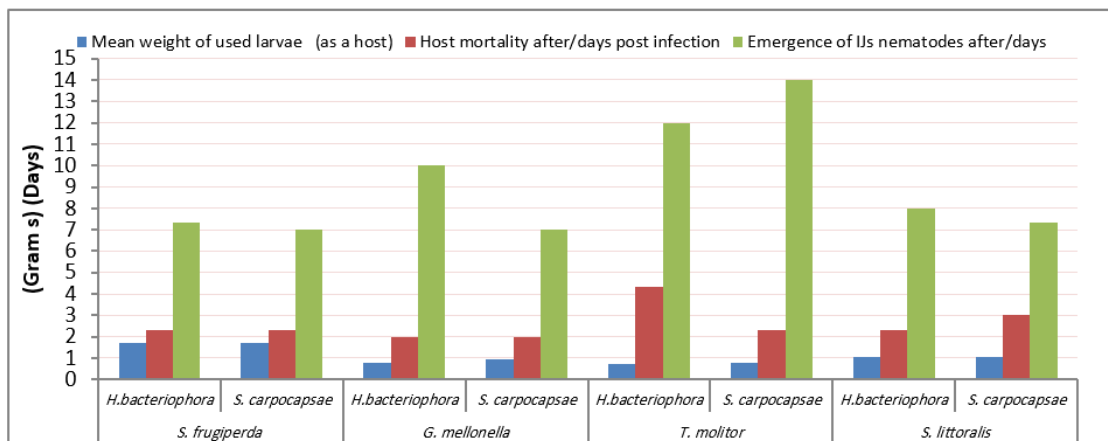
Data in Table, 1 and Figure,2 indicated that after infection of larvae of the four tested species with both EPN species of; *H. bacteriophora* and *S. carpocapsae* at the dose of 1000 IJs / 5 larvae, the mean period from exposure time until host mortality was, 2.33 days for *S. frugiperda* after infection with either of the two EPN species 2.00 days for *G. mellonella* after infection with *H. bacteriophora* and *S. carpocapsae* and mortality occurred after 4.33 and 2.33 days exposure respectively, while for *S. littoralis*, this period reached 2.33 and 3.00 days, respectively. Data in Table (1) and Fig. (1) indicated that the shortest post-infection period until mortality was only 2 days on *G. mellonella* larvae, while the longest duration until mortality reached 4.33 days recorded on *T. molitor* larvae after infection with *H. bacteriophora*.

Data in Table (1) and Figure (1), demonstrate the durations until the first emergence of IJs post-infection with 1000 IJs/5 larvae of the four insect species. Data showed that the first emergence of *H. bacteriophora* IJs occurred 7.33, 10.00, 12.00 and 8.00 days after infection of *S. frugiperda*, *G. mellonella*, *T. molitor* and *S. littoralis* larvae, respectively, while the first emergence of *S. carpocapsae* IJs on the same four hosts occurred after, 7.00, 7.00, 14.00 and 7.33 days, respectively. These data indicated that, generally, *H. bacteriophora* needed a longer period than *S. carpocapsae* to leave its host larvae.

**Table 1:** Mean weights of 4 host larval species and periods until mortality and the emergence of EPNs infective juveniles.

Insect Host	EPN species	Mean weight of used 5 larvae (as a host)	Post-infection period until host mortality	Post-infection period until IJs emergence
<i>S. frugiperda</i>	<i>H. bacteriophora</i>	1.70±0.02 <sup>a</sup>	2.33±0.33 <sup>bc</sup>	7.33±0.33 <sup>d</sup>
	<i>S. carpocapsae</i>	1.68±0.02 <sup>a</sup>	2.33±0.33 <sup>bc</sup>	7.00±0.00 <sup>d</sup>
<i>G. mellonella</i>	<i>H. bacteriophora</i>	0.80±0.04 <sup>d</sup>	2.00±0.00 <sup>c</sup>	10.00±1.53 <sup>bc</sup>
	<i>S. carpocapsae</i>	0.94±0.02 <sup>c</sup>	2.00±0.00 <sup>c</sup>	7.00±0.00 <sup>d</sup>
<i>T. molitor</i>	<i>H. bacteriophora</i>	0.75±0.01 <sup>d</sup>	4.33±0.33 <sup>a</sup>	12.00±0.00 <sup>ab</sup>
	<i>S. carpocapsae</i>	0.77±0.02 <sup>d</sup>	2.33±0.33 <sup>b</sup>	14.00±1.00 <sup>a</sup>
<i>S. littoralis</i>	<i>H. bacteriophora</i>	1.04±0.02 <sup>b</sup>	2.33±0.33 <sup>b</sup>	8.00±0.00 <sup>cd</sup>
	<i>S. carpocapsae</i>	1.07±0.04 <sup>b</sup>	3.00±0.00 <sup>ab</sup>	7.33±0.33 <sup>d</sup>
<b>LSD at 0.05</b>		<b>0.08</b>	<b>0.79</b>	<b>2.00</b>

a, b & c: There is nonsignificant difference ( $P>0.05$ ) between any two means, within the same column having the same superscript letter.



**Fig. 1:** Mean host larval weights and periods from exposure until larval mortality and IJs emergence of *H. bacteriophora* and *S. carpocapsae* on four insect hosts' larvae.

**Isolation and Counting of IJs Emerged from Larvae of The Four Hosts Larval Species After Infection by *H. bacteriophora* and *S. carpocapsae*:**

After the emergence of IJs from each host larva, the total number of emerged IJs/5 larva were counted after 3, 6, 9, 12, 15, 20, 25 and 30 days of infection by 1000 IJs/5 larva. The total counts of emerged IJs/larvae were calculated and recorded.

**Table.2:** Mean numbers of IJs of *H. bacteriophora* and *S. carpocapsae* emerged from four insect hosts larval species after successive days from first emergence/ larva up to one month.

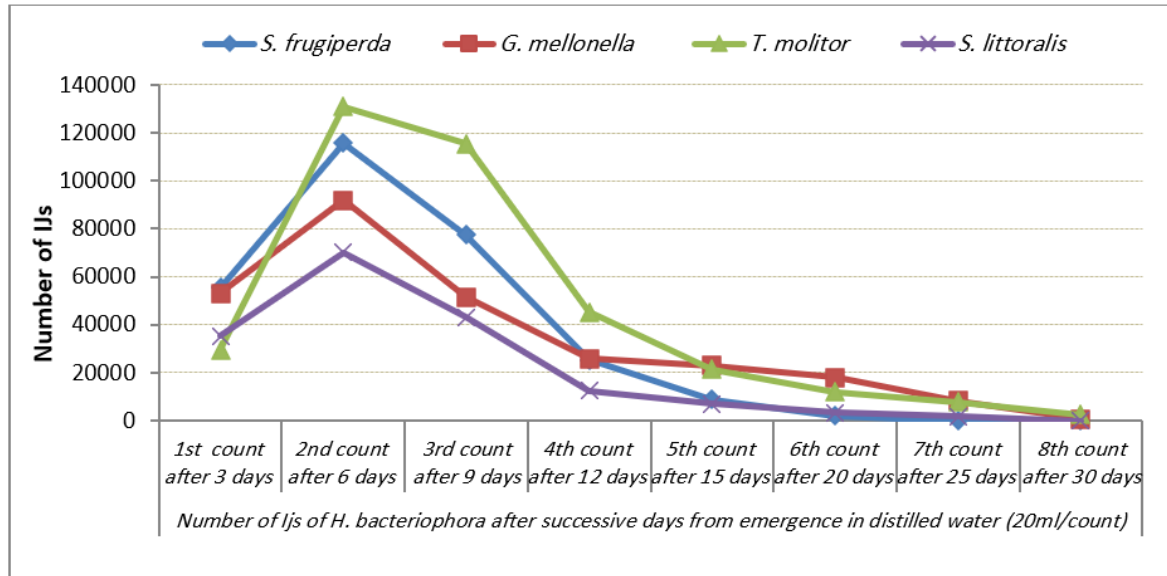
Insect Host	EPN species	Number of IJs after successive days from emergence in distilled water (20ml/count)								Total count of IJs
		1 <sup>st</sup> count after 3 days	2 <sup>nd</sup> count after 6 days	3 <sup>rd</sup> count after 9 days	4 <sup>th</sup> count after 12 days	5 <sup>th</sup> count after 15 days	6 <sup>th</sup> count after 20 days	7 <sup>th</sup> count after 25 days	8 <sup>th</sup> count after 30 days	
<i>S. frugiperda</i>	<i>H. bacteriophora</i>	55480 ±18	115733±35	77400±30	25267±11	8867±43	1933±71	0 ±00	0±00	284680±98
	<i>S. carpocapsae</i>	97663 ±33	70500±35	23467±12	28667±34	7867±36	21733±11	10400 ±22	±13	258363±70
<i>G. mellonella</i>	<i>H. bacteriophora</i>	53067 ±29	91817±66	51600±34	25800±14	22867±17	17900±12	8200 ±0.00	600 ±0.00	259817±13
	<i>S. carpocapsae</i>	116157±43	93970±49	47533±23	43093±97	16533±56	29067±76	9733 ±37	5333 ±22	341790±56
<i>T. molitor</i>	<i>H. bacteriophora</i>	29600±15	131000±15	115600±14	45267±13	21333±58	11867±26	7667 ±59	2433 ±38	364767±33
	<i>S. carpocapsae</i>	28333±26	26400±88	32133±58	18333±40	16133±39	11333±24	3333±35	1550 ±55	137033±43
<i>S. littoralis</i>	<i>H. bacteriophora</i>	35350±15	70250±27	43067±20	12533±38	7000±48	3200±0.00	1600 ±0.00	0 ±00	169800±29
	<i>S. carpocapsae</i>	26750 ±33	130083±32	37400±41	15667±35	26200±23	7667±71	2867 ±14	0±00	246633±43

**Volumes of harvested IJs from 5<sup>th</sup> instar larvae of 4 host species:**

**1-*H. bacteriophora*:**

Data in Table (2) and Figure (2), show that the first count of IJs emerged after 3 days from the first emergence were 55480, 53067, 29600 and 35350 IJs / host larval / 20 ml. distilled water for *S. frugiperda*, *G. mellonella*, *T. molitor* and *S. littoralis*, respectively. As for the 2<sup>nd</sup> count, those increased for the four host species as those reached 115733, 91817, 131000 and 70250 IJs /20 ml. for the same 4 host species, respectively. In the 3<sup>rd</sup> count (after 9 days), the numbers of *H. bacteriophora* IJs were 77400, 51600, 115600 and 43067 IJs /20 ml., respectively. The total count of IJs / larva indicated a decrease, being; 25267, 25800, 45267 and 12533 IJs /20 ml. from larvae of the 4 host insects, respectively. For the 5<sup>th</sup> count (a couple of weeks post-emergence), counts reached 8887, 22.867, 21333 and 7000 IJs/ 20 ml. water, respectively. Because of the decreased counts of harvested IJs, the periods between the counts were prolonged to five days, so after 20 days (the 6<sup>th</sup> count) indicated 1933, 8200, 7667 and 1600 IJs/20ml. in *S. frugiperda*, *G. mellonella*, *T. molitor* and *S. littoralis* larvae, respectively. Depending on this step it was the last count for *H. bacteriophora* on *S. frugiperda*. The 7<sup>th</sup> count revealed 8200, 7667 and 1600 IJs/20ml for *G. mellonella*, *T. molitor* and *S. littoralis*, respectively, that was the last count for *S. littoralis*. And finally, the last count (8<sup>th</sup>) revealed; 600 and 2433 IJs on *G. mellonella* and *T. molitor*, respectively, (Table, 2 and Fig.,2).

The total counts of *H. bacteriophora* IJs harvested during one month reached 284680 IJs / 120 ml. water from *S. frugiperda*, 259817 IJs / 160 ml. water on *G. mellonella*, 364767 IJS from *T. molitor* in 160 ml water and finally recorded 169800 IJs from on *S. littoralis* larva placed in 140 ml of distilled water Table (2) and Figure (2).



**Fig. 2:** harvested counts of *H. bacteriophora* IJs emerged from 4 species of larvae after 30 days of exposure to 1000 IJs.

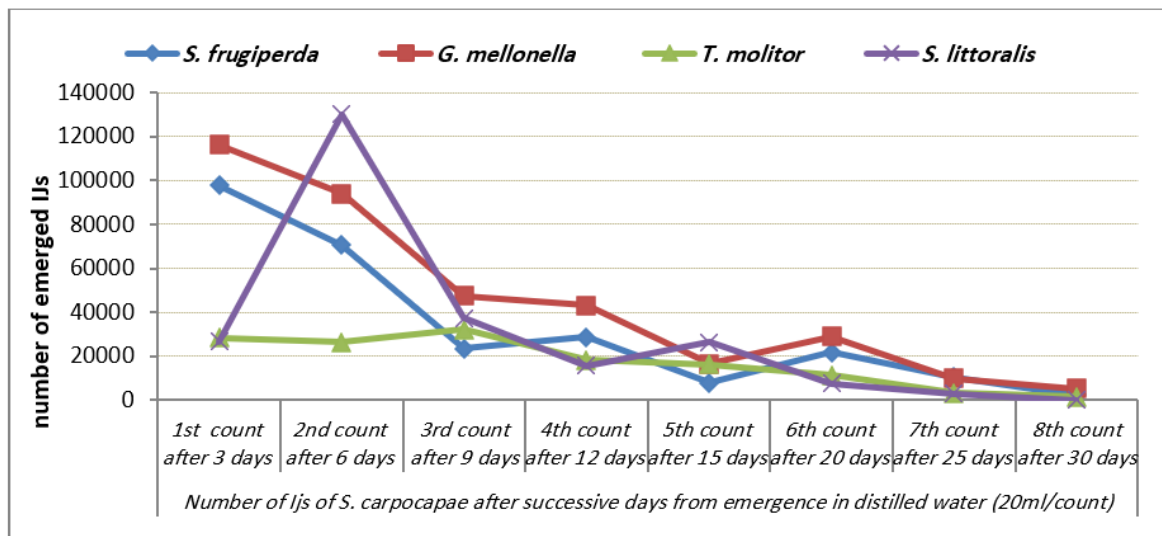
### 2-*S. carpocapsae*

Counts of *S. carpocapsae* IJs, 3 days after IJs emergence from 5<sup>th</sup> instar larvae of four host species were 97663, 116157, 28333 and 26750 IJs / 5 larvae of *S. frugiperda*, *G. mellonella*, *T. molitor* and *S. littoralis*, each in 20 ml. distilled water, respectively (Table, 2 and Fig.3). Numbers of the 2<sup>nd</sup> count (6 days after emergence) showed increased numbers to reach 70500, 93970, 26400 and 130083 IJs / 5 larvae 20 ml. water opposed to, 23467, 47533, 32133 and 37400 IJs/5 larvae in 20ml. water, respectively, after 9 days of IJs emergence (3<sup>rd</sup> count). While those of the 4<sup>th</sup> count was ; 28667, 43093, 18333 and 15667, and subsequently for the 5<sup>th</sup> count (two weeks after IJs emergence) were, 7867, 16533, 16133 and 26200 IJs/ 5 larvae of *S. frugiperda*, *G. mellonella*, *T. molitor* and *S. littoralis*, respectively, in 20 ml. distilled water. Twenty days after IJs emergence (the 6<sup>th</sup> count), these numbers were 21733, 29067, 11333 and 7667 IJs / 20ml. water, respectively. For the 7<sup>th</sup> count, the IJs of *S. carpocapsae* dropped down to 10400, 9733, 3333 and 2867 IJs/ 5 larvae in 20ml. water for the same host species, respectively, after the 7<sup>th</sup> count, infected larvae of *S. littoralis* did not reveal any more IJs, so the counted IJs for the last count (8<sup>th</sup>) were 2300, 5333 and 1550 IJs from *S. frugiperda*, *G. mellonella* and *T. molitor* larvae, respectively.

**Table 3:** Amounts of EPNs produced from different host larval species / gm. weight mean of different hosts:

Insect host	Weight (of 5 larvae) in gm.	EPN species	Total count of IJs/ 20 ml dis. Water / 5 larva	IJs total count / larva	IJs count/ gm.
<i>S. frugiperda</i>	1.70±0.02 <sup>a</sup>	<i>H. bacteriophora</i>	284680	56936	167459
	1.68±0.02 <sup>a</sup>	<i>S. carpocapsae</i>	258363	51672	153788
<i>G. mellonella</i>	0.80±0.04 <sup>d</sup>	<i>H. bacteriophora</i>	259817	51963	324771
	0.94±0.02 <sup>c</sup>	<i>S. carpocapsae</i>	341790	68358	363606
<i>T. molitor</i>	0.75±0.01 <sup>d</sup>	<i>H. bacteriophora</i>	364767	72953	486356
	0.77±0.02 <sup>d</sup>	<i>S. carpocapsae</i>	137033	27406	177965
<i>S. littoralis</i>	1.04±0.02 <sup>b</sup>	<i>H. bacteriophora</i>	169800	33960	163269
	1.07±0.04 <sup>b</sup>	<i>S. carpocapsae</i>	246633	49326	230498

From data presented in Table, 2 and Figure 3, the total number of *S. carpocapsae* IJs emerged during one month reached 258363 IJs / 160 ml. water from *S. frugiperda*, 341790 IJs in 160 ml. water on *G. mellonella*, 137033 IJS from *T. molitor* in 160 ml water and finally recorded 246633 IJs from *S. littoralis* isolated in 140 ml of distilled water. From data concerning the produced juveniles from each of the tested larvae, the suitability and validity of the 4 species for mass-production of *S. carpocapsae* juveniles may be arranged ascendingly as; *G. mellonella* (the recommended host larvae), followed by *S. frugiperda*, *S. littoralis* and *T. molitor* (lowest production rate of *S. carpocapsae* IJs). At the same time, in case of the absence of *G. mellonella* larvae for mass propagation of *S. carpocapsae* IJs, larvae of the other 3 insect species may be used descendingly.



**Fig. 3:** Numbers of emerged *S. carpocapsae* IJs from 4 insect hosts larval species throughout 30 days after infection with 1000 IJs.

In spite of the clear differences in numbers of EPN juveniles produced from either *H. bacteriophora* or *S. carpocapsae*, due to the differences in weights between 5<sup>th</sup> instar larvae of different tested host species, the volume of production of EPN juveniles was referred, mathematically, to one gram weight of larva from each of the tested host insect species. Data are clearly shown in Table (3), these data confirmed that the highest production of *H. bacteriophora* juveniles /gm. weight (means of 486356 juveniles) resulted by rearing on larvae of the mealworm, *T. molitor*, followed by 324771 juveniles /gm. weight of *G. mellonella* larva. In the case of *S. carpocapsae* the highest production (363606 juveniles /gm. weight) occurred by rearing on *G. mellonella* larvae, followed by 230498 juveniles /gm. weight) in case of rearing on *S. littoralis*.

## DISCUSSION

The greater wax moth larva, *G. mellonella* is the most common and widely used host for the production of EPNs. The highly susceptible nature and wide availability of *G. mellonella* make them a highly suitable candidate for the in vivo production of EPNs. Furthermore, *G. mellonella* is easily reared on artificial diets within a short time. These larvae produce a sufficient number of EPNs making them highly feasible for in vivo production. This conclusion, completely, agrees with the present results. Also, (Shapiro-Ilan and Gaugler, 2002; Blinova and Ivanova, 1987).



Indicated that *T. molitor* is also a promising host for mass-production of EPNs. That was clear in the present investigation in the case of mass production of *H. bacteriophora*. While, for *S. carpocapsae* production, *T. molitor* larvae seemed unfavorable as those produced the least total count of IJs. In the present study, the single *G. mellonella* produced an average of 51963 IJs of *H. bacteriophora* or 68358 IJs of *S. carpocapsae* while, (Poinar, 1979; Shapiro-Ilan *et al.*, 2002; Woodering and Kaya, 1988) reported 100,000 to 200,000 IJs as produced yield / *G. mellonella* larva.

In contrast to the present results Prabowo *et al.*, (2019) indicated that *S. carpocapsae* may be well mass-propagated on *T. molitor* last instar larvae, while the present results confirmed that this was true for *H. bacteriophora* not for *S. carpocapsae*.

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## ARABIC SUMMARY

الاكثار الكمي لنوعين من الـنيماتودا الممرضة للحشرات على يرقات أنواع مختلفة من الحشرات و تأثيرها علي عدد الأطوار المعديّة الناتجة

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تم تقييم يرقات العمر الخامس لاربعة أنواع من الحشرات لمعرفة قدرتها كعوائل للاكثار الكمي لنوعين من الـنيماتودا الممرضة للحشرات وهي *Heterorabditis bacteriophora* و *Steinernema carpocapsae* وذلك تحت الظروف المعملية عند درجة حرارة 25 درجة مئوية و  $65 \pm 5\%$  درجة رطوبة نسبية فوجد انه بعد تعرض اليرقات لتركيز 1000 IJs / 5 يرقات وعند إجراء فحوصات مجهرية لتعداد النوعين بشكل دوري بعد (3 ، 6 ، 9 ، 12 ، 15 ، 20 ، 25 ، 30 يوماً من بدء ظهور الأطوار المعديّة). فقد أظهرت يرقات دودة الشمع الكبيرة أقصر فترة حتى موت اليرقات والتي بلغت يومين ، مقابل 2.33 يوماً في حالة يرقات دودة الحشد الخريفية وكانت 2.33 و 3 أيام في حالة تعرض يرقات دودة ورق القطن للنوعين *H. bacteriophora* و *S. carpocapsae* على التوالي . وقد بلغت 4.33 و 2.33 يوم في حالة تعرض يرقات دودة الطحين مما يشير إلى انها بلغت أطول فترة بعد التعرض للإصابة حتى الموت بكلا النوعين السابقين على التوالي.

اما بالنسبة للفترة من وقت الإصابة حتى بداية ظهور الأطوار المعديّة، كانت أقصر فترة والتي بلغت 7 أيام في حالة دودة الحشد الخريفية المصابة بكلا النوعين . وكذلك دودة الشمع الكبيرة عند الإصابة بواسطة *S. carpocapsae* ، في حين كانت الفترة الأطول في حالة دودة الطحين المصابة بكلا النوعين والتي بلغت (12 و 14 يوماً ، لـ *H. bacteriophora* و *S. carpocapsae* على التوالي). بعد 30 يوماً من العدوي ، نتج أعلى اعداد من *H. bacteriophora*. ليرقات دودة الطحين والذي بلغ 364767 طور معدي. بينما في حالة *S. carpocapsae* كان أكبر عدد في يرقات دودة الشمع الكبيرة والذي بلغ 341790 طور معدي ، تليها يرقات دودة الحشد 258363 طور معدي ثم 246633 طور معدي ليرقات دودة ورق القطن. الخلاصة ، في حالة الاكثار الكمي لـ *H. bacteriophora* ، كان العدد الأكبر عند الاكثار علي يرقات دودة الطحين ، يتبعها دودة الشمع الكبيرة؛ اما في حالة *S. carpocapsae* كان أعلى إنتاج عند الاكثار علي يرقات دودة الشمع الكبيرة يليها دودة ورق القطن ثم دودة الحشد الخريفية.